Brain evolution in bats (Mammalia, Chiroptera): auditory, Olfactory and Sensorimotor systems

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Abstract

Data for brain structure volumes was analysed using multiple regression to test for correlated volumetric evolution in bats (Mammalia, Chiroptera). Significant partial correlations were found between major brain subdivisions, and between structures within the Auditory, Olfactory and Sensorimotor Systems that were predicted to have evolved together on the basis of anatomical connectivity and known functional relationships. Results were clearest in the auditory and sensorimotor systems and weakest for the olfactory system which included many limbic structures. Megachiroptera and microchiroptera were analysed separately; there was good general agreement between the patterns of correlated evolution in both of these clades. When compared to previous studies of correlated volumetric evolution in Insectivores and Primates, it was found that the pattern of correlations found in bats showed features that are unique to the order. These results strongly suggest that brain evolution in bats has proceeded in a mosaic fashion with individual functional systems being the targets of selection.

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Declaration

I confirm that no part of the material offered has previously been submitted by me for a degree in this or in any other University. Material from the work of others has been acknowledged and quotations and paraphrases suitably indicated.

1
Introduction

Introduction to the Bats

The bats (order Chiroptera) represent approximately one quarter of all mammalian species (Neuweiler, 2000). They belong to the superorder Archonta, along with the Primates, Scandentia (tree shrews) and Dermoptera (flying lemurs) (Nowak, 1999). Two suborders are recognised: the Megachiroptera (old world fruit bats) generally large bodied and frugivorous, and Microchiroptera, all echolocating, a mix of insectivorous, saguinivorous and frugivorous species (Altringham, 1996).

Although there is general agreement among biologists that bats are a monophyletic group, it has been proposed repeatedly that the bats might be polyphyletic, the Megachiroptera being more closely related to the Primates (Pettigrew et al, 1989) and Dermoptera (1990) than to the Microchiroptera. Most recently, similarities that were found in the retinotectal projection between Megachiroptera and Primates have been interpreted as evidence for diphyly (Pettigrew, 1986). This proposal has been described as "the flying Primate hypothesis".

The majority of phylogenetic reconstructions for bats have favoured monophyly. Evidence comes from the cranium (Starck, 1943; Wible & Novacek, 1988; King, 1991) and morphology of the ear (Starck, 1943; Habersetzer & Storch, 1992), masticatory system and gastrointestinal tract (Storch, 1968; Schultz, 1965 respectively) and morphology of the forelimb (Wible & Novacek, 1980; Novacek & Wyss, 1986; Baker et al, 1991). The vast majority of molecular taxonomies published to date clearly favour bat monophyly and these have been based on a range of molecules and phylogenetic methods (Adkins & Honeycutt, 1991; Allard et al, 1996; Ammerman & Hillis, 1992; Lapointe et al, 1999; McNiff & Allard, 1998; Stanhope et al, 1992; Teeling et al, 2000).

It has been argued that the postcranial anatomy of Megachiroptera and Microchiroptera, particularly those that relate to the wing, are likely to exhibit similarities in structure as required by their function and not related to phylogeny (Pettigrew et al, 1989). There are probably few ways that a mammalian pentadactyl limb can be converted into a wing (Altringham, 1996). Homoplasy would therefore be expected even if Megachiroptera and Microchiroptera were not closely related (Pettigrew, 1991a). Likewise, the high adenine and thiamine content of bat DNA may complicate molecular phylogenetic studies (Hutcheon et al, 1998; Kirsch & Pettigrew, 1998; Pettigrew & Kirsch, 1998).

Evidence for diphyly is largely confined to nervous and reproductive systems. Many claims have been made that the nervous system organisation of Megachiroptera shares many similarities with those of Primates.

In most mammals the tectum receives its entire projection from the contralateral retina. In Primates however it is well established that the tectum receives the contalateral hemifield from both eyes; it receives a projection both from the contralateral and ipsilateral retina. Pettigrew (1986) found that Megachiroptera too possess this pattern of projection and proposed it as a feature linking them with Primates. On the basis of these apparent subcortical similarities in the visual systems of Primates and Megachiroptera, Ichida et al (2000) compared the distribution of calcium-binding proteins in the visual cortex of Megachiroptera, Microchiroptera and Primates. The pattern of labelling suggests no special similarity between Megachiroptera and Primates. Even if the pattern of subcortical projections appear similar, the organisation and processing of the higher cortical systems may well be very different (Ichida et al, 2000).

It is well known that Megachiroptera use vision as their primary sense for locating food at a distance wheras in Microchiroptera the visual system is more rudimentary (Neuweiler, 2000). It is not surprising therefore that the visual system of the Megachiroptera appears well developed compared to that of Microchiroptera. The features of nervous system

organisation which provide for high levels of acuity or other measures of visual function may appear similar between mammals relying on a sense of sight, such as Megachiroptera and Primates. But there appears to be no good reason to consider this as anything other than of functional significance (Preuss & Kaas, 1999).

The Megachiropteran somatosensory cortex also exhibits some features in common with Primates but not Microchiroptera. Primates possess a number of parietal cortical areas related to somatosensation in addition to primary somatosensory cortex (S1). Many mammals only possess this primary somatosensory area (Hendry et al, 1999). Megachiroptera possess multiple fields (Krubitzer & Calford, 1992). The somatosensory homunculus of bats is unique compared to that of other mammals. Although showing a typical somatotopic organisation in most respects, the representation of the forelimb is the reverse of what would be expected (i.e. reversed compared to the rest of the body) from studies of other mammalian species (Hendry et al, 1999). It has been suggested that this reflects the habitual posture of the forelimb, caudal to the head when in flight and superior to the head when at rest (Calford et al, 1985). Both Megachiroptera and Microchiroptera possess this unusual arrangement (Wise et al, 1986).

Studies of the organisation of the hippocampus in Megachiroptera have shown that the relative size of the hippocampus is large in Megachiroptera compared with

Microchiroptera and share many features with Primates (Buhl & Dann, 1991). The size of the hippocampus is probably best explained by the demands of spatial memory that are made on the animal in its search for food (Neuweiler, 2000). The qualitative similarities described above might also be features suggestive of the functional demands on spatial memory in both Megachiroptera and Primates.

Despite the intriguing nervous system similarities between Megachiroptera and Primates the consensus of current studies strongly support monophyly (Simmons, 1993; Simmons & Geisler, 1998). However the controversy has stimulated more comparative brain studies on bats (Pettigrew, 1991b).

Volumetric Brain Evolution

In the history of the study of the evolution of the brain there have been two prominent theories that describe how brains might evolve. It has been argued that the composition of the brain is basically similar between species and that evolution proceeds through changes in overall size (Jerrison, 1973). As brain size and therefore the number of constituent neurons increases the capabilities of the brain improve. The alternative view is that brain evolution proceeds by a reorganisation of the structure of the brain itself (Preuss, 2000). The capabilities of a brain improve by changes in the connections made

by particular brain structures and the increasing complexity of the resulting neural networks (Galaburda & Pandya, 1982; Preuss, 2000).

The availability of large volumetric datasets such as the one used in this study on bats (Baron et al, 1996a) have led to the testing of many hypothesis that seek to explain how brains evolve. These datasets typically consist of the volumes of specific structures for a number of species. Although there is clearly a phylogenetic signal present in this data (since closely related species tend to share similar phenotypes) (Johnson et al, 1993, 1994; Jolicoeur et al, 1984) these are apparently not sufficiently strong to produce robust phylogenies (Lapointe et al, 1999). Rather, the relative proportions of volumes of structures within the brain seem closely related to role of those structures in behaviour and ecology (Barton et al, 1995; Clutton-Brock & Harvey, 1980; Jolicoeur & Baron, 1994; Lapointe et al, 1999). Therefore when testing hypotheses using volumetric data the behaviour and ecology of an animal should be taken into account.

Hypothesis

This work was intended to look for evidence of correlated volumetric evolution within functional systems of the bat nervous system. The hypothesis to be tested was as follows:

Brain evolution proceeds through coordinated size changes between structures linked by strong fibre connections and within functional systems.

This hypothesis should be proven correct if it is demonstrated that structures within the auditory, olfactory and sensorimotor systems show significant correlations with other structures within those systems when variation in the size of the rest of the brain is taken into account.

2
Materials and Methods

This section describes the data methods used throughout the remainder of the thesis. For a more complete account of the dataset and processing of specimens, see Baron et al (1996a).

Collection and Processing of Volumetric Brain Data - A Summary

Brain data for this study was taken from the published dataset of Baron et al (1996a). This was composed of volumetric measurements of brain structures from some 2500 specimens comprising 341 species of bat. In total, 17 of the 19 families of bats were represented in the dataset (Baron et al, 1996a). For a more detailed account see Baron et al (1996a).

The vast majority of the species were wild-caught in their natural environments and weighed immediately for fresh live body weight. Therefore the body weight

measurements in the Baron et al dataset are as reliable as can reasonably be expected, and the problem of taking average body weight from a species which may be different to the population sampled is negated. Fixation was undertaken within 4 hours of death to reduce the risk of brain shrinkage, which is said to be negligible if done quickly (Baron et al, 1996a).

720 brains representing 276 species were mounted in paraffin and serially sectioned using a uniform slice thickness of 20μm. Of these 694 brains from 272 species were used to calculate volumes. 250 sections from each brain at regular intervals were stained with either cresyl violet or gallocyanine to reveal their architectonic structure. One quarter of these stained sections (i.e. between 60 and 80 sections) taken at regular intervals were used to make volumetric determinations using stereological methods.

Comparison of whole brain volumes versus the sum of brain part volumes determined histologically indicated that in all specimens a degree of post-mortem shrinkage had occurred, probably due to the processes of fixation and mounting. A series of mathematical procedures was then undertaken to correct for the shrinkage.

Variation in Brain Measurements

When attempting to make interspecies comparisons as we do in this study, consideration must be made to the degree of intraspecies variation in the volumetric measurements of a brain structure. Out of the Baron et al (1996a) dataset, 232 of 272 species used for volumetric measurements were represented by more than one individual. The remaining 40 species were represented by single specimens. As is common for studies of intraspecies variation, some structures and some specimens seem more variable than others. The highest degree of variation in the Baron et al (1996a) dataset is to be found in the ventricles. These do not contain neural tissue and instead serve a structural function (Brodal, 1981). The other significant structure to show variation is what Baron et al (1996a) class as 'REST'. This consists of the stumps of the optic nerves, the trigeminal nerve with its gasserian ganglion, the hypophysis and remnants of the meninges adherent to the brain. The majority of REST constituents are not neural tissue. There presence in whole brain volume is important however as they were measured along with the rest of the brain.

Among neural structures the main and accessory olfactory bulbs show the highest degree of intraspecific variation. The AOB in particular is highly variable, being present in an irregular fashion among most new world and some old world bat species.

Baron et al (1996a) argue that the differences in variation seen between brain structures is genuine and does not reflect difficulties in the delineation of some structures during the volumetric determinations.

What do volumetric measurements represent?

