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INTERHEMISPHERIC TRANSFER IN A MARSUPIAL

A behavioural investigation of interhemispheric transfer of visual information in Trichosurus vulpecula.

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, to the University of Auckland, 1975.

Donald MacD. Webster
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ABSTRACT

Eight marsupial phalangers, (Trichosurus vulpecula) with mid-saggital section of the optic chiasma were trained monocularly in a visual discrimination task, as were four which had, in addition, mid-saggital sections of the anterior commissure, fasciculus aberrans and hippocampal commissure. All were tested for transfer to the untrained hemisphere. The results showed that those with the commissures intact transferred information to the hemisphere not trained directly, while those with commissures sectioned did not. It appears that transfer of visual information between cerebral hemispheres can take place in an animal which lacks a corpus callosum, and that the forebrain commissures may be functional equivalent, in this marsupial, of the corpus callosum in eutherians.

A further eight animals received mid-saggital section of the optic chiasma. Two had all other interhemispheric pathways intact, two had mid-saggital section of fasciculus aberrans and hippocampal and anterior commissures, two had fasciculus aberrans only sectioned, and two had fasciculus aberrans only intact. All were trained monocularly in a visual discrimination task and tested for transfer to the opposite hemisphere. Those with all commissural pathways intact and those with only fasciculus aberrans intact demonstrated transfer. Those with all commissures sectioned and those with only fasciculus aberrans sectioned did not show
transfer. It seems probable that integrity of fasciculus aberrans is a necessary and sufficient commissural condition for interhemispheric transfer of visual information in this marsupial.
INTRODUCTION

On the Corpus Callosum

Anatomists have for many years, believed the corpus callosum to be a structure of considerable importance. T.H. Huxley (1863) in "Man's Place in Nature" says,

"The subject requires careful re-investigation, but if the currently received statements are correct, the appearance of the 'corpus callosum' (sic) in the placental mammals is the greatest and most sudden modification exhibited by the brain in the whole series of vertebrated animals - it is the greatest leap anywhere made by Nature in her brain-work."

These sentiments were echoed by Sir Grafton Elliot Smith in 1894 in his preliminary communication upon the cerebral commissures of the mammalia to the Linnean Society. Nevertheless, our understanding of the functions of this structure could, until 1955, be summed up in the words of Stenson (1669) "certes, le corps calleux nous est si inconnu, que pour peu qu'on ayt d'esprit, on en peut dire tout ce qu'on veut."

Stenson's eminent contemporary, William Harvey, considered that "corpus callosum superior pars ventriculorum sustinet cerebrum". (Whitteridge, 1964) And such a view was being put forward - albeit wryly - as late as 1957 by Lashley (Sperry, 1964) although Bykov had much earlier (Pavlov, 1927) shown the independence of the cortices of the cerebral hemispheres following section of the corpus callosum. Only with the development of refined techniques for behavioural investigation during the past two decades, and principally as a
result of the work of Myers (1955, 1962, a,b) and Sperry (1961) has the functional significance of the commissural fibres been slowly elucidated.

The classical experiment is that of Myers (1955), who used cats as subjects. His basic preparation was an animal in which mid-saggital section of the optic chiasma had been carried out. Such a procedure cuts the decussating optic fibres but leaves intact those proceeding ipsilaterally. Thus, primary visual projection is homolateral in that impulses originating in, say, the left eye can, in the first instance, travel only to the left hemisphere and similarly for the right eye and right hemisphere. By occluding one eye, Myers was able to restrict the primary presentation of the visual information in a discrimination task to one hemisphere. When criterion performance was reached he could, by reversing the side of eye occlusion, challenge, via the previously closed eye, the hemisphere which had not had primary presentation of the visual task.

Myers found that if, (and only if) the corpus callosum was intact, then inter-ocular transfer took place. The subject could perform as well with the previously untrained eye as it could with the eye initially trained. In subjects with section of the corpus callosum, no inter-ocular transfer took place, and as long was taken for the second "eye" to master the task as had been taken by the first. Further, Myers showed that section of the corpus callosum after the task had been learned produced no deficit in inter-ocular transfer. From this it is inferred that, with the corpus callosum intact, memory traces are laid down in each hemisphere.
It is now clearly apparent that fibres joining the two hemispheres, particularly by way of the corpus callosum, but also via the anterior commissure and possibly other routes as well, (Trevarthen, 1965) play a major role in the inter-hemispheric transfer of learning based on sensory cues.

It is also apparent that the pattern of connexions between the two cerebral cortices is by no means a simple point to point one. Some cortical areas may send fibres simply to homotypic points, others may send fibres to – or receive fibres from – two quite divergent fields, while still others may be entirely without commissural connexions. (Myers, 1962 a; Ebner and Myers, 1965; Garey, Jones and Powell, 1968; Diamond, Jones and Powell, 1968; Pandya and Nignolo, 1969; Jones and Powell, 1968, 1969) The last pattern of connexions is of particular interest because in the primary somatic and visual sensory areas of the cortex, parts representing the distal parts of the limbs and the periphery of the visual field are not commissurally connected. (Choudhury, Whitteridge and Wilson, 1965; Berlucchi, Gazzeniga and Rizzolati, 1967; Hubel and Wiesel, 1967; Garey et al, 1968; Hughes and Wilson, 1969). On the other hand, those parts representing portions of the body in, and adjacent to, the midline, and the midline portion of the visual field are heavily connected. These connexions presumably ensure the fusion and overlap of sensory messages emanating from mid-line portions of a bilaterally symmetrical receptor apparatus. The reason for the lack of connexions between the representations of the hands and feet and of the periphery of the visual field is obscure. Perhaps it plays a role in ensuring that
confusion does not arise between sensory messages arriving from symmetrical but, in a sense, independent parts of the receptor apparatus.

Early attempts to study the function of the corpus callosum employed informal observation following callosal section. That little disturbance of ordinary behaviour results, has been borne out by split-brain studies in monkeys and humans. There has been opportunity to study humans who have undergone callosal section surgery for the relief of severe epilepsy. These patients show no noticeable disturbance of co-ordination, maintain their internal functions, are alert and active, respond to every-day situations in a usual way and perform just about as well as normal subjects in standard tests of learning ability, (Gazzaniga, Bogen and Sperry, 1962). Specially designed tests are required to reveal that the split-brain is not normal in its function, but consists of two halves which appear to be capable of behaving in a manner quite independent of each other. Trevarthen, (1962) was able simultaneously to teach one half of the eye-brain system in split-brained monkeys to recognise as positive and negative what the other half of the eye-brain system learned as negative and positive. Such a task to an intact monkey produces only irresolvable perceptual conflict. Hemispheres integrated by a corpus callosum are not necessarily superior to hemispheres working independently. Gazzaniga and Young (1967) also with monkeys as subjects, found that in some cases, the two hemispheres, working separately but simultaneously, could perform at a higher level than the hemispheres of commissure-intact controls in the processing
of visual information. Studies in humans (Gazzaniga, Bogen and Sperry, 1963) have shown a similar independence and mutual lack of accessibility to information between the half-brains in commissurotomised man.

The philosophical implications of having two entities capable of independent activity in the one body have not escaped notice. (Sperry, 1966) The role of the corpus callosum in integrating the activity of the two cerebral hemispheres in so-called phyletically higher organisms is well established.

**Comparative Anatomical Considerations**

But the corpus callosum is a unique feature of the eutherian brain. It is not found in monotremes, marsupials or other vertebrates. Absence of a corpus callosum notwithstanding, it is obvious that the cerebral hemispheres in Prototheria and Metatheria are joined. The connexion most readily apparent is the group of fibres passing by way of the anterior commissure, (Smith, 1894; Abbie, 1939; Ebner, 1969) and it has been shown (in monkeys) that this pathway can transmit information essential to the establishment of duplicate memory in each hemisphere. (Sullivan and Hamilton, 1973).

The neuroanatomy of marsupials has long been of interest and has given rise to a substantial body of literature. The context of most studies has been that of classical anatomy, embodying precise and detailed descriptions of the morphology of individual species, together with consideration of position in the traditional phylogenetic series. An understanding has
been sought of the paths by which the structures present in "higher" brains have come to be.