Brains are not homogenous structures. Different parts of the brain have been demonstrated to serve quite different functions (Brodal, 1981), and the histological structure of a region may vary considerably from that of its neighbours (Baron et al, 1996a). Likewise, not all parts of brain tissue serve information processing functions. Neurons form complex networks within the brain. These networks are formed from synapses between the axon of one neuron and the dendritic field of another (Zigmond et al, 1999). While a neuron may only have one cell body, the distribution and complexity of its neuropil (i.e. its dendritic and axonal parts) may vary considerably. Thus an increasing complexity of brain function might result not from an increase in neuron number, but in the quantity and distribution of neuropil per neuron (an increase in white

matter relative to grey matter) (Barton & Harvey, 2000). Neurons are supported by a wide range of glial cells, such as oligodendrocytes which myelinate CNS axons, microglia which respond immunologically to the presence of foreign matter within the brain, and astrocytes which anchor neurons to their nearest capillaries, thus ensuring a stable blood supply (Zigmond et al, 1999). Blood is supplied by a range of small blood vessels. The intracellular spaces between cells of the nervous system contain tissue fluid that allows for metabolic exchange. All of these parts have a volume. A perceived change in the volume of a structure may be result in wholly or in part from changes in the number or size of these constituent parts, yet only some of them function to process information (Armstrong, 1982).

Interpretation of the function of particular parts of the brain is dependent on an understanding of the connectivity of the nervous system, i.e. how different parts are connected together to interact and exchange information. Although it is commonly assumed that all mammals are basically similar in the patterns of brain connectivity they exhibit, a careful study of the relevant literature would appear to suggest that this is not universally true (Preuss, 2000). As with all phenotypic characters it is likely that brain connectivity is related to phylogenetic proximity, closely related species being more similar. For this reason connectivity data from Archontan mammals (mostly Primates or Scandentia) is to be preferred. Relatively few tract tracing experiments have been

performed in bats aside from studies of the auditory pathway (for example Covey & Casseday, 1986). In most cases the generalised mammalian plan will be adequate for analysis, although it should be kept in mind that some departures from the general mammalian plan might be expected given the unique demands placed on the bat brain (Neuweiler, 2000).

Whilst it is desirable to attempt the kind of neuroanatomical studies of tracts that can be attempted in vivo with the appropriate tracers such as horseradish peroxidase, HRP), they are expensive to perform and present difficulties in obtaining sufficient live specimens; not to mention the practical obstacles in investigations of novel species. Despite this major gaps are present in our understanding of how the brain varies with phylogeny. These issues mean that comparative analyses using volumetric measurements must continue to be conducted. The knowledge may provide a framework for future experimental investigations which will provide a more detailed understanding of how brain structure varies with phylogeny.

Why use Phylogenetic comparative methods?

Phylogenetic comparative methods (P.C.M.s) are primarily used to counter the problem of statistical dependence when undertaking comparative studies (Martins, 1996). The

accuracy of parametric statistical tests depend on the number of independent data points available for analysis. Closely related taxa are generally more phenotypically similar than they are to more distantly related taxa, as a result of them sharing a common evolutionary history (Harvey & Pagel, 1991). PCMs modify comparative data in such a way as to render it statistically independent so that it can be analysed by standard statistical techniques. Use of untransformed data significantly increases the risk of detecting strong statistical relationships where in fact none exists (type 1 error). The risk of rejecting a true null hypothesis is nine times more likely when using non-phylogenetically corrected data (Harvey & Rambaut, 1998). Likewise the ability to discriminate genuine relationships is much reduced compared to phylogenetic comparative analyses (Nunn & Barton, 2001).

Most comparative methods require a representative phylogeny. While it is debated whether it is possible to reconstruct "perfect" phylogenies (Harvey & Pagel, 1991), it is worth noting that even imperfect phylogenies have been shown to be preferable to performing analyses without taking phylogeny into account (Nunn & Barton, 2001).

For the reasons set out above it was necessary to use the comparative method to analyse the dataset of Baron et al (1996a). The method chosen was Independent Contrasts

(Felsenstein, 1981), as implemented by the computer program CAIC (Purvis & Rambaut, 1995).

Independent Contrasts

The independent contrasts method was originally proposed by Felsenstein (1985) as a means of compensating for statistical dependence with continuously varying characters. The evolution of traits is modelled as being a process akin to Brownian Motion, a stochastic process in which the evolution of characters within a given time interval have a normal distribution (Harvey & Pagel, 1991). Variance in the character is directly proportional to the time interval and independent of the state of the character at the beginning of the time interval (Harvey & Pagel, 1991). Brownian motion underlies much of the theory of population genetics (Martins et al, 1996), and as such is directly applicable to evolutionary problems, especially since contrasts represent the evolutionary change that has occurred in two species since they last shared a common ancestor (Nunn & Barton, 2001). Comparative data derived from species values are not statistically independent, but contrasts (i.e. the difference between two species or ancestral nodes) are (Felsenstein, 1981). Contrasts are always taken beginning with the tips of the phylogenetic tree, as this maximises the number of contrasts obtained.(Felsenstein, 1985;

Nunn & Barton, 2001). Contrasts are standardised according to the phylogeny to have the same variance (homoscedasity) and a mean which equals 0. This is done by dividing each contrast by the summed total of its branch lengths (Purvis & Rambaut, 1995). In this way, ancestral node states, which are less reliably known, exert less influence on the slope because they are set closer to the origin (Nunn & Barton, 2001). Regressions involving contrasts are always forced through the origin (Purvis & Rambaut, 1995).

Independent contrast methods are guaranteed to correct for statistical dependence only if the phylogenetic relationships and assumptions underlying the method are correct (Martins et al, 1996). The method assumes that intraspecific variation in a character is negligible, that the phylogenetic relationships between taxa used in the analysis are known, and that the evolutionary process approximates brownian motion (Felsenstein, 1985). It is therefore necessary to test that these assumptions have been met after the data has been phylogenetically transformed.

The method has been criticised because of the difficulties in obtaining true phylogenies, including accurate tree structures and branch lengths. However, even an imperfect phylogeny will be preferable in most cases than ignoring phylogeny altogether, assuming a star-like speciation model with a single common ancestor for all species in the analysis (Nunn & Barton, 2001). An additional (but unfounded) criticism is that contrast methods

weaken the statistical power to detect relationships by reducing the number of degrees of freedom compared to analyses based on species values. In fact, Felsenstein's method yields n-1 contrasts from species values, and loses an additional degree of freedom from calculation of the slope (Nunn & Barton, 2001). In comparison, analyses based on species values also lose two degrees of freedom: one for calculation of the slope and another for the y-intercept (contrasts are forced through the origin and have no intercept) (Dunn & Clark, 2001; Nunn & Barton, 2001).

Comparative Analysis by Independent Contrasts (CAIC)

The computer program CAIC, developed by Purvis & Rambaut (1995), was used to implement the method of independent contrasts. The algorithm for continuously varying characters ("Crunch") is true to Felsenstein (1985). CAIC is currently at version 2.6.9. Data output from CAIC were analysed using SPSS for Macintosh version 10.0.

Phylogeny

In order to implement CAIC a phylogeny was required. The most comprehensive bat phylogeny currently available is that developed by Dr Kate Jones of the University of Virginia (contact kate.jones@virginia.edu), which has previously been used for

comprehensive investigations of bat life-history evolution (Jones, 1998). The updated version used for this analysis has yet to be published (Jones et al, in press). The Jones phylogeny does not at present have branch lengths, and these were set to a uniform value to represent a speciational model (Harvey & Pagel, 1991). The latest version of CAIC will automatically make this adjustment for phylogenies that do not have a branch length file.

Allometry

Many anatomical, physiological or behavioural properties of organisms show predictable relationships with body size. Allometry describes changes in a character associated with changes in body weight (Harvey & Pagel, 1991). Most such relationships may be described by the power function:

$$Y = aX^b$$

This may be expressed linearly by the equation:

$$\log(Y) = \log(a) + b\log(X)$$

Where X is body weight, Y is the character thought to be associated with X, alpha and beta are properties of the power function (Harvey & Pagel, 1991).

In cases where beta does not equal one, then the ratio of Y/X varies with changes in body weight. When beta is equal to one (a rare and special case - Isometry) then changes in Y are directly proportional to changes in X. Hence in most cases "bigger animals are not just scaled-up versions of smaller animals" (Harvey & Pagel, 1991).

In most cases, the ratio Y/X will decrease as X increases (Negative allometry), however in a few cases the ratio Y/X may increase as X increases (Positive allometry). Three types of allometry are normally recognised: ontogenetic (or growth) allometry is relevant to longitudinal studies, intraspecific allometry examines relationships between adult members of a single species, while interspecific allometry is concerned with relationships between adult members of different species. Since this study is based on adult bats from a large number of species, we are only concerned with interspecific allometry.

Brain-Body Allometry

Brain and body weights scale with negative allometry, since increases in the size of the brain across species do not keep pace with increases in body size. Different scaling

exponents have been proposed, most significantly that of .67 (Stephan et al., Jerrison, 1973) and .75 (Martin, 1983). The exponent of .67 has been proposed because of the relationship between the brain and its receptors and effectors, which are intimately tied to body surface area (Jerrison, 1973). The assumption is that across all mammals the density of receptors per unit surface area is constant, however there appears to be little empirical support for such a notion (Hendry et al, 1999). Most recent studies have favoured an exponent of .75, since this corresponds closely to the exponent for basal metabolic rate (Harvey & Pagel, 1991).

Testing statistically for the coordinated evolution of brain components

A major objective of the thesis was to test for coordinated evolution between brain parts within functional systems. This had already been demonstrated on a more limited set of structures for Primates and Insectivores by Barton & Harvey (2000). Independent contrasts of the volume of each brain structure were tested against other structures in separate multiple regressions. Regressions were forced through the origin as is required for independent contrasts. Structures were chosen for analysis on the basis of established patterns of anatomical connectivity. Megachiroptera and microchiroptera were analysed separately in order to test for differences in their patterns of brain evolution which might be expected either from their very different sensory organisations (Neuweiler, 2000) or

the possibility of diphyly (Pettigrew, 1986). All data was logarithmically transformed in order to normalise variance in the sample prior to analysis (Dunn & Clark, 2001).

Multiple regression is a multivariate statistical method which allows the relationship to be determined between one dependent and two or more independent variables. These variables may be continuous, as is the case for brain volumes (Dunn & Clark, 2001). The independent variables may also show some degree of correlation between one another, although very strong correlations may produce unreliable results, a situation referred to as multicollinearity (Pedhazur, 1973). The dataset under investigation (Baron et al, 1996a) fulfils the basic criteria required for the multiple regression to be used: the data constitutes a random sample having a normal distribution and the relationship between dependent and independent variables are expected to be linear.

The overall regression can be considered by examining the "goodness of fit" of the data points to the regression line, known as R^2. In multiple regression R^2 is known to increase as the number of independent variables increases and it is therefore common to consider instead an adjusted R^2 which takes into account the number of independent variables. The F statistic assesses whether the independent variables taken together are significantly associated with the dependent variable. Correlation coefficients indicate the relationship between the dependent and one independent variable whilst holding constant the values

of the other independent variables. In this case we are mostly interested in the standardised correlation coefficients (beta weight) which can indicate which independent variable has the greatest effect on the dependent variable. The statistical significance of these correlations can be determined by examining the t values associated with the correlation coefficients (Dunn & Clark, 2001; Pedhazur, 1973).

In each analysis, a variable was calculated as the net brain volume, minus the volumes of structures included in the multiple regressions ("rest of brain"). This was done so that the relationship between specific structures and other parts of the brain that do not form functional networks could be taken into account. Significant relationships with the rest of brain variable suggest that the dependent variable is evolving with structures not included in that multiple regression. This might be expected for structures that have very widespread connections within the brain (for example the amygdala or hippocampus) (Brodal, 1981). It has been proposed that testing for changes in one brain structure relative to changes in the rest of the brain in this way may introduce autocorrelation if individual brain structures are especially large (e.g. neocortex) (Stephan et al, 1991).