Marsupials occupy a position of particular interest since they, together with Prototheria - the monotremes - provided a bridge, conceptually at least, between the lower vertebrates and the higher animals more readily recognisable as mammals. Outstanding in the field of marsupial neuroanatomy are the systematic studies of hippocampal and precommissural areas and the evolution of the corpus callosum by G. Elliot Smith. Particularly influential was his contribution to the Catalogue of Brains in the Museum of the Royal College of Surgeons of England, which, together with other works elsewhere (Smith, 1896, 1897) constitute the basis of viewpoints still held by most neuroanatomists.

Thus, what follows is both a summary of Smith's work and a brief statement of what is known in the field of the comparative neurology of the cerebral commissures.

The commissures of the telencephalon are usually defined according to the areas of that part of the brain they join together. In eutherian mammals there are three commissures. 

(1) An anterior commissure which connects the two sides of the rhinencephalon. It contains interbulbar fibres carrying olfactory impulses, intertemporal fibres joining the pyriform lobes and amygdaloid nuclei, and in some proto- and metatheria, which lack a true corpus callosum, a small neopallial component. The anterior commissure is always anterior, and in mammals, ventral to the lateral ventricles.
(2) A hippocampal commissure which carries fibres joining the two hippocampi, or the two hippocampal cortices in reptiles. In reptiles this is situated dorsally, and in mammals, where it is called the psalterium, posterior, to the ventricles. In some metatheria, a few neopallial fibres join through the hippocampal commissure.

(3) A corpus callosum which carries only fibres joining the two sides of the neopallium. This is always situated dorsally to the ventricles and forms their roof. Anteriorly it forms a genu in the lamina terminalis and posteriorly a splenium above the psalterium. Reptiles, having no neopallium, unless a small area of general cortex is thus described, do not have a corpus callosum. The principal novel feature of the reptilian brain is the appearance of the hippocampal gyrus, the two hippocampal cortices being joined by a new structure, the hippocampal commissure. Prototherian and Metatherian brains show various stages in the development of the corpus callosum.

(1) The platypus (Ornithorhynchus anatinus) brain is essentially the same as that seen in reptiles, except that a small neopallium is present, the fibres of which cross in the anterior and hippocampal commissures. This hippocampal commissure is situated dorsal to the ventricles. The hippocampi are situated on the medial walls of the hemispheres dorsal to the hippocampal commissure being bounded dorsally by the hippocampal fissure. The hippocampus has almost no descending limb; the commissure is therefore fairly straight with
only a slight posterior hook.

(2) The hippocampus of a bandicoot (Perameles) is larger than that in the platypus and is bent posteriorly downwards and forwards. The hippocampal commissure extends around with this descending limb assuming a crescent-like shape. Smith describes this as the splenium of the hippocampal commissure.

(3) In the higher metatheria, such as the kangaroo (Macropus), the angle of the splenium becomes more acute and the hippocampal commissure assumes the shape of a two-layered structure, the dorsal and ventral layers being united posteriorly at the splenium.

(4) In Eutheria, the dorsal of the two layers of the metatherian hippocampal commissure is composed wholly of callosal fibres, the ventral layer becoming the psalterium. Higher eutherian mammals show this structure fully, but in some lower members of the group such as the mole and the bat, the corpus callosum is composed of neopallial fibres which find a bed in the psalterium.

With progress up the phyletic series from prototheria to eutheria, the hippocampus moves from its dorsal position ventrally and laterally; the dorsal portion of the hippocampal commissure being replaced by the corpus callosum, the splenium of which is continuous with the psalterium in higher eutherians; there often being no distinguishable boundary between them. The ontogenetic development of the corpus callosum in the higher eutheria takes place by the
interstitial growth of callosal cells in the dorsal layer of the psalterium. As the corpus callosum develops caudally, the psalterium, the hippocampus, and the dentate gyrus are compressed, being forced laterally and ventrally. A small part of the original dorsal layer of the hippocampal commissure remains above the newly formed corpus callosum as the induseum griseum or longitudinal stria. The condition of these structures early in foetal life is similar to that seen in some primitive metatheria. Smith believed all three commissures to be serially homologous originating in the lamina terminalis. More recent research on the origin of the corpus callosum and the fate of structures related to it (Abbie, 1939) has added little to Smith's findings. The development of the corpus callosum appears to parallel that of the neopallium through the mammalian series, with the exception that, in those mammals with highly convoluted cerebral hemispheres, the corpus callosum is well developed, whereas in those with smooth hemispheres, it is poorly developed. The contrast is, however, not marked. A comparison of the cross-sectional area of the corpus callosum with the total surface area of the brain in rat and Man (Suitsu, 1920) finds in the rat it is 3.29% and in Man, 4.43%. In some prototheria (echidna) fibres joining the neopallial cortices are carried in the anterior commissure which is highly developed, the cross-sectional area being six times that of the human anterior commissure relative to brain surface area. This increase is almost entirely due to neopallial fibres. In rabbits and bats neopallial fibres are carried in both the anterior commissure via the internal capsule and the corpus callosum.
A small dorsal corpus callosum is present in bats but most neopallial fibres cross in a large ventral commissure. It appears that there is a gradient in the number of neopallial fibres which pass through the anterior commissure from the highest to the lowest mammals. It is possible that a small remnant of neopallial fibres is present in the temporal pole component of the anterior commissure even in the highest forms. Smith's investigations led him to the now generally held conclusion that "the appearance of a corpus callosum can hardly be considered...such a sudden event...as Professor Huxley's remark....seems to imply."

Further, the corpus callosum is not the only brain structure which makes a sudden appearance in the mammals. The appearance of areas in the cerebral cortex with more than three cell layers, that is the neopallium, is equally sudden. Some reptiles have three cortical fields which can be broadly described as medial hippocampal, lateral pyriform (both olfactory) and dorsal general cortex. All three of these areas are composed of simple three-layered cortex. In Didelphis marsupialis the olfactory cortex is still three-layered, but the new non-olfactory cortex is thicker than other archipallium, and may be composed of from four to ten layers; although in area it is not relatively larger than the general cortex in some reptiles. From constituting a small cap to the cerebral hemispheres in primitive metatheria, the neopallium has increased in size relatively to the rest of the brain so that in Man it represents about half the total weight of the brain and contains slightly more than two-thirds of all brain cells. This contrast may be less than it appears because functional value is the result, not
only of an increase in the number of brain cells, but also of the complexity of their interconnections. It follows from this that the increase in the number of interconnections provided by the corpus callosum must increase the functional capacity of the mammalian brain above what would be achieved simply by increasing the size of the neopallium. This proposition presented 19th Century biologists with the temptation to say that the corpus callosum arose specifically for this purpose; a temptation which many of them were not able to resist. In this regard both Smith and Huxley show Lamarkian tendencies when they suggest that the corpus callosum arose to facilitate a shorter connection between the emerging neocortices. It may be true that wherever eutherian mammals have invaded an area, they have replaced most metatheria but in the American continent, where many of the larger metatheria were eliminated by eutherian carnivores, one of the surviving metatheria, the oppossum, does not have a corpus callosum. It appears that natural selection does not necessarily favour a more highly integrated nervous system. Also, some lower eutheria (e.g. the bat and the hedgehog) with quite large neopallial cortices have only a small corpus callosum, most neopallial fibres being carried in the anterior commissure.

The 19th Century view of vertebrate animals was still that of the "scala naturae" and implied attempts were made to trace homologous structures through this series. The problems and fallacies inherent in this approach have been fully discussed by Hodos and Campbell (1969). No present day fish ever was, nor could have been, an ancestor of a
present day amphibian; nor were the metatheria the ancestors of the eutheria. Rather, the evolution of both groups has been parallel from pantotherian ancestors. We know nothing about the soft brain structure of these small, rat-like, arboreal, Jurassic animals. During their evolution, and until the time when they and their descendents emerged as the dominant terrestrial animals at the end of the cretaceous about 100 million years later, their survival against competition from powerful and predatory archosaurs may have been, in large part, dependent on their small size and arboreal habitat. Unfortunately, such features do not readily lead to the deposition of fossil remains and many of them are known only by their teeth. Evolutionary "trees" may prove to be of some use in comparative studies, but comparisons across families and orders must be made very carefully because of the role of adaptation and specialization in evolution. Ontogeny recapitulates phylogeny only in a loose sense.

A Marsupial Phalanger, Trichosurus vulpecula

Friant (1959) has summarised the morphology of the prototherian and metatherian brains. Her comparison of the Polyprotodonta and Diprotodonta again draws attention to the existence in the latter sub-order exclusively of the neopallial pathway named by Smith (1902), fasciculus aberrans.

One Diprotodont in particular, Trichosurus vulpecula, has claimed a fair share of attention, and the neuroanatomy of this animal is probably as well understood as that of any Australian marsupial. In fact, within Metatheria it is
Fig 1

Midsagittal section of the head of Trichosurus vulpecula. The relatively small brain size is apparent as is the absence of a corpus callosum. The scale is in centimeters.
Fig 2

The brain of Trichosurus vulpecula, dorsal surface. The anterior commissure is clearly visible. Scale in cm.

Fig 3

The brain of Trichosurus vulpecula, ventral surface. The optic chiasma can be seen. Scale in cm.
perhaps exceeded only by Didelphis in the nature and extent of neurological investigations. The visual system in Trichosurus vulpecula, for example, has been the subject of extensive investigation by Packer (1941) and Hayhow (1966, 1967) and data on other, particularly somesthetic, pathways is available. (Clezy, Dennis and Kerr, 1961; Dennis and Kerr, 1961 a,b; Megerian, 1968, 1970; and Martin, Megerian and Roebuck). The anatomy of the interhemispheric pathways in Trichosurus vulpecula was investigated in an experimental degeneration study by Heath and Jones (1971).

(On the other hand, published behavioural studies appear to be restricted to that of Pollard and Lysons (1967) who included Trichosurus vulpecula in their series of investigations using the closed field test.)

Trichosurus vulpecula presents the primitive features of, inter alia, relatively small brain size and absent (Fig 1) corpus callosum, (Fig 2, 3). Nevertheless, certain parts of the nervous system, particularly the central connexions of the visual apparatus, (Golby, 1941; Hayhow, 1967) appear to conform to a more highly developed pattern - even approximating that of the higher primates (Hayhow, 1967). Further, as a Diprotodont, Trichosurus vulpecula has the additional neocortical commissure, fasciculus aberrans, (Elliott Smith, 1902; Abbie, 1939). This structure bypasses the anterior commissure and apparently represents an interhemispheric pathway which is an alternative to (but not an anatomical precursor of) the corpus callosum of Eutheria, (Heath and Jones, 1971). That this is so, rests on evidence from anatomical studies of normal and experimental (degeneration) material.
It seems, in this animal, particularly apposite to ask - considering its well developed visual system and absence of any corpus callosum - whether there might be evidence from behaviour that it can, in the manner of eutherians, transmit visual information between its cerebral hemispheres.
EXPERIMENT 1

Introduction

The role of the corpus callosum in the transmission of visual information between cerebral hemispheres in eutherian mammals is well established. The absence of a corpus callosum in metatherian mammals was first noted by Smith (1894), raising a question as to the existence of a metatherian homologue to the corpus callosum. Abbie (1939), from anatomical studies and Putnam, Megirian and Manning (1968) from electrophysiological studies have suggested that the anterior and hippocampal commissures in metatheria form interhemispheric connexions which may serve the same function as the corpus callosum in eutheria. However, whatever the similarity of the two sets of structures with respect to anatomy and neurophysiology, conclusions regarding similarity of behavioural function demand behavioural evidence. Thus, this study is addressed to two questions:

Part 1. Can transfer of learning based on visual cues take place between the cerebral hemispheres in a marsupial, specifically the phalanger Trichosurus vulpecula; or is this animal, as has been suggested (Kirby 1969), naturally "split-brained"?

Part 2. Are the structures suggested by Abbie (1939) and Putnam et al (1968), namely the anterior and hippocampal commissural complex, functionally equivalent to the corpus callosum in the sense that interhemis-
pheric transfer is prevented by midline sagittal section of these structures?

Part 1

A procedure by which both these questions can be investigated is provided by the paradigm adopted by Myers (1955).

![Diagram of the visual projection of an animal which has undergone mid-sagittal section of the optic chiasma. The site of section is shown at S.](image)

**Fig 4**
The visual projection of an animal which has undergone mid-sagittal section of the optic chiasma. The site of section is shown at S.

In mammals, information entering each eye normally travels to primary projection areas in both hemispheres. Information
travelling to the ipsilateral projection area is carried by fibres which remain on that side, whereas information travelling to the contralateral projection area is carried by fibres which decussate in the optic chiasma. Mid-saggital section of the optic chiasma severs the decussating fibres, thus producing a preparation in which information entering one eye can proceed to the primary projection area of only one hemisphere. Figure 1 illustrates the visual projection of an animal which has undergone mid-saggital section of the optic chiasma. This method is suitable for use only in animals in which an adequate proportion of non-decussating fibres exist, since only they remain after mid-saggital section to provide the animal with vision. A study of chiasmatic degeneration patterns by Hayhow (1966) found, in Trichosurus vulpecula, that approximately 25% of the axons in the optic nerve proceed ipsilaterally. An earlier study by Packer (1941) found the ratio of crossed to uncrossed fibres to be approximately 3:1. It is thus clear that section of the decussating fibres in this animal still leaves a substantial proportion of optic fibres intact.

By occluding one eye in such a preparation it is possible to provide to one hemisphere only, the information necessary to carry out a visual discrimination. When such a visual discrimination task has been learned, it is possible to test the opposite hemisphere of the brain for retention by changing occlusion to the opposite eye, and presenting the subject with the same task. The extent to which discrimination performance is maintained is thus a measure of the extent of interhemispheric transfer.
Fig 5

Preliminary training apparatus.

Fig 6

Discrimination training and testing apparatus.
Method

Three male and five female specimens of Trichosurus vulpecula served as subjects. All were feral, taken as adults and adapted to captivity in the laboratory for at least two weeks before surgery.

Procedure

In all subjects the optic chiasma was transected saggitally in the midline, to cut all decussating fibres. The surgery was carried out under direct vision and the surgical procedures are fully described in Appendix II. Following recovery, usually in 2-3 weeks, subjects were adapted to a 23-hour food-deprivation schedule and pre-training was begun. This employed the apparatus shown in Fig 5 which was mounted on the front of the subject's home cage. Preliminary training consisted of placing pieces of carrot, cut into rough segments, on the platform of the apparatus and allowing the subject to reach through with either forelimb and take the food. Sessions were given daily and continued until the subject had consumed his daily ration of carrot, or until he failed to respond by not taking food for 30 seconds. The apparatus was then removed and the subject given the remainder of his daily food ration.

When the subject had become accustomed to the procedure and was able to draw in the morsels of food readily, one eye was occluded by closing it with mattress sutures, (see Appendix III). Discrimination training was then begun. The apparatus used is illustrated in Fig 6. It was
Fig 7

Discrimination training and test apparatus. Picture shows a typical "correct" response.
based on the Wisconsin General Test Apparatus, (WGTA) and was, like the pre-training apparatus, mounted on the front of the home cage. The task required the subject to discriminate correctly between a vertical (positive) and a horizontal (negative) piece of carrot, Fig 7. The vertical piece of food could be drawn into the cage; the horizontal could not.*

A trial consisted of placing the discriminanda in position (according to a Gellerman (1933) schedule for left-right randomisation) with the blind closed. The blind was then raised. A trial was scored correct only if the subject's forelimb first passed through the slot on the side on which the vertical piece of carrot was placed. If the subject responded incorrectly, he was allowed to take the food on a second "reach". The blind was then closed. Trials were continued to a total of 100 or until the subject failed to respond within 10 seconds of the blind being raised on two successive trials. Sessions were given daily, until the subject reached a criterion of more than 85% of trials correct on three successive days. (The chance probability of such an event = \(< 81 \times 10^{-18}\))

* The nature of the task requires comment: pilot studies were undertaken to determine a task which was within the animal's capacity to master. These are mentioned in Appendix I. In brief, it was found that learning to an acceptable criterion occurred within a reasonable time only when there was close contiguity of positive discriminandum, manipulandum and reward. For this reason, all three consisted of the same piece of accessible food.
Fig 8  Each graph represents data from one subject in Part 1. Each point is from one day. All subjects had chiasma section; all forebrain commissures were intact.
When a subject reached criterion, the previously sutured eye was opened, and the previously opened eye was sutured shut in preparation for the following two (test) days. The procedure on the two test days was identical to that on the training days.