The volume of most brain structures is strongly correlated with body weight, and this may in some cases increase the correlation coefficient of two structures included in a regression (Stephan et al, 1991). Although it is possible to hold constant the effects of

body size by including body size as a variable in the multiple regressions this might risk adding a significant source of multicollinearity (Pedhazur, 1973). Body size variation was not corrected for in Baton & Harvey (2000) and to permit comparisons with this paper body size was not included in the analyses.

As noted above, strong correlations among independent variables may lead to multicollinearity, although the strength of the correlation needed to produce adverse effects is not always clear. Multicollinearity produces counterintuitive results as a result of adverse effects on the standard errors of regression coefficients, their significance levels and confidence limits. For example, a negative correlation may be indicated where a positive one is expected (Pedhazur, 1973).

Although impressive in scope, in some cases there were insufficient sample sizes for some structures in the Baron et al (1996a) dataset which placed limitations on the analyses that could be performed. These are noted in the text.

3
Correlated Evolution Among Major Brain Components

Introduction

Mosaic brain evolution is expected to produce correlated size changes in structures that are functionally connected, independent of size changes in the rest of the brain. Barton & Harvey (2000) examined the mosaic evolution of major brain components (medulla oblongata, mesencephalon, diencephalons, neocortex and cerebellum for primates and insectivores. They found significant partial correlations suggesting that pairs of structures show correlated evolution and that the patterns of this evolutionary change are very common in both primates and insectivores. Here the issue of correlated evolution among major brain components is examined for megachiropteran and microchiropteran bats.

Neurobiology of the Major Brain Components

Of the five structures chosen for this initial analysis all are highly heterogenous. The medulla oblongata, mesencephalon and diencephalons are each subdividable into a large range of structures that might be otherwise attributed to a specific functional system. The neocortex was chosen in place of the telencephalon because the latter structure varies

Figure 1:
Multiple regression of major brain structures in Megachiroptera to show correlated volumetric evolution

	DIE	MES	CER	OBL
NEO	1.427 7.583 P<0.0001	-0.228 -1.552	0.089 0.417	-0.295 1.312
DIE		0.185 2.170 P<0.05	0.070 0.538	0.215 1.595
MES			0.009 0.027	0.378 1.101
CER				0.661 3.208 P<0.005

Standardised correlation coefficients (top row) shown with t-values (middle) and p-value if results are significant. Structures in the left colum were regressed on structures in the top row as for Barton & Harvey (2000).

Significant results indicate the two structure exhibit correlated volumetric evolution with changes in the other structures taken into account.

Figure 2:
Multiple regression of major brain structures in microchiroptera to show correlated volumetric evolution.

	DIE	MES	CER	OBL
NEO	0.834 10.172 P<0.0001	0.206 2.491 P<0.05	0.218 3.571 P<0.001	-0.263 2.503 P<0.05
DIE		0.068 0.938	-0.92 1.687	0.411 4.962 P<0.0001
MES			-0.98 1.296	0.672 6.225 P<0.0001
CER				0.920 6.609 P<0.0001

Standardised correlation coefficients (top row) shown with t-values (middle) and p-value if results are significant. Structures in the left colum were regressed on structures in the top row as for Barton & Harvey (2000).

Significant results indicate the two structure exhibit correlated volumetric evolution with changes in the other structures taken into account.

enormously in its functional organisation (Barton & Harvey, 2000) (it includes much of the limbic system), but even the neocortex is divisable into primary projection and association areas involved in sensory, motor and cognitive activities. Although more homogenous in its internal organisation the cerebellum exhibits localisation of function, the pattern of this is still not well understood (Voogd & Glickstein, 1998).

Results

These results show that significant partial correlations exist between even these crude brain subdivisions, as was previously found by Barton & Harvey (2000).

Comparing these results with those shown in Barton & Harvey (2000), it is clear that both of these plans deviate from the pattern shown by Insectivores and Primates. The pattern for Megachiroptera shows correlations previously described between the neocortex and diencephalon, mesencephalon and diencephalon and cerebellum and medulla oblongata. It is likely that the lack of additional correlations (for example between mesencephalon and medulla obongata) are due to the small sample size available for megachiroptera (n=14 independent contrasts).

The pattern of correlated volumetric evolution shown by the microchiroptera includes all but two of the correlations described by Barton & Harvey (2000) plus some additional correlations not previously described. Medulla correlates with neocortex and diencephalon, but mesencephalon fails to correlate with diencephalon. It is unlikely that this result has occurred because of sample size limitations (n=59 independent contrasts). It is possible that the strong relationship between sensory nuclei of the medulla (proprioceptive, vestibular, auditory) would cause this result since the cortical representations of these nuclei make up such a large part of the neocortex of the bat (O'Neil, 1995).

It is also notable that unlike for primates and insectivores, the basic anatomical chain of structures (medulla oblongata-mesencephalon-diencephalon-neocortex) is not represented in these results despite functional connections being present between each of these subdivisions.

4
The Auditory System

Introduction

The bat auditory system is highly specialised compared to most other mammals: the detection of echolocation calls represents the primary navigation sense for many species. It is also the most studied part of the brain since bats are useful experimental models for the auditory system (for example Covey et al, 1987; Dallard, 1965). As well as the normal auditory range detected by most mammals, bats must also detect and process ultrasound (with frequencies greater than 20 kHz), which is outside of the normal human hearing range (Neuweiler, 2000).

At its simplest, the echolocation mechanism of a bat consists of a transmitter and receiver (Neuweiler, 2000). Echolocation calls are generated by the larynx in Microchiroptera (Grinnel, 1995). Only one genus of Megachiroptera, *Rousettus*, uses echolocation and its calls are produced by tongue clicking (Altringham, 1996). Laryngeal innervation comes predominantly from the Vagus nerve (cranial nerve X) and the tongue is controlled by the hypoglossal nerve (cranial nerve XII) (Baron et al, 1996c). Sound waves produced by

either of these mechanisms radiate out from an area centred on the bats mouth and nose until they contact an object. At this point the object reflects a part of the sound wave and the bat detects this "echo" through the ear mechanism and auditory system of the brain (Grinnel, 1995). The physical properties of the contact object determine the amount of sound that is reflected but also the modification to that sound in terms of frequency and amplitude that is returned (Neuweiler, 2000). In this way the bat is able to derive a mental image of the shapes, textures and distances of objects in the surrounding environment that surpasses merely location (Neuweiler, 2000).

Despite the obvious advantages of being able to navigate in total darkness, echolocation has some notable limitations (Neuweiler, 2000). Unlike vision, which is essentially passive, the generation of echolocation calls requires that energy be expended. Each emission is highly focussed and provides information on a small area and the distance over which an echo can be returned is limited (Grinnel, 1995). Moreover the time taken for an echo to return may be too long to avoid obstacles at close range during fast flight. The biggest limitation however is that an echolocating bat can only obtain sensory feedback at discrete time intervals, unlike vision which produces a continuous stream of information (Neuweiler, 2000). The auditory system of the bat must be specially adapted to cope with this limitation, but it is also not surprising that vision still plays an important role in obstacle avoidance (Chase, 1981, 1983) and navigation (Barbour et al, 1966).

Neurobiology of the Auditory System

Sounds travel as vibrations of air particles. These vibrations travel through the mammalian ear to the cochlea, where the displacement of hair cells is translated into a series of action potentials in the auditory nerve (Neuweiler, 2000). The auditory pathway can be divided into monaural (from one ear) and binaural (from both ears) pathways that proceed in an essentially linear fashion from the cochlear nuclei to the inferior colliculus, some pathways going by way of the superior olive. The inferior colliculus in turn projects to the medial geniculate body and the auditory cortex. Although it is in the neocortex that the most sophisticated analysis of auditory information occurs, the lower auditory pathway is heavily involved in discerning the location of a sound source. Through connections to the superior colliculus these structures can produce orienting movements of the head and neck.

Tonotopy is a characteristic of the auditory system. Rather than a purely topographical organisation, inputs in many auditory structures a map exists that is organised according to frequency and other sound characteristics, so that high frequency sounds may be represented at the rostral end of a nucleus, and low frequency sounds at the caudal end, for example.

Dorsal Cochlear Nucleus (DCO)

The dorsal cochlear nucleus exhibits a 3 layered organisation of molecular, intermediate and polymorphic layers (Covey & Casseday, 1995), although the extent of differentiation of the nucleus and the cell densities of the individual layers varies taxonomically especially between Megachiroptera and Microchiroptera (Baron et al, 1996a). The nucleus appears best differentiated in Megachiroptera, but also in the Microchiroptera families Megadermatidae, Desmodontidae and Phyllostomidae. Conversely the Microchiroptera families Mormoopidae and Noctilionidae have the least differentiated DCO (Baron et al, 1996b). How this relates to the function of the nucleus in the different groups is not clear, however. In the families Hipposideridae and Rhinolophidae it is possible to discern dorsal and ventral components of the nucleus (Schweizer, 1981). The granular cochlear nucleus, located between DCO and VCO was included in the DCO volume by Baron et al (1996a). The DCO appears particularly sensitive to sound frequencies in the normal hearing range of most mammals (Brown, 1999).

Ventral Cochlear Nucleus (VCO)

The VCO of bats is divisible into posteroventral (PVCO) and anteroventral (AVCO) components (Baron et al, 1996a). PVCO is subdivided into caudal, lateral and ventral divisions (Schweizer, 1981; Zook & Casseday, 1985). As with the DCO there is taxonomic variation in the differentiation of the nucleus. In Hipposideridae, Rhinolophidae, Mormoopidae and Natalidae the nucleus is one folded cell layer. In Vespertilionidae the nucleus is two layered; in Rhinolophidae and Hipposideridae the nucleus is composed of two to three convoluted layers of cells (Baron et al, 1996b). Conversely Nycteridae have the least developed PVCO (Baron et al, 1996a).

AVCO is subdivided into caudal and rostral parts (Baron et al, 1996a). There is a notable caudorostral gradient, the rostral AVCO having a higher cell density than the caudal part (Zook & Casseday, 1985). VCO appears especially sensitive to high frequency sounds, including ultrasound.

Superior Olivary Complex (OLS)

The superior olivary complex is located in the ventral part of the medulla oblongata, caudal to the pons (Covey & Casseday, 1995). It is composed of three major nuclei (medial OLSM, lateral OLSL nuclei and the nucleus of the trapezoid body CTM) plus a group of periolivary nuclei which were included in the OLS volume by Baron et al (1996a). OLS extends from the rostral end of the facial nucleus to the ventral nucleus of the lateral lemniscus (Baron et al, 1996a).

The medial superior olivary nucleus (OLSM) shows the greatest taxonomic variation of the OLS structures (Baron et al, 1996c). In most Microchiroptera two cell layers are present (e.g. *Myotis, Rhinolophus, Hipposideros* and *Tadarida*) (Baron, 1972; Schreiber, 1982; Casseday et al, 1988a,b). OLSM may be subdivided into ventral and dorsal portions. They are equally developed in Rhinopomatidae and Emballonuridae. The dorsal portion is better developed in Megadermatidae, Vespertilionidae and Natalidae; in Mormoopidae and Furipteridae the ventral portion is better developed (Baron et al, 1996b). The lateral part of OLS is large in Noctilionidae and Desmodontidae (Baron et al, 1996a). A functional interpretation of these differences has not been ventured.