Following testing, each subject was sacrificed with an overdose of Nembutal, perfused successively with isotonic saline and 10% formalin; the head was removed, placed in 10% formalin for at least 4 weeks, the brain was removed, examined visually for gross evidence of separation and then sectioned frontally at 60 μ with a freezing microtome. Representative sections through the transected areas (optic chiasma and hippocampal commissures) were printed at about 10x using a photographic enlarger.

Results

Figure 8 presents the data of performance on sessions prior to testing and after testing had begun. In the procedure followed, testing is, of course, training. For this reason, the data for the first session in all cases following change of side of occlusion are the results of the first 25 trials. In no case did any subject perform at or about the chance level (50%) on either test session.

During training, most subjects demonstrated a paw preference. No formal record of this was made, however, from informal observations, it was clear that no major change in paw preference took place with the change in side of occlusion.
Discussion

These results are similar to those of Myers (1955) in his preparations in which only the decussating fibres in the optic chiasma were cut. When the major interhemispheric pathways are intact (the corpus callosum in the cat, the anterior and hippocampal commissures and fasciculus aberrans in Trichosurus vulpecula) then inter-ocular transfer occurs. Thus, it appears that visual information passing primarily to only one hemisphere is, in this marsupial at least, available to the other.

PART 2

Advantage can be taken of the same basic paradigm as was used in Part 1 to compare the functional equivalence of the eutherian corpus callosum and the metatherian forebrain commissural complex. Myers (1955) has shown that section of the corpus callosum prior to training abolishes interocular transfer in chiasma-sectioned cats. A similar investigation was undertaken in Trichosurus.

Method

Two male and two female specimens of Trichosurus vulpecula served as subjects. As in Part 1, they were laboratory adapted feral adults.

Procedure

In all four subjects the optic chiasma was sectioned midsaggitally. In addition, in all four subjects, the anterior and hippocampal commissures together with fasciculus
Fig 9

Each graph presents data from one subject in Part 2. All four subjects had complete section of all the forebrain commissures as well as chiasma section.
Fig 9a

Drawing of a section from a representative subject (Ts B2) from Experiment 1, Part 2.
aberrans were similarly sectioned. (The surgery was carried out under direct visual control and is fully described in Appendix II) Following recovery from surgery, pre-training, eye occlusion and discrimination training were carried out in the same way, with the same apparatus, and to the same criterion, as previously described in Part 1. The same test procedure was followed except that it was continued until the subject reached the criterion of more than 75% trials correct on three successive days. \( p = < .0001 \) At the completion of testing the same procedures were followed as in Part 1.

Results

Data from each of the four subjects is presented in Fig 9. The figure represented by the first test day data point is, as in Fig 8 from the first 25 trials only. On the first test day, all subjects performed at or about the chance (50%) level. All took between 7 and 15 days to reach criterion.

Informal observation of paw preference showed that no change had taken place.

Discussion

It is clear that interocular transfer as measured by maintained performance, did not take place. The major inference is that when the forebrain commissures are severed, visual information does not pass from the hemisphere receiving the primary projection to the opposite hemisphere.
It is, however, probably incorrect to say that no information is available relating to the task with which the untrained hemisphere is challenged. It can be seen from Figure 9 that considerably fewer sessions are required to regain criterion (when the second hemisphere is being trained) than were first taken to achieve it in the first hemisphere. Such savings presumably represent some non-stimulus specific component of learning which may have either transferred by other pathways to the second hemisphere, or to which the second hemisphere has access. This aspect of the results is consistent with, although less dramatic than, the findings of Russell and Ochs (1963) who, using spreading depression in rats, reported transfer following only one training trial with the previously naive hemisphere. Such transfer of training need not necessarily be explained in terms of information specifically related to the stimulus. (Thomas, Miller and Svindicki, 1971)

A difficulty arises in the interpretation of the results of this experiment. Animals tended to develop a preference for the use of one forelimb rather than the other. This did not relate in any systematic way to the hemisphere being trained. It was as often the ipsilateral as the contralateral limb. Nor did it appear that there was any systematic difference in the time taken to reach criterion as a function of the ipsilateral or contralateral limb being preferred. Further, the original paw preference appeared to persist following the change of side of occlusion.

Nevertheless, an apparently unresolvable question remains regarding the equivalence of the training and testing tasks.
It will be recalled that the forelimbs do not have strong, if any, ipsilateral representation. It is by no means evident that, for example, training using the left limb and the left eye is properly tested by having the subject use the left limb and the right eye. Nor does it seem defensible to require the subject to use an opposite limb to that originally trained.

It may be possible to slip between the horns of this dilemma by finding a task which has no marked inherent asymmetry.

The above difficulties and qualifications notwithstanding, it is possible to answer the two original questions:

(1) It does appear that learning based on visual cues can transfer between the hemispheres in Trichosurus vulpecula.

(2) Within the paradigms used, there seems to be functional equivalence between the eutherian corpus callosum and the metatherian forebrain commissures.
Fig 9b
Frontal section to show the hippocampal commissure, (h.c.)
The scale is in millimeters.
Fig 10(a).

A section anterior to Fig 9 shows fasciculus aberrans, f.a.
Fig 10(b)
A section anterior to Fig 10(a) shows both the anterior commissure (a.c.) and fasciculus aberrans (f.a.). Scale in millimeters.
EXPERIMENT 2

Introduction

It is clear from the preceding study that in the marsupial phalanger, transfer of visual information between hemispheres can take place in the absence of a corpus callosum. It seems most probable that some structure or structures within the group of forebrain commissures mediate such transfer.

Three main commissural pathways exist in the forebrain of this animal. In the dorsal aspect of the septum, the two fimbriae of the hippocampal formations come together and decussate as the hippocampal commissure. (Fig 9) In the more ventral aspect of the septum lies the anterior commissure, the fibres of which are mainly continuous with those in the internal capsule. (Fig 10, a,b) Immediately applied to the dorsal aspect of the anterior commissure proper is fasciculus aberrans, whole contributing fibres appear to be mainly continuous with the internal capsule; it thus forms a shorter route for fibres passing between the more dorsal aspects of the hemispheres. From their anatomical studies using the Nauta technique, Heath and Jones (1971) suggest that fasciculus aberrans represents a Diprotodont alternative to the corpus callosum.

This study was designed to investigate, by differential sectioning of the forebrain commissures, the role of fasciculus aberrans in the interhemispheric transfer of
learning dependent on visual cues.

The problem of selection of an appropriate task by which interhemispheric transfer of visual information can be examined, has already been raised. A desirable characteristic in a suitable paradigm is that the response demanded of the subject should be symmetrical in the sense that it should not depend on skeletal and muscular structures having unilateral cortical representation. It was believed that tail withdrawal to a mild electric shock might have been an appropriate conditioned response; however, it was not found possible to condition withdrawal to a visual stimulus. Although it is well known that the speech function in humans is lateralised in the left hemisphere and lateralisation of bird song has been reported, (Nottebohm, 1971, 1972), there was no known reason to believe that the neural structures involved in vocalisation in marsupials is situated in only one hemisphere. A vocal response was therefore considered.