The nucleus of the trapezoid body (CTM) is the most medial part of the superior olivary complex. No obvious differences are apparent in its relative size among the different taxonomic groups of bats (Baron et al, 1996a).

Eight periolivary nuclei were named for *Pteronotus* (Zook & Casseday, 1982a). Those present commonly in bats are the dorsal, dorsomedial, ventral, ventromedial, anterolateral and the lateral and ventral nuclei of the trapezoid body (Baron et al, 1996a).

The superior olive is known to integrate inputs from the cochlear nuclei in order to allow for low level auditory discrimination (Brown, 1999).

Nuclei of the Lateral Lemniscus (NLL)

There are three nuclei of the lateral lemniscus: ventral (VLL), intermediate (ILL) and dorsal (DLL) (Brown, 1999). VLL is the most taxonomically variable. In Megachiroptera the VLL has a similar cytoarchitecture to the insectivores (Stephan et al, 1991). In Microchiroptera it can be divided into two parts medial (VLLM) and lateral (VLLL). The cell packing is apparently less dense in VLLL in Rhinopomatidae and Hipposideridae; in Mollosidae the division into VLLM and VLLL is not clear (Baron et al, 1996a).

Vespertilionidae exhibit a different organisation with the VLL being divisable into dorsal and ventral components (VLLD and VLLV) (Baron et al, 1996a).

ILL forms a prominent mass on the lateral surface of the brain stem in all Microchiroptera but there is little taxonomic variation (Baron et al, 1996a). DLL, located dorsomedial to ILL is best developed in Megachiroptera. Among Microchiroptera, Megadermatidae and Phyllostomidae also have well developed DLL, but among most Microchiroptera it is rather small (Baron et al, 1996a).

Inferior Colliculus (INC)

Along with the superior colliculus, the inferior colliculus forms the midbrain tectum, bordered by the cerebellum, DLL and parabrachial nuclei and covered by the superior colliculus (Pollak & Park, 1995). The brachium of the inferior colliculus was included in the INC volume by Baron et al (1996a).

The INC consists of a central nucleus (INCC) bordered by a pericentral area of dorsal and external pericentral nuclei. Paracentral nuclei usually seen in the cat appear not to be present in bats with the exception of the nucleus of the rostral pole (Morest & Oliver, 1984). In *Rhinolophus ferrumequinum* the INCC was divided into dorsomedial,

dorsolateral and ventromedial parts (Schweizer, 1981) but anterolateral, medial and dorsal parts in *Pteronotus parnelli* (Zook & Casseday, 1982a).

The dorsal pericentral nucleus (INCP) lies above the INCC and is well developed in some Microchiroptera (*Myotis, Noctilio, Pteronotus*) (Baron et al, 1996a) but is very small in *Rhinolophus* (Schrieber, 1982). In Megachiroptera the nucleus is very small (Baron et al, 1996a). Lateral to the INCC lies the external pericentral nucleus (INCE). The brachium is large in Phyllostomus but small in Molossus (Baron et al, 1996a).

Medial Geniculate Body (CGM)

The CGM lies caudolateral to the diencephalons immediately below the superior colliculus, with the hippocampus lateral and CGL rostrolateral. In bats the CGM can be divided into 3 nuclei (Baron et al, 1996a). The ventral nucleus (GMV) is subdivided into medioventral and lateroventral parts. The medial "magnocellular" nucleus (GMM) is better developed in Microchiroptera than it is in Megachiroptera. The dorsal nucleus (GMD) consists of the suprageniculate (SG) nucleus, which exhibits a characteristic high cell density, and another (unnamed) component at the dorsal end of the nucleus (Baron et al, 1996a). The CGM represents on of the higher order auditory structures and is

intimately connected with the auditory cortex (O'Neil, 1995). GMV is known to be involved in auditory discrimination (Winer, 1985).

Connectivity of the Auditory System

Because of the interest in bat echolocation there is some usable connectivity data based specifically on bats. The species must intensively studied include *Rhinolophus ferrumequinum* (Schweizer, 1981), *Pteronotus parnelli* (Zook & Casseday, 1982a,b, 1985) and *Eptesicus fuscus* (Covey & Casseday, 1986; Covey, 1993).

The cochlear nuclei receive projections from the cochlear via the auditory nerve and give rise to three major pathways: the trapezoid body arising from the AVCO, the intermediate acoustic stria arising from the PVCO and the dorsal acoustic stria from the DCO (Baron et al, 1996a).

The AVCO receives afferents from the ascending branch of the auditory nerve and projects to the lateral superior olive ipsilaterally, the medial nucleus of the trapezoid body contralaterally and the medial superior olive bilaterally (Zook & Casseday, 1985; Casseday et al, 1988ab). These fibres ascend as the lateral lemniscus pathway (Brown, 1999).

The connections of the PVCO are essentially the same as for the AVCO (Baron et al, 1996a). One interesting detail of the bilateral projections from this nucleus was observed in *Rhinolophus sp.* Where the projection was shown to favour the contralateral side (i.e. it is asymmetric) (Casseday et al, 1988a,b). In contrast the projection in the cat is symmetrical (Cant & Casseday, 1986). The functional significance of this difference is unclear but it may represent an adaptation for improved localisation of sound (Brown, 1999). PVCO sends direct efferent fibres to the contralateral inferior colliculus (Schweizer, 1981).

The DCO receives afferents from the descending branch of the auditory nerve and projects to the dorsal and lateral nuclei of the lateral lemniscus. It also provides a bilateral projection to the inferior colliculus (Baron et al, 1996a). Both the DCO and VCO project to the periolivary nuclei (Zook & Casseday, 1985).

The superior olive projects strongly to the ipsilateral inferior colliculus (Pollak & Park, 1995). The CTM projects to the intermediate and ventral nuclei of the lateral lemniscus. CTL both receives and projects to the AVCO and DCO, while CTV receives fibres from AVCO, PVCO and DCO, sending weak efferent fibres to AVCO and DCO but a strong projection to PVCO (Covey & Casseday, 1995).

The subnuclei of the NLL each have their own pattern of connections. VLL receives fibres from the contralateral AVCO and PVCO and ipsilateral CTM, projecting to the ipsilateral inferior colliculus. ILL receives afferents from the DCO and VCO bilaterally and the OLS ipsilaterally. It also receives a projection from the VLL. The ILL projects to the inferior colliculus bilaterally and the contralateral ILL across the corpus callosum. The DLL receives afferents from the ipsilateral medial inferior olive, VLL, ILL and lateral superior olive bilaterally. The DLL also receives projections from the contralateral VCO and DCO. DLL projects to the superior colliculus bilaterally (Covey, 1987).

The inferior colliculus receives projections from all auditory nuclei except the MGB and CTM (Baron et al, 1996a). The INCC receives topographically organised projections from the DCO bilaterally (although the contralateral projection is stronger) and the PVCO and AVCO contralaterally (Zook & Casseday, 1985). DCO primarily targets the dorsomedial part of the INCC while the AVCO targets the ventral 2/3 of the nucleus. There is a tonotopic organisation in which high frequencies reside on the anterolateral part of the INC and low frequencies on its medial part (Pollak & Park, 1995). Indirect projections from the cochlear nuclei come via the superior olive and nuclei of the lateral lemniscus (Baron et al, 1996a). Ventral OLSM and medial OLSL project to the ventromedial part of the INCC. Dorsal OLSM and the lateral OLSL project to anterior

and lateral INCC. Of the projections from NLL, only DLL projects bilaterally to the INC, the ILL and VLL projecting contralaterally. These projections overlap with those from the AVCO and OLS. There are also projections from the auditory cortex to the INC, both to the pericentral area and the central nucleus (Schweizer, 1981). Additional afferents arise from the pontine reticular formation, midline raphe nucleus and funicular nuclei (Baron et al, 1996a).

Efferent projections from the INC pass to the auditory cortex via the MGB, and to the cerebellum via the pontine nuclei (Pollak & Park, 1995). A direct projection from INC to the cerebellum has been demonstrated in *Tadarida* (Henson et al, 1968) but not in Rhinolophus. There are also projections to the superior colliculus and the central grey (Schweizer, 1981).

The connectivity of the CGM can be divided into four distinct pathways (Oliver, 1982). The central pathway, the fastest and most direct, passes from the INCC to the GMV and from there to the primary auditory cortex (O'Neil, 1995). Another pathway originates in the pericentral nuclei and passes to GMD which in turn projects to cortical areas bordering the primary auditory cortex. GMD, along with SG, also receives projections from the midbrain tegmentum and superior colliculus (Baron et al, 1996a). SG is known

to project to the frontal lobe (Kobler et al, 1987). The final "widespread" pathway originates widely from midbrain structures and projects to the CGM (Baron et al, 1996a).

The Auditory System, Behaviour and Ecology

Among bats there is a significant diversity in the structure of echolocation calls both in terms of the call design (of which there are a number of basic variants) and components (FM, CF, broadband). Calls vary in frequency (usually in a range of 20-120 kHz), amplitude and duration (range 0.2-100 ms).

The main purpose of the bat echolocation system is to find and identify targets (Grinnel, 1995). The properties of the target determine the modification that occurs to the returned "echo" sound wave. Moving targets, for example flying insects, produce Doppler shifts and the returning echo is frequency modulated (Brown, 1999). Changes in target size and shape will produce amplitude changes as the surface area of the target changes shape. Reflected sound from complex surfaces tends to produce complex interference patterns which give the echo a characteristic spectra which is distinct from that of other targets (Brown, 1999). A common method for assessing such acuity is to use holes drilled in a metal surface. *Eptesicus fuscus* has been shown to be able to detect a difference 0.6-0.9 mm (Simmons et al, 1974) while *Myotis myotis* can detect 0.8-1 mm differences

(Habersetzer & Vogler, 1983). Range to the target can typically be determined to within an accuracy of 10-45 mm (Simmons & Verron, 1971) and represents the difference between the time of emission and the time at which the echo is received by the bat. Orientation to a sound is achieved because each ear receives a slightly different pattern from sounds not occurring in the midline (Brown, 1999).

Many bat echolocation calls are primarily intended to detect flying insects (Bellwood, 1988). A variety of echolocation calls are suitable for this task. *Rhinolophus sp.* And *Pteronotus parnelli* typically use long duration constant frequency (CF) calls which allows them to detect the wing beat of prey insects (Goldman & Henson, 1977). Hipposideridae however prefer short duration CF calls (Baron et al, 1996b). Bats that use long duration CF-FM calls are also capable of discriminating fluttering targets. Most bats use a variety of call structures that are suited to different situations (Neuweiler, 2000). Broadband short duration calls are best for discriminating targets, while long duration constant frequency calls are most sensitive to velocity. However it is possible to identify species of bat solely by their echolocation call structures (Altringham, 1996). Because of the tonotopic organisation of the auditory cortex, bats with different call structures have slightly different topographical maps in the auditory cortex. For example, species with constant frequency call will have a large constant frequency responsive area in the auditory cortex (O'Neil, 1995).

Results

Multiple regressions were performed for ten pairs of structures within the auditory pathway that are known to possess strong anatomical connections, whilst controlling for variation in the volume of the rest of the brain. Megachiroptera and Microchiroptera were analysed separately. Significant partial correlations were found in six out of ten regressions for both Megachiroptera and Microchiroptera. Five out of these six correlations were common to both clades. It is also notable that for those pairs of structures that were found to show partial correlations, the predicted relationship was the strongest in all cases when compared to the rest of the brain.