Both classical and operant conditioning procedures have been used to show that frequency of vocal responding by animals can be brought under the control of specific aspects of the environment. Operant conditioning of a vocal response using food as the reinforcing stimulus has been demonstrated in a number of species including the budgerigar (Geisbury, 1960), the cat, (Molliver, 1963), the dog, (Salzinger and Wallis, 1962) and the chicken (Lane, 1960). Lal, (1967) has also shown that vocalisation which is elicited by an electric shock, can be brought under control of a reinforcing stimulus. However, few studies have demonstrated classical
conditioning of a vocal response.

Cowles and Pennington (1943) conditioned a vocal response in rats using tail shock as the unconditioned stimulus.

It was observed that Trichosurus vulpecula vocalised readily following a mild electric shock to the tail. Since this response met the requirement of symmetry and since it proved possible to condition it to a previously neutral stimulus, viz. a rotating disc, it was adopted. A flashing light was considered as a suitable stimulus but was rejected when preliminary studies showed that transretinal illumination readily produced interocular transfer. The movement of a slowly rotating disc (12 RPM, 13 cm in diameter) provided a cue which did not transfer transretinally. The disc carried a spiral pattern rotated in such a direction as to encourage central fixation. The disc and driving mechanism were mounted in a sound-proof box, and preliminary studies showed that no learning based on sound or vibrational cues took place.

The general procedure was similar to that of Cowles and Pennington (1943) except that the stimulus preceding shock could be terminated by a vocal response, and thus the animal could avoid shock.

Method

Eight adult specimens of Trichosurus vulpecula served as subjects. These were divided into four groups, each of two subjects, as follows:

1. Optic chiasma sectioned midsaggittally, all commissural fibres intact. (S1, S2)
Fig 11

The experimental situation in Experiment 2.
(2) Optic chiasma sectioned midsagittally, as well as hippocampal and anterior commissures and fasciculus aberrans. (S3, S4)

(3) Optic chiasma, hippocampal and anterior commissure sectioned midsagittally, fasciculus aberrans intact. (S5, S6)

(4) Optic chiasma and fasciculus aberrans sectioned midsagittally, hippocampal and anterior commissures intact. (S7, S8)

The surgical procedures were the same as those in Experiment 1 and are presented in detail in Appendix II.

The experimental situation is illustrated in Figure 11. The animal was held in a restraining box which enabled the head to be positioned in front of a screen. Two electrodes were clipped on to the distal end of the tail about 3 cm apart. The maximum intensity of tail shock was 1.0 mA but most animals received much lower intensities.

A white disc 13 cm in diameter was situated about 15 cm in front of the animal's nose. The screen was at the back wall of a three-sided box with a matt black surface. The microphone of a voice key was mounted in a cone at the top of the box about 10 cm above the animal's nose. The experiment was conducted in a darkened room. The white disc could carry a spiral and be made to rotate, or could be covered by a white translucent screen and illuminated from behind by a flashing 6-watt bulb. A speaker located 12 cm from the animal's nose on one side delivered white noise of approximately 80 db A as measured by a Precision sound level meter. This effectively marked all equipment noise.
Relay equipment controlling experimental events was in an adjacent room.

Initial training began using a flashing light behind the translucent screen, paired with a sound cue consisting of a 100 Hz tone, presented on an alternating 0.5 second on and 0.5 second off variable interval schedule with a mean of 30 seconds. The flashing light preceded the shock for 8.5 seconds and was then accompanied by 1.5 seconds of tail shock. Vocalisation exceeding 80 dB could, via the voice key, terminate the flashing light and prevent the onset of shock. Vocalisation after the onset of shock could terminate neither the light nor the shock. Thus the situation was one in which avoidance, but not escape was possible. (It early became evident that subjects allowed to escape would do so reliably and very rapidly, but would not learn to avoid.)

During the early stages of training, the flashing light did not elicit vocalisation. However, most, but not all, animals emitted a screech in response to the shock. The remaining subjects soon began to vocalise following prompting by the experimenter who emitted a screech-like noise. When vocalisation was reliably established to the sound and flashing light cues, one eye was occluded with an opaque contact lens (following insertion of which, the eye was taped shut; see Appendix III). The translucent screen was removed, revealing the spiral disc which rotated simultaneously with the flashing light. First the sound cue, then the flashing light cue were faded out over a period of several days until only the rotating disc remained as a
discriminative stimulus. Training in this mode was continued until subjects learned to make an avoidance response on at least 95% of trials with no more than 1% of the total number of vocalisations being given in the absence of the rotating stimulus. (During early training, subjects tended to adopt the strategy of greatly increased frequency of vocalisation. It was found, in a pilot study, that vocalisation could be restricted to the presence of the discriminative stimulus if the inter-trial interval was sufficiently long.) A mean inter-trial interval of 30 seconds proved appropriate. Subjects took varying times to reach criterion. Because fading out of the additional cues was done intuitively, data on the number of trials to criterion are not comparable; however, all subjects received at least two weeks' training in the final condition.

When subjects had reached criterion, a test condition was instituted. Immediately following at least 10 correct trials, the occluding contact lens was changed to the other eye, and the shock generator was turned off; otherwise conditions were identical to those during training. Following 20 test trials, the previously untrained eye was again occluded and the trained eye presented with the stimulus for a further 10 trials, still with the shock generator turned off.

Following testing each subject was sacrificed with an overdose of Nembutal, perfused successively with isotonic saline and 10% formalin; the head was removed, placed in 10% formalin for at least 4 weeks, the brain was removed, examined visually for gross evidence of separation, and sectioned

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Fig 12a: Drawings of sections from representative subjects from the three lesion groups.
Fig 12
Cumulative vector diagram comparing performance of eight subjects during the final 10 training trials, 20 test trials of the previously untrained eye and 10 retest trials of the originally trained eye. All had mid-sagittally sectioned optic chiasmata. Of the major commissures, S5 and S6 had only fasciculus aberrans intact, S7 and S8 only fasciculus aberrans sectioned, S1 and S2 had no commissural section and S3 and S4 had fasciculus aberrans, anterior and hippocampal commissures sectioned.
frontally at 60μ with a freezing microtome. Representative sections through the transected areas (optic chiasma and anterior and hippocampal commissures) were printed at about 10x using a photographic enlarger.

Results

The data from the four groups are presented as a cumulative vector diagram (see footnote)*, Figure 12. Group 1 (all commissures intact) and Group 3 (fasciculus aberrans intact) show a tendency to continue with correct 'avoidance' responses during the first half of the test trials while Group 2 (all commissures sectioned) and Group 4 (fasciculus aberrans sectioned) do not. During the second half of the test trials, Groups 1 and 3 ('intact' groups) tend to cease giving 'avoidance' responses, and in the retest period, give no, or few, such responses. Groups 2 and 4, on the other hand,

* CUMULATIVE VECTOR DIAGRAM

The presentation of data as a cumulative vector diagram may be unfamiliar. It is suited to binary data from sequential events.

The occurrence of a positive event is recorded as a line, one unit in length, at an angle of 45° to the ordinate. A negative event is represented by a similar line 135° from the ordinate. Thus time, or sequence of events, is represented by the abcissa, while a sequence of positive events is shown as a continuous upward slope, a negative sequence by a downward slope. This method of display has the virtue of losing no data while making clear trends of any sequences within a series of events. It is believed to have been developed by J.S. Pollard, Department of Psychology and Sociology, University of Canterbury. The writer is indebted to I.L. Beale, Department of Psychology, University of Auckland, who drew it to his attention. No published references to the method are known to the writer.
Fig 13

Sonagrams of the vocal responses of one subject.
A: unvoiced screech; B: voiced speech; C: snort;
D: snort followed by chatter
(those with at least fasciculus aberrans sectioned), although making few 'avoidance' responses during the test trials, continue to give such responses during the retest period.

It was of interest to observe, during training, the progressively earlier occurrence of vocalisation, so that typically after 300 to 600 trials, more than 70% of vocalisations occurred during the period of flashing light preceding the onset of shock. Since measurement of learning rate was not of primary concern, animals were advanced to the next phase of training (the rotating disc) at various levels of performance. Further, the prompting by experimenters was given intuitively. Thus, no data on rate of acquisition of the vocal avoidance response is presented.

Presenting trials on a variable interval schedule with a mean of at least 30 seconds ensured that almost all responses were confined to the period in which the rotating stimulus was presented.

When vocalisation was elicited by shock, it was a voiced or unvoiced screech (a uniform high-pitched sound of short duration). The vocalisation that most commonly occurred at the onset of the flashing light was a voiced or unvoiced screech similar to that elicited by shock, but usually of much lower intensity. However, on many occasions, other calls were emitted at the onset of the flashing light. These were snorts and chatters; sometimes a snort was followed by a chatter. Infrequently, a chatter followed the screech elicited by the shock. Figure 13 shows sonagrams of typical vocalisations made by one subject. The voiced and unvoiced screeches are typical of those elicited
by shock and also of those which terminated the flashing light and prevented shock. The snort and snort-and-chatter are also typical of those which resulted in shock avoidance. Characteristic of all sonograms is the high amplitude of some frequencies up to 3000 Hz and the relatively high intensity of all bandwidths sampled. This latter feature makes the screech in particular, resemble a white noise.

The calls used for communication in Trichosurus vulpecula depend on age, sex, and the situation in which the animal finds itself. (Winter, 1973) The screech is common to all of the species and in the natural environment, probably occurs in 'danger' situations. It is, therefore, not unexpected to find that the vocal response elicited by shock was a screech, or that a screech, often softer and unvoiced, was made at the onset of the flashing light. However, other calls, the chatter and snort, also resulted in shock avoidance. In the natural state, a chatter is frequently a warning to other animals of approaching danger. Thus, the chatter might be expected to occur in situations where danger, like the shock in this experiment, was signalled by some stimulus, and might be avoided. In his classification of vocal responses in Trichosurus vulpecula, Winter (1973) makes no mention of the snort; as this often occurred preceding a chatter, it may be emitted in similar situations.

Discussion

Both the Test Trials and Retest sessions may be considered as extinction conditions, since there is no penalty for failing to make an 'avoidance' response. It is apparent in most cases,
that in Groups 1 and 3 (fasciculus aberrans intact) 'avoidance' responses have virtually ceased by the beginning of the Retest period; this is not the case in Groups 2 and 4 (those with at least fasciculus aberrans sectioned). Thus, by the beginning of the Retest period, Groups 1 and 3 can be regarded as having learned that responding no longer leads to avoidance of an aversive event. Groups 2 and 4 have not learned this.

The failure of Groups 2 and 4 to continue making 'avoidance' responses is evidence for the lack of transfer of information to the untrained hemisphere. Further support for this interpretation comes from consideration of the performances during the Retest period. Some subjects, Groups 1 and 3, had learned, via the previously untrained hemisphere, by the end of the Test Trials that 'avoidance' responses were no longer necessary. The performance of these subjects during the Retest period suggests that the originally trained hemisphere, which had not had direct training in 'not responding', nevertheless had access to the information and thus behaved as though responding during the Retest period was inappropriate. However, in the case of Group 2, which had fasciculus aberrans and hippocampal and anterior commissures sectioned, and Group 4, which had only fasciculus aberrans sectioned, no such transfer appears to have taken place since almost as many 'avoidance' as 'non-avoidance' responses were made during the Retest period. The 'transfer of extinction' and the transfer of the originally taught task took place in opposite directions. This implies that some symmetrical relationship exists between the two hemispheres. Thus,
it is clear that in Trichosurus vulpecula, integrity of fasciculus aberrans is a necessary condition (of the commissures) for the transfer of visual information between hemispheres.

The continuation of 'avoidance' responses by Groups 1 and 3 after the first condition change may be interpreted as evidence for the transfer of information to the 'untrained' hemisphere. In the case of Group 1, all the cerebral commissures were intact. In Group 3, fasciculus aberrans only was intact. It thus seems clear that the integrity of fasciculus aberrans is a sufficient condition for transfer to occur in this situation.
CONCLUSION

These studies have shown that interhemispheric transfer of learning based on visual cues can take place in the marsupial phalanger, and that the marsupial forebrain commissures (in toto or in part) and the corpus callosum are functionally equivalent insofar as the paradigms employed by Myers (1955) and those used in the present work are comparable. Further, behavioural evidence is presented that the integrity of fasciculus aberrans is both a necessary and sufficient condition of the forebrain commissures for the interhemispheric transfer of visual information, a position quite consistent with the anatomical findings of Heath and Jones (1971).

The problems of homological inferences and the dangers of considering some presently existing forms as being comparable to the antecedents of other contemporary species, need not be elaborated here. It is the case, however, with only one major (though some minor) exceptions, that metatherians have, throughout the world, been replaced in virtually every ecological niche by placental mammals. A wide variety of forms demonstrate the capacity of marsupials to fill niches in ways highly comparable with eutherians. The extent to which quite remarkable convergences can be seen between marsupials on the one hand, and eutherians of similar life pattern on the other, have often been noted (Keast 1968). They include the wombat and the woodchuck, the kangaroo and the antelope, the marsupial mole (Notoryctes typhlops) and the mole, the thylacine and the dog, the
Tasmanian devil (Sarcophilus ursus) and the wolverine, and the water opossum and the otter. The morphological similarity between the marsupial and the eutherian in many of the above pairs, notwithstanding a genetic separation of 100 million years, suggests that biological success depended on something additional to external physical characteristics. The crucial difference may well have been one of superior organisation of the central nervous system, typified by efficiency of interhemispheric transmission via those pathways noted by T.H Huxley as being so dramatically increased in size. It is, perhaps, significant that wherever they have been in competition, animals possessed of a corpus callosum have survived those not so endowed.

This study, while demonstrating that some interhemispheric transfer is possible in marsupials, leaves open the range of questions which might be asked concerning the qualitative aspects of that transmission. Trichosurus vulpecula presents a visual system which, in structure with respect to certain central connexions, conforms to the pattern of much more highly developed animals, even the higher primates, (Hayhow, 1967). However, it does not, of necessity, follow that because the structure in lower forms bears close similarity to that of more advanced forms that visual information is in fact, processed in the same way. There is presently no evidence on this point. This animal offers a preparation in which questions might be asked relating to the processing of information by a well-developed visual system without the massive interconnexions provided by a corpus callosum. In particular, attention might be
directed to such questions as the speed of processing simple and complex information, and the nature, (in terms of amount and complexity), of the information which the system can handle. Some light might then be shed on the precise nature of the advantages bestowed by the possession of a Great Cerebral Comissure.
APPENDIX 1

Laboratory Care and Management of Trichosurus vulpecula

General

When one elects to use as experimental subjects species other than those commonly accepted as "laboratory animals", often much needs to be learned about their day-to-day care and management. This can be a time-consuming and expensive process. The following points gained in several years experience with this animal may be of interest and perhaps save future workers some frustration.

Trichosurus vulpecula is a native of Australia and has, in New Zealand, the common name of "oppossum". This name, however, applies strictly to the American oppossum (Family, Didelphidae) which belongs to the polyprotodont suborder of marsupials. The oppossum-like animals of Australia, (Family, Phalangeridae), are diprotodont, i.e., they have a two-incisored condition of the lower jaw. According to Troughton (1946), the diprotodonts were formerly purely insectivorous but gradually adapted to a vegetarian diet. During this process, they have lost some of their incisors and so do not possess a full set of little front teeth. The remaining incisors have increased in size as is usual in herbivorous animals. Trichosurus vulpecula is arboreal and nocturnal or crepuscular.

The first of at least 219 recorded releases, covering virtually all areas in New Zealand, took place about 1840,
and releases continued until 1950, (Pracy, 1962). Acclimatisation was, in general, highly successful; almost all forested areas in New Zealand are now heavily infested.

Trichosurus vulpecula is now classified as a noxious animal and thus cannot legally be kept in captivity without a government permit. Application for such a permit is made to the Conservator of Forests, New Zealand Forest Service. There are Conservators of Forests in Auckland, Rotorua, Palmerston North, Nelson, Hokitika, Christchurch and Invercargill. We have had no difficulty in obtaining permits. In Auckland no problems have been experienced in obtaining a plentiful supply of subjects. The animals abound in the suburbs where there are plenty of trees. Their diet, (which includes citrus and other fruit, and the buds and blossoms of roses) and the readiness with which they take up residence in ceilings of houses, is sufficient to ensure the collaboration of most householders in their capture. We have found it sufficient to have a number of box traps available and to let it be known that they can be borrowed. Fruit or bread, treated with a few drops of oil of aniseed or oil of raspberry is an effective bait. We find people prefer to return the trap with the animal inside, rather than deal with it themselves.