These results are shown in full in Figures 3 and 4.

Figure 3:
Multiple regressions of functionally connected brain structures and rest of brain volume for Megachiroptera.

Dependent Variable	Independent Variables	R^2_{adj}	Df	F	F sig.	Beta	t	Sig
AUD	OLS	0.948	2,15	137.96	0.0001	0.937	8.898	**0.0001**
	R. of Brain					0.048	0.457	0.655
DCO	OLS	0.797	2,15	30.45	0.0001	0.892	4.286	**0.001**
	R. of Brain					0.019	0.089	0.930
VCO	OLS	0.945	2,15	130.80	0.0001	0.864	7.996	**0.0001**
	R. of Brain					0.132	1.224	0.243
VCO	INC	0.931	2,14	95.27	0.0001	0.676	3.664	**0.003**
	R. of Brain					-0.310	-1.683	0.118
DCO	INC	0.922	2,14	84.130	0.0001	0.517	4.338	**0.001**
	R. of Brain					-0.507	-4.257	**0.001**
OLS	INC	0.909	2,14	71.032	0.0001	0.472	1.984	0.071
	R. of Brain					-0.503	-2.116	0.056
FUN	INC	0.932	2,14	96.696	0.0001	0.305	0.940	0.366
	R. of Brain					-0.670	-2.065	0.061
CER	INC	0.936	2,14	104.194	0.0001	0.659	1.109	0.289
						-0.315	-0.530	0.606
SUC	INC	0.961	2,14	173.777	0.0001	0.855	5.934	**0.0001**
	R. of Brain					-0.136	-0.942	0.365
NEO	INC	0.952	2,14	141.086	0.0001	-0.571	-0.755	0.465
	R. of Brain					-1.548	-2.044	0.064

Figure 4:
Multiple regressions of functionally connected brain structures and rest of brain volume for Microchiroptera

Dependent Variable	Independent Variables	R^2_{adj}	df	F	F sig.	Beta	t	Sig
AUD	OLS	0.972	2,64	1120.751	0.0001	1.085	23.879	**0.0001**
	R. of Brain					0.113	2.482	**0.016**
DCO	OLS	0.823	2,64	149.924	0.0001	0.069	0.776	0.441
	R. of Brain					-0.854	-9.683	**0.0001**
VCO	OLS	0.890	2,64	259.553	0.0001	0.871	10.547	**0.0001**
	R. of Brain					-0.085	-1.025	0.309
VCO	INC	0.932	2,59	405.544	0.0001	0.878	11.650	**0.0001**
	R. of Brain					-0.098	-1.300	0.199
DCO	INC	0.865	2,59	190.588	0.0001	0.194	2.123	0.038
	R. of Brain					-0.762	-8.358	**0.0001**
OLS	INC	0.870	2,59	198.335	0.0001	0.622	6.141	**0.0001**
	R. of Brain					-0.339	-3.346	**0.001**
FUN	INC	0.803	2,59	121.524	0.0001	0.111	0.526	0.601
	R. of Brain					-0.793	-3.754	**0.0001**
CER	INC	0.820	2,59	135.339	0.0001	0.000	-0.002	0.998
	R. of Brain					-0.909	-4.121	**0.0001**
NEO	INC	0.870	2,59	199.171	0.0001	-0.491	-1.700	0.095
	R. of Brain					-1.417	-4.903	**0.0001**

Although the connections of the medial geniculate body and nuclei of the lateral lemniscus were of interest these structures could not be examined because of insufficient sample sizes (n=2 independent contrasts for Megachiroptera, n=7 independent contrasts of Microchiroptera).

The superior olive exhibits strong correlations with the auditory nuclei as a whole (Megachiroptera R^2_{adj} =0.948, df=2,15, p=0.0001; Microchiroptera R^2_{adj} =0.972, df=2,64, p=0.0001) and with the ventral cochlear nucleus (Megachiroptera R^2_{adj} =0.945, df=2,15, p=0.0001; Microchiroptera R^2_{adj} =0.890, df=2,64, p=0.0001). In Megachiroptera the dorsal cochlear nucleus was found to be strongly correlated with the superior olive (R^2_{adj} =0.797, df=2,15, p=0.001), a result not found in Microchiroptera (R^2_{adj} =0.823, df=2,64, p=0.441). The importance of the DCO for the processing of sounds within the average hearing range of most mammals has been previously discussed (Neuweiler, 2000). It has been noted previously that the DCO is well developed in Megachiroptera whilst in Microchiroptera the nucleus tends to be small and undifferentiated (Baron et al, 1996a). Microchiroptera are known to be relatively insensitive to low frequency sound (Dalland, 1965), and this may provide some explanation for the observed result.

In Microchiroptera the superior olivary nucleus was found to correlate strongly with the inferior colliculus (R^2_{adj} =0.870, df=2,59, p=0.0001) and this result was not observed in Megachiroptera (R^2_{adj} =0.909, df=2,14, p=0.071). There are two possible explanations for this. Firstly it will be noted that the sample size available for Megachiroptera was quite small (n=14 independent contrasts) compared to Microchiroptera (n=59 independent contrasts). The absence of a correlation could therefore be the result of a lack of statistical power. Alternatively echolocation may produce a need for a greater interaction between this structures that is unnecessary for Megachiroptera.

Neither the dorsal column nuclei (Megachiroptera R^2_{adj} =0.932, df=2,14, p=0.366; Microchiroptera R^2_{adj} =0.803, df=2,59, p=0.601) nor the cerebellum (Megachiroptera R^2_{adj} =0.936, df=2,14, p=0.289; Microchiroptera R^2_{adj} =0.820, df=2,59, p=0.998) showed a significant correlation, despite the latter structures significant cerebellar input in Microchiroptera (Baron et al, 1996c). However a significant correlation was found between the superior colliculus and the inferior colliculus (Megachiroptera R^2_{adj} =0.961, df=2,14, p=0.0001; Microchiroptera R^2_{adj} =0.845, df=2,59. p=0.0001). In bats the superior and inferior colliculi are tightly integrated and function in head-orienting reflexes to auditory stimuli (Covey et al, 1987).

The presence of statistically significant partial correlations between structures within the auditory pathway strongly suggest correlated evolution between those structures. Moreover many of these correlations were stronger than those with the rest of the brain. Those partial correlations that do exist occur principally among structures with large but topographically limited projections (i.e. the lower auditory pathway); in most cases the role of these connections and their functional significance are well understood. These results strongly suggest that structures in the auditory pathway have evolved together in a coordinated fashion independent of the rest of the brain.

The presence of statistically significant negative correlations between dependent variables and the rest of the brain was unexpected. One explanation for these results is the presence of trade-offs between the different functional modalities. In the case of echolocating bats the auditory system might have expanded at the expense of olfactory or visual brain structures. It is also possible that these unexpected negative correlations result from multicollinearity, however.

5
The Olfactory System

Introduction

Olfaction was perhaps the first sensory system to evolve, and plays a role in diverse behaviours such as locating food, communication, mate choice and predator-prey interactions (Smith & Shepherd, 1999). The role of the olfactory system is to detect and discriminate between odour molecules, identify novel odours and orient an animal toward a odour source (Hendry et al, 1999). Due to the need to accomplish these tasks, the functional architecture of the olfactory system is said to be highly conserved between phyla (Baron et al, 1996c). There is a strong relationship between olfaction and diet. Olfaction in bats is made more difficult in that, as flying animals, they are frequently exposed to concentrations of odour molecules that are lower than those encountered by terrestrial or arboreal animals (Altringham, 1996). Despite this many bats have highly developed olfactory systems (Baron et al, 1996a).

The accessory olfactory system is best considered as a parallel pathway and is only present in some bats (Baron et al, 1996a). Its function is still unclear but is widely believed to be involved with the detection and processing of pheremones that are detected

by the vomeronasal organ (Neuweiler, 2000). In Hamsters and Rabbits pheremones have been shown to trigger endocrine and behavioural responses related to reproductive behaviour. It has been shown that mice that have their vomeronasal organ removed surgically while immature will fail to generate the typical vocalisation cues to reproductively viable females when old enough to mate; the rat accessory olfactory bulb has been shown to be driven by sexually active substances (Smith & Shepherd, 1999). The vomeronasal organ itself is fluid filled and contains receptor cells with microvilli, rather than the cilia of main olfactory receptors. It opens into the nasal cavity by way of a small canal and into the mouth via the nasopalatine duct (Neuweiler, 2000). For this reason it has been alternatively proposed that the accessory olfactory system is involved in sampling food taken into the mouth (Baron et al, 1996c).

Neurobiology of the Olfactory System

Transduction of odour molecules entering the nose is accomplished by receptor cells within the olfactory epithelium (Smith & Shepherd, 1999). The area and thickness of the olfactory epithelium may be related to the olfactory capability: in megachiroptera the thickness of the epithelium is 250 μm, whereas in microchiroptera it is only 50 μm (Bhatnagar & Kallen, 1974). It is the megachiroptera that are generally credited with the superior sense of smell (Neuweiler, 2000). Receptor cells project to glomeruli,

spheroidal regions of neuropil with only a few glial cells, in which the axons of olfactory receptor cells synapse with the dendtrites of mitral and tufted cells (Smith & Shepherd, 1999). In the rabbit, each glomerulus is estimated to receive projections from 25000 receptor cells. The high degree of convergence increases stimulus intensity thresholds and is a key feature of highly developed olfactory systems (Hendry et al, 1999). Differential activation of glomeruli generate neural images of the odour stimulus which is analysed by higher brain centres (Smith & Shepherd, 1999).

The olfactory system was considered to consist of the main and accessory olfactory bulbs, palaeocortex, schizocortex, parts of the amygdala and ventral striatum. Projections to the neocortex and hippocampus were also examined.

Main Olfactory Bulb (MOB)

The main olfactory bulb is the most rostral component of the telencephalon, lying in continuity with the cribriform plate of the ethmoid through which it receives projections from the olfactory epithelium (unmyelinated fibres collectively termed the olfactory nerve) (Baron et al, 1996a). It appears that megachiroptera possess significantly more perforated cribriform plates than microchiroptera, which are most densely perforated in the dorsal half of the cribriform plate (Bhatnagar & Kallen, 1974). It would seem

therefore that megachiroptera olfactory bulbs receive a greater level of projections than microchiroptera, but this is a crude measure of olfactory performance and has not been directly related to the odour discriminating capabilities of individual species (Baron et al, 1996c).

The main olfactory bulb is generally considered to consist of between six and seven distinct layers. In small olfactory bulbs these layers are often indistinct, but may easily be resolved in larger specimens (Baron et al, 1996a). The main olfactory bulb generally has a round appearance. In species with well developed olfactory bulbs, the layers may surround an olfactory ventricle which is visible in the Megachiroptera, and some New World Microchiroptera (the Phyllostomidae and Desmodontidae) (Baron et al, 1996a).

Accessory Olfactory Bulb (AOB)

The accessory olfactory bulb is a highly variable structure and is only present in certain groups of bats i.e. Desmodontidae, Miniopterinae, most Phyllostomidae and some Mormoopidae (Bhatngar & Meisami, 1998). With the exception of the Miniopterinae, all of these bats are neotropical (Nowak, 1999). No megachiropteran bat appears to possess an accessory olfactory bulb. The principal afferent of the accessory olfactory bulb is the vomeronasal (sometimes called Jacobson's) organ (Neuweiler, 2000).