Trichosurus vulpecula has a robust jaw and powerful temporalis muscles, and is capable of inflicting a painful bite, even through heavy leather (welding) gloves. Fore and hind limbs are armed with long, sharp, and non-retractable claws which are actively used as weapons. Although generally
Fig I-1

An efficient and safe method of restraint.
shy, Trichosurus vulpecula is not timid when cornered.

These animals can, however, be managed safely and confidently, provided certain skills are learned. Handling and restraint for such purposes as transfer or medication are similar to practices used with small monkeys. The animal is pinned to the floor or side of the cage by a quick grasp above the shoulders and sustained pressure of the fore-arm along the animal's back. The hand grasping the shoulders is then slipped down to hold both the forelimbs behind the back. The other hand grasps the base of the tail. (see Fig I-1)

An alternative method of catching and transfer is simply to pick the animal up quickly by the tail and swing it to and fro - but sufficiently far from the operator's legs so the animal cannot grasp them with his claws. It is necessary to swing the animal so that he remains extended by centrifugal force, otherwise he may turn on his prehensile tail and climb up the arm of the holder.

We have found that animals captured as adults adapt readily to laboratory conditions in about two to three weeks; animals reared in captivity, on the other hand, tend to become more intractable and difficult to handle. Thus, for reasons of economy and convenience, we prefer fully grown animals.

Animals are kept in sheet metal cages with a wire grille front and floor. One animal is housed in each cage - we have noted persistent fighting where animals (regardless of sex) are housed together. We provide a tree branch in each cage; animals seem to prefer resting or sleeping on a branch rather than on the floor of the cage.
Although predominantly vegetarian, Trichosurus vulpecula is omnivorous. In the wild, in addition to leaves, shoots and berries, small birds and eggs are eaten. In the laboratory, animals can be maintained in a healthy state indefinitely on a diet of standard laboratory (rat) pellets, although fresh vegetables, e.g. carrots and cabbage, are preferred. When animals are fed fresh vegetables, additional water seems to be unnecessary. We have observed no difficulty with ectoparasites, however, Trichosurus vulpecula can carry bovine tuberculosis which can be transmitted to humans. It has, therefore, been our practice to arrange regular tests for those workers in contact with them. In general, Trichosurus vulpecula is a healthy laboratory animal.

Discrimination Tasks

A series of pilot studies were carried out to determine an appropriate discrimination task. We found a runway of the type used by Lander (Lander, 1962) to be unsatisfactory because of laterality preferences. It was not readily possible to train an animal to an acceptable criterion within a reasonable time.

It was found relatively easy to shape a bar press response in Trichosurus vulpecula, using a perspex manipulandum with a hemispherical recess on the upper surface into which diluted sweetened condensed milk could be delivered. However, five months of daily one-hour sessions were necessary to establish a discrimination between a 30 cm x 3 cm black line placed vertically as a positive stimulus and
Fig I-2

Discrimination learning apparatus using pigeon chamber type operant keys. Candy could be delivered to the centre tray.
horizontally as a negative stimulus. The subject in this study was intact and learning was assisted by pairing a tone (which was subsequently faded out) with one condition.

A further pilot study used the apparatus shown in Fig I-2. Stimuli from multiple stimulus projectors could be projected on to the two screens which were large versions of an operant chamber pigeon key, readily operated by pressure from the subject's nose. Choice of the stimulus designated "correct" produced an aniseed flavoured candy ball in the centre tray. The stimuli were randomised left-right in accordance with a Gellerman schedule (Gellerman, 1933). It was uniformly found with the six animals used in this study that magazine training proceeded rapidly, as, apparently, did discrimination learning, to a level of about 60% trials correct; which level was never exceeded. It became evident that all subjects adopted a position-based strategy: "respond on the key which was last rewarded, if no reward, change key." Little reflection is needed to recognise that this tactic will give a success rate of about 60% when S+ is disposed left-right on a Gellerman schedule. A variety of counter-measures failed to break this habit, or to produce an alternative to a position habit in naive subjects.

We have consistently found that motor responses, even in complicated sequences, are very readily shaped in Trichosurus vulpecula, but have encountered considerable difficulty in shaping any discrimination based on visual cues.

At this point in the preliminary studies, consideration was given to the point made by Jarvik, (1953)
relating to contiguity of stimulus, reward, and response manipulandum, and ease of learning. It was found, when the stimulus, reward, and response manipulandum were combined in one object, e.g. a vertical piece of carrot, that training in a visual discrimination task was not only possible, but proceeded to an acceptable criterion within a feasible number of sessions.
APPENDIX II

SURGICAL PROCEDURES

Since there is little published information on experimental neurosurgical techniques with Trichosurus vulpecula and since some special problems arise, a detailed recording of the surgical procedures found satisfactory seems justified.

The optic chiasma was transected in the mid-sagittal plane. The transbuccal approach used by Myers (JCPP 48, 1955, 420-423) was inappropriate with Trichosurus vulpecula because of the limited jaw opening and because of a venous sinus immediately ventral to the optic chiasma.

A transtemporal approach was found to be successful, the method being adapted from the technique for removing the hypophysis devised by Dandy and Reichert (1925) and elaborated by Markowitz et al. (1964).

Preparation

1.0 to 1.5 ml of ketamine hydrochloride, 50 mg/ml (Ketalar, Parke Davis) is given intramuscularly to the animal in its home cage. Within 10-15 minutes the tranquillising and paralysant effect is such that the animal can be readily handled.

Acetyl promazine has little or no effect on Trichosurus even at twenty times the usually recommended dose while morphine analogues such as diethylthiambutene hydrochloride
A cuffed endotracheal tube is entered with the assistance of a gag.
Fig II-2

A 20 g butterfly needle is entered in the cephalic vein.

Fig II-3

The limb is immobilised with a metal splint.
(Themalon; Burroughs, Wellcome & Co.) appear to lead to excitation similar to that produced in cats (Kreuger, Eddy, and Sumwalt, 1941).

Halothane with oxygen and nitrous oxide is administered via a conical mask over the animal's muzzle and deep anaesthesia induced. An endotracheal catheter (Magill's cuffed tube oral, Franklin DV, 3 mm, Fig II-1) is introduced with the aid of a gag and laryngoscope, anaesthesia is then maintained with halothane, oxygen and nitrous oxide. The closed circuit mode of the Gillies' apparatus is employed without using soda lime. Subcutaneous electrodes are inserted at this time (one over the sternum and the other in one forelimb) connected to a cardiac monitor.

One forelimb is prepared on the anterior aspect to give access to the cephalic vein. A tourniquet is applied to distend the vein. A 20 g butterfly needle (Abbott No 4492) is entered, threaded up the vein so the hub is at the site of venipuncture, (Fig II-2) taped in place and the limb immobilised with a three-inch by one inch vee-shaped metal splint, (Fig II-3). Prior to insertion, the needle and tube are filled with a 1% solution of sodium citrate. This prevents clotting which tends to occur very rapidly with these animals - to the extent, in fact, that the flow of blood relied on to indicate successful venipuncture is stopped.

An intravenous administration of mannitol (15% w/v) is now begun at at a rate sufficient to infuse 50 ml over a period of 10-15 minutes. (Mannitol, a high molecular weight plant sugar, acts osmotically to reduce total brain volume
Fig II-4

The subject is positioned in a stereotaxic frame, using modified eye bars.
Fig II-5

The operative site prepared by clipping, shaving, and treatment with depilatory cream.

Fig II-6

The site draped and marked for incision.
Fig II-7
The skin flap reflected. The sagittal crests and temporalis muscles can be seen.