A six-layered structure characterises the accessory olfactory bulb and is typical of all mammals examined (Bhatngar & Meisami, 1998). The first and most superficial layer is derived from the vomeronasal nerve which courses over the medial surface of the main olfactory bulb.

The main and accessory olfactory bulbs share much in common in terms of structure and the differences reflect the functional specialisations of the two systems and provide a means for their determining the boundaries of each structure in Nissl stained sections (Baron et al, 1996a).

Palaeocortex (PAL)

The palaeocortex is a heterogenous structure consisting of the structures related to the anterior piriform lobe. These include the retrobulbar region, olfactory tubercle and prepiriform region (Brodal, 1981). In the Baron et al. (1996a) dataset palaeocortex also includes the lateral olfactory tract, anterior commisure and substantia innominata. However the periamygdaloid region was included with the amygdala (Baron et al, 1996a).

The retrobulbar region topographically links the olfactory bulb with is respective hemisphere. An intrabulbar division can be discerned in large brained bats with well developed olfactory systems (Bhatngar & Kallen, 1974). The retrobulbar region appears to have two layers, an outer cell-poor and an inner cell-dense layer. Megachiroptera may also exhibit a cell-poor polymorphic layer (Baron et al, 1996a).

The olfactory tubercle lies caudal to the retrobulbar region and medial to the prepiriform region. It is designated as being part of the olfactory cortex, but has close topographical and functional relations with the ventral striatum (i.e. nucleus accumbens and substantia innominata). Large brained bats with well developed olfactory systems typically exhibit three distinct layers: a broad cell poor layer, a folded cell-dense layer and a polymorphic layer between which may be found the "islands of Calleja" which typify this part of the palaeocortex. In bats with less developed olfactory bulbs these layers may be quite indistinct (Baron et al, 1996a).

The Prepiriform region is caudal to the retrobulbar region but lateral to the olfactory tubercle. It too exhibits three layers, of which the cell-poor and cell dense layers are well developed in all bats, the polymorphic layer exhibits a particularly high cell density (Baron et al, 1996a). The structure varies little between large and small brained bats, as has also been found in Scandentia and Primates (Stephan, 1970). Physiologically the

prepiriform region may be divided into frontal and temporal parts which reflect differences in the pattern of projections. The prepirifrom region is the most extensive recipient of main olfactory bulb projections (Switzer et al, 1985).

Schizocortex (NEO)

Also called the parahippocampal region, the schizocortex is considered to consist of entorhinal (area 28), perirhinal (area 35) and presubicular (areas 27 and 49) cortices. The schizocortex as a whole has a distinctive cytoarchitecture with obvious cell-poor layers. The schizocortex is organised into six layers which form external and internal principle laminae.

Numerous subdivisions of the schizocortex are possible based on cytoarchitectonic and histochemical criteria. The entorhinal cortex may be divided into lateral and medial parts. Perirhinal cortex has a transitional cytoarchitecture: the region closest to the entorhinal cortex has features in common with it, but the remainder shows features in common with neocortical areas (Baron et al, 1996a).

Connectivity of the Olfactory System

The Main Olfactory Bulb receives its principle afferent projection from the cells of the olfactory epithelium. Efferent projections pass to the anterior olfactory nucleus, prepiriform cortex and olfactory tubercle (Heimer, 1978); these are included in the palaeocortex (Baron et al, 1996a). Additional projections pass to the amygdala and entorhinal cortex (included in schizocortex). The accessory olfactory bulb receives its afferent projection from the vomeronasal organ (Neuweiler, 2000). Fibres originating in the main olfactory bulb pass through the accessory olfactory bulb and some synaptic activity is thought to result (Smith & Shepherd, 1999). However the main destination of efferent fibres from the accessory olfactory bulb are to the amygdala, both its medial and cortical parts receiving projections (Switzer et al, 1985). The olfactory bulb receive projections from the structures to which they project: these are thought to modify sensory input (Smith & Shepherd, 1999).

Retrobulbar palaeocortex receives an additional afferent projection from the hippocampus (Brodal, 1981). Reciprocal projections exist with the septum, amygdala and hypothalamus (Luskin & Price, 1983a,b). The olfactory tubercle has a complex projection pattern. In addition to the projections from the main olfactory bulb, it receives projections from the hippocampus, schizocortex, amygdala and mesencephalon (Skeen & Hall, 1977). Additional efferent fibres pass to the ventral globus pallidus and substantia nigra. Reciprocal connections exist between olfactory tubercle and the thalamus (Brodal, 1981).

The entorhinal cortex, in addition to fibres from the main olfactory bulb also receives projections from the hippocampus and neocortex. The parahippocampal region as a whole receives projections from the entire telencephalon, but particularly the amygdala, septum and mesencephalon. In terms of efferents, the hippocampus receives a significant projection, since the schizocortex is thought to act as a relay between the main olfactory bulb and hippocampus. The schizocortex also projects to the dentate gyrus of the neocortex, the striatum and diencephalon (Switzer et al, 1985).

Prepiriform palaeocortex receives the majority of projections from the main olfactory bulb (Heimer, 1978). It also receives afferents from the brainstem. Efferent projections are to the striatum and hippocampus. Reciprocal projections exist with the amygdala and diencephalon (hypothalamus and medial thalamus) (Switzer, 1985). Via the thalamus the projections reach the medial and lateral orbitofrontal neocortex (Brodal, 1981).

Role of Olfaction in Behaviour and Ecology

The olfactory system is clearly very variable within the order Chiroptera, making up a largest proportion of the telencephalon in megachiroptera (Baron et al, 1996a). Likewise the anatomy of the nasal cavity is also variable between species, for example in terms of volume and the number of turbinate bones (Neuweiler, 2000). Partly this may reflect the

dual role of the bat nasal cavity: in many echolocating species sonar emissions are released through the nose (Grinnel, 1995). In an analysis of the ethmoidal cribriform plate, Bhatnagar & Kallen (1974a) noted that the number of perforations created by the olfactory fibres were strongly related to dietary habits of the species under investigation as well as to the overall size of the olfactory bulb. For example the area of the cribriform plate ranged from 12-23 mm^2 in insectivorous species compared to an average of 54 mm^2 in frugivorous species. Likewise the number of perforations ranged from 30-100 in insectivorous bats and 75-145 in frugivorous species. In addition, the volume of olfactory epithelium in significantly greater in frugivorous bats than in insectivorous microchiroptera, but body size may also be a factor. Receptor:glomerular ratios in *Artibeus jamaicensis* (frugivorous) and *Desmodus rotundus* (sanguinivorous) are reportedly much higher than in *Eptesicus fuscus*, an insectivorous bat (Bhatngar, 1977). High olfactory receptor: glomerular ratios indicate a higher degree of convergence in olfactory signalling, meaning that odour molecules can be detected at weak concentrations (Smith & Shepherd, 1999).

Behavioural studies support the view that olfaction is of considerable importance to frugivorous bats in locating food sources. In a conditioning test, *Rousettus aegyptiacus* were trained to take bananas from either black or white boxes. Regardless of previous conditioning, individuals would always correctly identify the box containing the food

reward by olfactory cues alone. When both boxes were empty the bat would ignore them both. Hence *Rousettus* were demonstrably able to detect 50-100 mg of banana whilst flying at 15 km per hour (Baron et al, 1996c). It was also capable of choosing between banana paste and banana oil. *Carollia*, a frugivorous microchiropteran was shown to be able to locate a source of bananas solely by odour; this ability has also been demonstrated by *Phyllostomus hastatus*. Additionally, individuals were able to discriminate between ripe and unripe fruit. *Carollia* appear to make qualitative odour choices whilst searching for food in reinforced choice tests (Laska, 1990). Rieger & Jakob (1988) baited mist nets with bananas using either olfactory cues only, olfactory, visual and echolocation cues, echolocation and visual cues or no cues at all (as a control). The numbers of bats caught indicate a non-random distribution of responses. Most bats were caught when only olfactory cues were presented whilst the fewest were caught when no bait was present (Rieger & Jakob, 1988). Sternoderminae showed a significant trend in this respect, but Glossophaginae and Carollinae did not, perhaps reflecting differences in the ways olfactory cues are used to guide feeding behaviour. Plants may form symbiotic relationships with bats in order to fulfil needs for pollination or seed dispersal. This is often facilitated by the use of attractive odours (Sussman & Raven, 1978).

Insectivorous microchiroptera are also able to use olfactory cues to detect food insects with remarkable acuity (Baron et al, 1996c; Neuweiler, 2000). It has been noted that

when stalking, vampire bat species visit the same victim repeatedly and they may use olfactory cues to locate their prey (Schmidt, 1988).

The sense of smell may also be used to facilitate social communication or to demarcate territory (Neuweiler, 2000). Most bats possess a range of exocrine glands and some of these are thought to release pheromones (Altringham, 1996). They may be used to mark roost sites; young bats left while the mother forages may be located again partly by odour. Bats may possess a combination of facial, pararhinal, pectoral, cervical, gular, scapular, elbow, anal, preputial, frontal and caudal glands. *Noctilio* possesses bacteria laden recesses in the groin (Baron et al, 1996c). Not all of these glands need serve communication functions. For example, the pararhinal glands are thought to function primarily to lubricate the wing membranes (Cernova, 1989). *Pteropus* species regularly rub glandular secretions over their entire bodies, but whether or not this is related to olfactory signalling is unclear (Nelson, 1965). The variability in the olfactory systems and gland distribution of bats could indicate the significance of social communication within a species (Bhatngar & Meisami, 1998). Exocrine glands appear to find their greatest expression in colonial species (Cernova, 1989). *Pteropus poliocephalus* scent mark tree roosts during the mating system, using a highly developed scapular gland. Members of the same social group often smell each others scapular region, perhaps as a means of identification (Nelson, 1965). *Mollosus* too have well developed scent glands.

Dominant male *Molossus* mark other harem members (including subordinate or immature males) with excretions of a throat gland. Similarly they mark strategic locations within their home range (Schmidt, 1985). Nose touching behaviour has been documented in *Myotis lucifugus* and *Desmodus rotundus* returning to their social groups (Thomas et al, 1979). Mother-young interactions also appear predominantly to utilise odour. Female *Rousettus aegyptiacus* and *Pteropus poliocephalus* identify young by their odour (Nelson, 1965). Female *Myotis myotis*, *M. nigricans* and *Antrozous pallidus* also appear to identify young by smell, perhaps in combination with acoustic stimuli (Balcombe, 1990). *Tadarida brasiliensis* females mark young with their own odour in order to facilitate recognition (Gustin & McCracken, 1987).

Results

Multiple regressions were performed for ten pairs of structures within the olfactory system that are known to possess strong anatomical connections, whilst controlling for variation in the volume of the rest of the brain. Separate analyses were performed for megachiroptera and microchiroptera with, and without, an accessory olfactory bulb. For those species with an accessory olfactory bulb two additional regressions were performed to test for correlations between the accessory and main olfactory systems, and the accessory olfactory bulb and amygdala. Significant partial correlations were found in all

Figure 5:
Multiple regressions of functionally connected olfactory brain structures and rest of brain volume for Megachiroptera

Dependent Variable	Independent Variable	R^2_{adj}	df	F	F sig.	Beta	t	Sig
MOB	PAL	0.984	2,18	546.278	0.0001	0.474	4.384	**0.0001**
	R. of Brain					-0.529	-4.893	**0.0001**
MOB	AMY	0.976	2,18	364.100	0.0001	0.518	4.384	**0.0001**
	R. of Brain					-0.484	-4.099	**0.001**
MOB	SCH	0.981	2,18	464.476	0.0001	-0.057	-0.486	0.634
	R. of Brain					-1.046	-8.865	**0.0001**
PAL	HIP	0.986	2,18	630.188	0.0001	0.227	1.648	0.119
	R. of Brain					-0.771	-5.606	**0.0001**
PAL	STR	0.987	2,18	706.726	0.0001	0.175	0.911	0.376
	R. of Brain					-0.821	-4.278	**0.001**
PAL	AMY	0.990	2,18	865.469	0.0001	0.935	7.259	**0.0001**
	R. of Brain					-0.062	-0.480	0.637
PAL	SEP	0.987	2,18	664.664	0.0001	0.071	0.441	0.665
	R. of Brain					-0.924	-5.724	**0.0001**
SCH	HIP	0.989	2,18	796.530	0.0001	0.595	4.009	**0.001**
	R. of Brain					-0.404	-2.719	**0.015**
SCH	NEO	0.996	2,18	2523.165	0.0001	0.186	1.424	0.174
	R. of Brain					-0.813	-6.210	**0.0001**
SCH	STR	0.985	2,18	581.568	0.0001	-0.257	-0.938	0.362
	R. of Brain					-1.248	-4.556	**0.0001**

Figure 6:
Multiple regression of functionally connected brain structures and rest of brain volume for Microchiroptera lacking an accessory olfactory system

Dependent Variable	Independent Variable	R^2_{adj}	df	F	F sig.	Beta	t	Sig.
MOB	PAL	0.967	2,56	813.803	0.0001	0.523	8.387	**0.0001**
	R. of Brain					-0.481	-7.701	**0.0001**
MOB	AMY	0.953	2,56	571.979	0.0001	0.065	1.277	0.207
	R. of Brain					-0.923	-18.103	**0.0001**
MOB	SCH	0.970	2,56	906.347	0.0001	0.099	2.203	**0.032**
	R. of Brain					-0.899	-19.927	**0.0001**
PAL	HIP	0.887	2,56	220.396	0.0001	0.254	2.751	**0.008**
	R. of Brain					-0.714	-7.738	**0.0001**
PAL	STR	0.971	2,56	949.388	0.0001	0.076	1.528	0.132
	R. of Brain					-0.918	-18.562	**0.0001**
PAL	AMY	0.933	2,56	393.056	0.0001	0.213	2.890	**0.006**
	R. of Brain					-0.744	-10.518	**0.0001**
PAL	SEP	0.973	2,56	1013.899	0.0001	0.435	8.557	**0.0001**
	R. of Brain					-0.576	-11.327	**0.0001**
SCH	HIP	0.895	2,56	239.631	0.0001	0.715	4.580	**0.0001**
	R. of Brain					-0.241	-1.542	0.129
SCH	NEO	0.974	2,56	1061.346	0.0001	-0.418	-4.328	**0.0001**
	R. of Brain					-1.391	-14.400	**0.0001**
SCH	STR	0.966	2,56	788.076	0.0001	0.60	0.688	0.495
	R. of Brain					-0.926	-10.641	**0.0001**

Figure 7:
Multiple regression of functionally connected brain structures and rest of brain volume for Microchiroptera possessing accessory olfactory systems.

Dependent Variable	Independent Variables	R^2_{adj}	df	F	F sig.	Beta	t	Sig
MOB	PAL	0.986	2,40	1395.548	0.0001	0.522	7.999	**0.0001**
	R. of Brain					-0.482	-7.390	**0.0001**
MOB	AMY	0.953	2,40	407.042	0.0001	-0.40	-0.605	0.549
	R. of Brain					-1.012	-15.123	**0.0001**
MOB	SCH	0.970	2,40	650.610	0.0001	0.076	0.997	0.325
	R. of Brain					-0.915	-12.023	**0.0001**
MOB	AOB	0.770	2,40	67.834	0.0001	-0.907	-4.900	**0.0001**
	R. of Brain					-1.629	-8.802	**0.0001**
AOB	AMY	0.944	2,40	341.226	0.0001	0.053	1.117	0.271
	R. of Brain					-0.940	-20.016	**0.0001**
PAL	HIP	0.894	2,40	169.175	0.0001	0.193	1.161	0.253
	R. of Brain					-0.763	-4.584	**0.0001**
PAL	STR	0.976	2,40	828.241	0.0001	-0.017	-0.236	0.815
	R. of Brain					-1.005	-13.796	**0.0001**
PAL	AMY	0.935	2,40	273.379	0.0001	-0.024	-0.235	0.815
	R. of Brain					-0.989	-9.651	**0.0001**
PAL	SEP	0.943	2,40	334.366	0.0001	0.192	1.566	0.126
	R. of Brain					-0.789	-6.445	**0.0001**
SCH	HIP	0.933	2,40	280.032	0.0001	1.043	5.264	**0.0001**
	R. of Brain					0.077	0.390	0.699
SCH	NEO	0.976	2,40	823.786	0.0001	-0.289	-2.740	0.009
	R. of Brain					-1.268	-12.011	**0.0001**
SCH	STR	0.974	2,40	747.749	0.0001	-0.090	-0.911	0.368
	R. of Brain					-1.074	-10.852	**0.0001**

three groups (four for megachiroptera, seven for microchiroptera without an AOB, four for microchiroptera with an AOB) but only two were common to all groups. These were the main olfactory bulb and the palaeocortex (Megachiroptera R^2_{adj}=0.984, df=2,18, p=0.0001; Microchiroptera without AOB R^2_{adj}=0.967, df=2,56, p=0.0001; Microchiroptera with an AOB R^2_{adj}=0.986, df=2,40, p=0.0001), and the schizocortex and the hippocampus (Megachiroptera R^2_{adj}=0.989, df=2,18, p=0.001; Microchiroptera without an AOB R^2_{adj}=0.895, df=2,56, p=0.0001; Microchiroptera with an AOB R^2_{adj}=0.933, df=2,40, p=0.0001). Main olfactory bulb is known to project directly to the palaeocortex in mammals. In microchiroptera the partial correlation between main olfactory bulb and palaeocortex was larger than to the rest of the brain, although this was not true for megachiroptera. The schizocortex is known to be the structure responsible for relaying information from the main olfactory bulb to the hippocampus (Switzer et al, 1985). In all three groups of bats the partial correlation between schizocortex and hippocampus was greater than that for the rest of the brain, particularly so in microchiroptera.

In megachiroptera a strong partial correlation was observed between main olfactory bulb and amygdala (R^2_{adj}=0.98, df=2,18, p=0.0001) that was not found in microchiroptera (without AOB R^2_{adj}=0.953, df=2,56, p=0.207; with an AOB R^2_{adj}=0.953, df=2,40, p=0.549). The palaeocortex was also strongly correlated with amygdala in

megachiroptera (R^2_{adj}=0.99, df=2,18, p=0.0001) and microchiroptera lacking an accessory olfactory system (R^2_{adj}=0.93, df=2,56, p=0.006) but not in microchiroptera possessing an accessory olfactory bulb (R^2_{adj}=0.935, df=2,40, p=0.815). The accessory olfactory bulb projects principally to the amygdala but the correlation between these structures was also not significant (R^2_{adj}=0.944, df=2,40, p=0.271).

Microchiroptera lacking an accessory olfactory system exhibited a number of correlations not found in other groups. A strong partial correlation was found between palaeocortex and hippocampus (R^2_{adj}=0.887, df=2,56, p=0.008) and between palaeocortex and septum (R^2_{adj}=0.973, df=2,56, p=0.0001).

A negative partial correlation was observed in microchiroptera between schizocortex and neocortex (microchiroptera without AOB R^2_{adj}=0.97, df=2,56, p=0.0001; microchiroptera with AOB R^2_{adj}=0.976, df=2,40, p=0.009). This was not found in megachiroptera (R^2_{adj}=0.996, df=2,18, p=0.174). This trend in microchiroptera might suggest a trade-off with another sensory system, for example auditory or visual structures. Evidence for another trade off was found in a significant negative correlation between the main and accessory olfactory bulbs (R^2_{adj}=0.770, df=2,40, p=0.0001). Given that these structures are thought to process odours in parallel, a trade-off was not unexpected.

In many cases the partial correlations with the rest of the brain were negative. As with the auditory system, this could represent trade-offs between sensory systems or be a symptom of multicollinearity in the data.

Many of the structures included here in the olfactory system are also part of the limbic system. As such they are very heavily interconnected (Baron et al, 1996a). Such heavy interconnections between structures might make it difficult to detect correlated evolution statistically because projections from each structure are so widely dispersed.

6
The Sensorimotor System

Introduction

In order for animals to move in a coordinated way they must not only initiate fixed motor programs, but also use information gathered by the senses to guide and modify movements generated by their motor centres (Thach, 1999). This is the role fulfilled by the sensorimotor system. Information from all sensory modalities are used to guide movement of the body and its parts. The animals own movement will generate sensory feedback (proprioception) which is relayed to the somatosensory system (Hendry et al, 1999); visual, auditory and even olfactory cues are used to guide movement. The sensorimotor system is here considered to consist of the motor structures and those sensory modalities directly related to movement, i.e. the somatosensory and vestibular brain structures.

For a bat, the difficulties of coordinating locomotion are multiplied many times. Flight is a continuous balancing act between the aerodynamic generation of lift and the constant pull of gravity (Altringham, 1996). A flying bat must avoid obstacles and find its way to food, in many cases flying insects and small vertebrates. At the same time it must

generate a wing beat suitable for the generation of thrust and adjust the position of the wing to produce whatever directional motion (such as turning or changes in altitude) are required. Finally, the bat must constantly make adjustments for buffeting by the wind or by other environmental factors such as the presence of thermals, that might affect its flight pattern (Bilo, 1992). It should be of little surprise that a large part of the brain in bats is dedicated to the control of flight (Neuweiler, 2000).

Despite the wing being a defining characteristic of bats, it places significant limitations on their agility either when moving terrestrially or arboreally (for example in the case of flying foxes which clamber in amongst branches for fruit) (Dietz, 1973). The evolution of the wing would have placed massive demands for adaptations in the control of movement by the brain (Neuweiler, 2000).

Neurobiology of the Sensorimotor System

The sensorimotor system is complex and consists of a large number of brain structures. Not all of these are represented in the Baron et al (1996a). dataset. There is little understanding at present of the ways in which the bat motor systems are adapted to the control of powered flight, although it would be expected that parts of the brain involved with control of the forelimb musculature (i.e. the wing) will be particularly well

developed (Kennedy, 1991). Despite the obviously specialist nature of bat locomotion, it is likely that the organisation of the motor system in bats is basically similar to that found in other mammals (ten Donkelaar, 2001). Of the common experimental animals, it would be expected that the primates would represent the best comparison group, based both on phylogenetic proximity (Adkins & Honeycutt, 1991) but also the highly developed forelimb control common to all primate species (Porter & Lemon, 1993). The somatosensory cortex of the megachiroptera has been compared to that of primates, with which it shares many features (Krubitzer & Calford, 1992).