Fig II-8
Left temporalis muscle is excised.
by the extraction of intra-cellular water, thus aiding retraction) At the same time, the infusion of a 0.3 per cent solution of sodium thyamyral (Surital, Parke Davis) is commenced at a drip rate of about 1 drop per 50-60 seconds. The halothane is gradually stopped but the oxygen and nitrous oxide are continued. Anaesthesia is maintained with Surital and the oxygen-nitrous oxide gas mixture for the remainder of the operation. Occasional apnea is dealt with either by "bagging" or the use of a small animal ventilator.

The animal is now positioned in a stereotaxic frame, (David Kopff, No 1204, Fig II-4) using standard cat ear bars. The only modification which has been found necessary is the provision of curved eye bars to accommodate the more laterally placed eyes of Trichosurus vulpecula.

The operative site is now prepared by clipping, shaving, and treating with depilatory cream, an area about 7 cm x 5 cm over the cranium, (Fig II-5). The skin is treated with a 1:100 solution of benzalkonium chloride (Zephiran), sterile drapes are clipped in place and the incision line marked (Fig II-6). A U-shaped incision is made over one temporalis muscle with the base of the "U" extending as far laterally as the ear will allow. The use of diathermy to cut the fascia reduces bleeding. The skin is reflected, (Fig II-7). Temporalsis muscle is incised at its origin on the sagittal crest and stripped downwards to the base of the skull, freed from the coronoid process by blunt dissection, and excised entirely, (Fig II-8). The posterior two-thirds of the zygomatic arch is removed with rongeurs. A gag is
Fig II-9

The bone covering the left hemisphere is removed with rongeurs.
Fig II-10

Skull of Trichosurus vulpecula; the lower picture shows the extent of bone removal during surgery. The approximate axis of view to the optic chiasma is shown by the pointer.
Fig II-11

The dura is reflected.

Fig II-12

The animal is rotated to almost a supine position.
Fig II-13

The introduction of isotonic saline solution frees the hemisphere from the floor of the skull.
Fig 14(a,b) Two views of the exposure of the chiasma. In (a) the chiasma is more readily seen, (b) shows the direction of approach of the instrument.
placed in position to prevent the coronoid process from obscuring access to the lower part of the cranium. Bleeding is controlled by diathermy, irrigation with a 1:20,000 adrenalin solution, and packing. It has not been found necessary to tie bleeding points.

By this time, the infusion of mannitol is usually complete and an intravenous drip of 6% dextrose in isotonic saline is begun and continued at a rate of about 20 drops per minute throughout the operation.

A burr hole is made in the cranium and enlarged with rongeurs, (Fig II-9). (In some cases, a bone flap has been removed for later replacement but this practice is time-consuming and appears to have no advantage.) The areas of bone removed are illustrated in Fig II-10. The dura is incised and reflected, (Fig II-11). The animal is now turned almost completely upside down to a supine position by rotating the stereotaxic frame about its longitudinal axis. (Fig II-12)

The cerebral hemisphere is parted from the floor of the cranium, gravity being assisted by introducing isotonic saline solution between the ventral surface of the hemisphere and the floor of the cranium through a large-bore hypodermic syringe, (Fig II-13). Gentle traction is applied. (A strip of brass 5 mm wide, bent to distribute pressure over as great an area as possible, serves as a retractor. The brain is protected by a strip of cottonoid.) This procedure exposes to view the optic nerve and the optic chiasma, (Fig II-14). An operating microscope (in our case Zeiss Opmi No 1) with a coaxial lighting system is invaluable since simultaneous
Fig II-15

Knife fashioned from silver wire.

Fig II-16

The animal is rotated through about $270^\circ$, so gravity assists separation of the hemispheres.
Fig II-17

The viewing axis is in the horizontal plane. The retraction due to gravity separates the hemispheres so that the commissures can be seen.
lighting and observation through the narrow aperture is somewhat difficult. Any bleeding inside the cranium is controlled by topical thrombin solution although this is not usually necessary if great caution in retraction is exercised. Care is necessary to avoid damage to the internal carotid and anterior cerebral arteries and the hypophysis. Breaking the internal carotid or anterior cerebral arteries produces bleeding which is difficult to control and which obscures the optic chiasma. Furthermore, it is particularly important in an experiment of this nature, that any risk of brain damage, such as might result from breakage of a cerebral artery, be kept to an absolute minimum.

The chiasma is sectioned in the midline, using a knife made from 18 swg silver wire held in a dental broach holder, (Fig II-15).

If partial or complete commissural section is elected, the animal is, at this stage, rotated axially through about 270 degrees by moving the stereotaxic frame (Fig II-16) so that the exposed hemisphere falls away from the midline, (Fig II-17). Gentle retraction with the assistance of gravity, clearly exposes to view the hippocampal and anterior commissures. Section of the commissures to the extent desired can be carried out using a blunt needle with a slightly roughened end - used with a brushing action - and aspiration via a 22 g hypodermic needle.

The animal is now returned to a prone position. The dura is replaced as far as possible but is not sutured.
Fig II-18

Interrupted sutures are used to close the incision.

Fig II-19

Recovery from anaesthesia takes place in an incubator.
A thin piece of polythene film* is cut to extend about 1 mm beyond the area of exposed cortex. The film can be given a permanent set by pulling over the forefinger, to make a shape which conforms to the hemisphere being covered. The film is laid over the dura and the edges tucked between the dura and the skull. Gelfoam soaked in isotonic saline is placed in the cavity, the skin is drawn together and the incision is closed with interrupted sutures, (Fig II-18). Complete closure is regarded as important for asepsis since no external dressing is applied. The wound is dusted with Tolid (Bayer) powder.

**Post-Operative Care**

Chloramphenicol (Intramycetin, Parke Davis) is given intramuscularly, about 10 mg per kg daily for 5-7 days.

A sedative, e.g. Hemineurin, is given to ensure a smooth recovery from anaesthesia, and the animal is placed in an incubator, (Fig II-19).

Twelve hours after surgery about 25 mls of Complan solution (5 gms to 25 mls water) is tube fed twice daily for 2-4 days. The animal is then offered a Complan and Farex (proprietary invalid/baby foods) mixture and is given

* Polythene film does not adhere to the underlying leptomeninges and cerebral cortex and the dura can regenerate between the polythene film and the arachnoid. There is no tissue reaction to this material. (Brown, Grindlay, and Winshell, 1948)
access to its regular diet of carrot and cabbage. Animals have generally returned to their normal diet in 7-10 days. In a small proportion of cases CSF leaks have developed. It has been our experience that this condition clears up over a period of a few days following aspiration at intervals determined by the build-up of pressure.

We have found that a high standard of aseptic procedures throughout, equivalent to that generally held to be appropriate with cats, is an adequate safeguard against infection.
Fig III-1

Two mattress sutures inserted for eye closure. The suture on the left has been completed, that on the right has yet to be drawn up and tied.

Fig III-2

For insertion of eye sutures, a modified cat head holder has been found appropriate.
APPENDIX III

EYE OCCLUSION

Occlusion of the eye in Experiment 1 was accomplished by the insertion of two mattress sutures, using 5/0 braided polyester yarn, (Markowitz et al, 1964). Since the suture was expected to remain in place for some weeks, additional support was provided by 2-3 mm lengths of polythene tubing placed as shown in Fig III-1. For the placement of sutures, the head was held in a modified cat head-holder, (Fig III-2). The procedure was carried out under general anaesthesia using halothane and a nitrous oxide/oxygen mixture, following premedication with Ketalar as detailed under Surgery, Appendix II.

To occlude the eye in Experiment 2, an opaque acrylic contact lens, 12 mm in diameter and obliquely fenestrated to provide ventilation, was used. When the animal was placed in the restraint box, a drop of 4% Xylocaine solution was instilled in the eye. After a few seconds the lens was placed in position using a conventional suction lens holder. The eye was then held shut with polythene adhesive tape. This was necessary because Trichosurus vulpecula has a nictitating membrane, the action of which can force the contact lens out of the eye. Periodic examination of the subjects' eyes by staff of the Department of Optometry found no adverse consequences of this procedure although it was carried out daily for periods up to several weeks.
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