Movements of the body are brought about by the contraction of skeletal muscles, which are composed of large numbers of muscle fibre cells. Groups of muscle fibres are innervated by motoneurons, located in the brainstem and ventral horn of the spinal cord (Thach, 1999). Descending motor pathways originating in the motor cortex and red nucleus (corticospinal and rubrospinal tracts) synapse onto motoneurons either directly (in Primates) or indirectly (Porter & Lemon, 1993).

Just as motoneurons innervate muscles, so sensory afferents arise from individual muscles, and may project locally within the spinal cord (e.g. to coordinate reflexes) or to higher centres such as the cerebellum or somatosensory cortex. Such proprioceptive

feedback provides the animal with information about how its various parts are moving, the forces and stresses incurred (Hendry et al, 1999; Kirkpatrick, 1994).

The somatosensory system is concerned with the detection of stimuli directly making contact with the body, for example touch or heat. Somatic sensation may be divided into three primary types: Exteroceptive functions include mechanoreception (skin stretch), thermoreception (temperature) and nociception (pain). Proprioceptive functions involve sensing the position and movement of body parts by means of receptors in muscles, tendons and joints (Thach, 1999). Interoceptive functions monitor the internal state of the body e.g. the viscera (Hendry et al, 1999). Mechanoreception covers several types of stimulus, including form perception (judging the shape of objects that contact the skin), texture and vibration. Somatic sensory receptors are specific to the type of stimulus they detect. Mechanoreceptors include Merkel cells which detect pressure, form and texture. Meissner corpuscles detect low frequency vibration and motion, while Ruffini corpuscles detect the stretching of skin and Pacinian corpuscles respond to higher frequency vibration. Mechanoreceptors tend to be distributed superficially within the skin, and in higher densities on glabrous (non-hairy) compared with hairy skin (Hendry et al, 1999). Proprioceptors are also divided into types. Bare Ruffini or Paciniform endings detect extension movements at joints, relaying this information to prevent hyperextension. Ruffini endings can also detect changes in joint angle. Golgi tendon organs detect muscle

tension and muscle spindles detect muscle length and velocity of contraction. All of this information is monitored by the sensorimotor system to regulate the generation of appropriate movements (Hendry et al, 1999).

Information from these peripheral sensory receptors is conveyed to the CNS via dorsal root ganglion cells which form ascending pathways including the dorsal root-medial lemniscus pathway and the spinocerebellar tract (Baron et al, 1996a).

The somatic sensory pathways are characterised by modality segregation and as such preserve a distinct functional and topographical organisation throughout their length, culminating in the homuncular pattern in primary somatosensory cortex (S1). The gracile fasciculus receives input from dermatomes T7-Cox1 (i.e. the hindlimb and lower trunk) while the cuneate fasciculus receives its dermatomal contribution from C1-T6 (i.e. forelimb and upper body). These run to the gracile and cuneate nuclei (the dorsal column nuclei) of the medulla oblongata. Of importance to this study are the nucleus fascicularis gracilis (FGR) which receives projections from the hindlimb, nucleus fascicularis cuneatus medialis (FCM) and the nucleus fascicularis cuneatus externus (FCE) (which receive projections from the forelimb. Efferent projections from these nuclei form the medial lemniscus pathway which projects to the venteroposterior nucleus of the

Thalamus, where they are sorted by place and modality to form the cortical area S1 in the parietal lobe (Hendry et al, 1999).

The organisation of the Trigeminal system is reminiscent of that for the rest of the body but supplies the orofacial region. The projections from the trigeminal (semilunar) ganglion run as three nerves (supraorbital, infraorbital and mandibular) which terminate in the head and orofacial region as the same sensory receptors found in the rest of the body (e.g. mechanoreceptors and proprioceptors) (Hendry et al, 1999). As with dorsal root ganglia, these pathways are modality segregated. Axons from the mechanoreceptors and proprioceptors terminate in the trigeminal primary sensory nucleus or descend in the trigeminal spinal tract to the spinal trigeminal nucleus, where it decussates to join with the medial lemniscus pathway entering the medial venteroposterior nucleus of the thalamus (Baron et al, 1996a).

The cerebellum regulates the function of descending motor pathways, and damage to it produces erratic movements and potentially severe deficits (Porter & Lemon, 1993). The cerebellum receives input from all brain regions. Its output is directed to the descending motor pathways via the red nucleus, motor and premotor cortices (via the thalamus). It affects the excitability of motoneurons either directly to the descending tracts or indirectly via the motor cortex. Whilst the understanding of the exact functions of the

cerebellum are disputed, a consensus view is that the cerebellum is involved in the sensory guidance of movement (Thach, 1999). Sustained flight is a behaviour likely to require considerable cerebellar input (Neuweiler, 2000). Additionally, there is much interest in the auditory functions of the cerebellum in bats and the role it has in echolocation (Baron et al, 1996c). The cerebellum has a homogenous cytoarchitecture, but there is increasing evidence for the localisation of function within it (Glickstein, 2000).

Trigeminal Sensory Nucleus (TR)

The trigeminal nucleus (TR) can be divided into three sensory components: the mesencephalic sensory nucleus (TRM), principle somatosensory nucleus (TRP) and nucleus of the spinal tract (TRS). In bats it is not possible to measure the TRM volume as it is a narrow strip of dispersed cells located in the lateral border of the central grey (Baron et al, 1996a). In small brained species the separate components of the trigeminal system are hard to differentiate. The TRP and TRS measurements are combined into a single value for TR by Baron et al (1996a).

The TRS lies in continuity with the dorsal funiculus of the spinal cord and also the TRP. It is divided into three subnuclei: caudalis, interpolaris and oralis; varying in their fibre

connections (Olszewski, 1950). The caudalis extends from the 1st cervical root to the obex, the interpolaris from the obex to the rostral limit of the hypoglossal nucleus (Astrom, 1953) and the oralis extending from their to the rostral limit of the facial nucleus (Baron et al, 1996a).

TRP extends from the lateral side of the rostral pole of the motor trigeminal nucleus. Its measured volume includes part of the reticular formation at this level (Baron et al, 1996a). The supratrigeminal nucleus (Darian-Smith, 1973) is also included within the TRP, being located at its dorsomedial margin (Baron et al, 1996a).

Dorsal Column Nuclei (FUN)

The dorsal column nuclei are located in the rostral end of the dorsal columns: their constituent cells are second order neurons receiving synapses from primary sensory afferents (Hendry et al, 1999). The fibre tracts begin in the dorsal columns: the contralateral and adjunct medial lemniscus pathways and an ipsilateral projection to the ipsilateral inferior olive and cerebellum (Massopust et al, 1985).

Gracile Fascicular Nucleus (FGR)

Extends from the first cervical segment to the caudal limit of the medial vestibular nucleus, where it appears continuous with the fasciculus gracilis (Baron et al, 1996a).

Medial Cuneate Fascicular Nucleus (FCM)

The FCM extends from the caudal limit of the FGR to the caudal limit of the intermediate vestibular nucleus, bordering the FGR and TR, ventral to the fasciculus cuneatus. The nucleus may appear to blend with the latter, and with the FCE and FGR (Baron et al, 1996a).

External Cuneate Fascicular Nucleus (FCE)

Begins caudally at the obex and reaches to the caudal end of the intermediate vestibular nucleus. FCE lies dorsal to the trigeminal tract extends into the adjacent inferior cerebellar peduncle (Baron et al, 1996a).

Lateral Reticular Nucleus (REL)

This nucleus protrudes from the ventral surface of the medulla obongata. It consists of two parts: a main part and a much smaller subtrigeminal part. These are completely

separate and present in all species of bats examined (Baron et al, 1996a; Wahlberg, 1952), although the subtrigeminal part may be very small. The main part is located between the trigeminal complex laterally and the inferior olive medially, ventral to the nucleus ambiguus and the reticular formation. The subtrigeminal part is ventromedial to the trigeminal complex, and is the most rostral part of the REL. Although described in a number of other species (Brodal, 1943), a distinct parvocellular region appears not to be present in bats (Baron et al, 1996a).

Inferior Olivary Nuclear Complex (INO)

Located in the ventromedial medulla oblongata, the INO lies dorsal to the pyramidal tract. It extends from the caudal end of the REL to the caudal end of the facial nucleus. INO is medial to REL, ventral to the reticular formation. There are three major subdivisions: medial accessory olive, dorsal accessory olive and the principle olivary nucleus (Kooy, 1916); these can be differentiated in bats by virtue of their topography. There are also four subnuclei which are functionally related to the medial accessory nucleus and which are included in INO by Baron et al (1996a).

Vestibular Complex (VC)

The vestibular complex consists of four nuclei plus a series of associated cell clusters (Brodal, 1974) which were included in the nuclei volumes by Baron et al (1996a). It is important to note that each nucleus exhibits a unique pattern of connections; each may be involved in different reflexes or movements of a specific body part.

Medial Vestibular Nucleus (VM)

The medial vestibular nucleus lies between the FGR caudally, its rostral end just caudal to the locus coeruleus and adjacent to the PRP and TSO. The reticular formation is found ventrally (Baron et al, 1996a). VM exhibits an homogenous cytoarchitecture (Wilson & Jones, 1979). Based upon previous physiological studies and on connections, VM appears to integrate movements of the eyes and head (Meesson & Obzewski, 1949).

Inferior Vestibular Nucleus (VI)

The inferior vestibular nucleus is found between VM and VL and adjacent to the restiform body and cuneate nuclei (Brodal, 1981). Its volume as recorded in Baron et al (1996a) includes a series of small cell groups 'f', 'z' and 'x' which are functionally interconnected with VI (Brodal & Pompeiano, 1957).

Lateral Vestibular Nucleus (VL)

The lateral vestibular nucleus is located between VI and VS and covered dorsally by the white matter of the cerebellum (Baron et al, 1996a). In terms of cytoarchitecture the nucleus can be divided into dorsocaudal and rostroventral parts (Haines, 1975; Gacek, 1977). The volume for VL also includes the nucleus interstitialis nervi vestibularis (Baron et al, 1996a). VL is involved in the generation of the vestibulo-occulomotor reflexes (Mehler & Rubertone, 1985); it is of particular interest in bats because it processes vestibular information relevant to forelimb control (Brodal, 1984).

Superior Vestibular Nucleus (VS)

The superior vestibular nucleus is found between VL and the parabrachical nuclei (Baron et al, 1996a). It is deeply involved in the sacculo-occulomotor reflex and the control of eye movements (Carpenter & Cowie, 1985). VS appears to be particularly strongly involved in the generation of optokinetic and smooth pursuit eye movements (Langer et al, 1985).

Striatum (STR)

The striatum is a subcortical motor structure and part of the basal ganglia (Brodal, 1981). In Insectivores the striatum is undifferentiated (Stephan et al, 1991) but in bats (Baron et al, 1996a) and Primates (Porter & Lemon, 1993) it can be clearly divided into two components, the caudate nucleus and putamen, which have separate input/output organisations (Parent, 1986). These two parts of the striatum communicate via projections which traverse the internal capsule (Brodal, 1981). In the dataset Baron et al (1996a) the volume also includes the nucleus accumbens, part of the olfactory tubercle and ventral pallidum (these last two compose the largely olfactory 'ventral striatum') and part of the internal capsule. Although functionally related the pallidum is not included (Baron et al, 1996a).

On morphological grounds the caudate can be divided into a head, which makes up part of the wall of the lateral ventricle, body, which lies along the dorsocaudal border of the thalamus, and tail, which forms part of the roof of the temporal horn of the lateral ventricle (Brodal, 1981). The putamen lies ventrolateral to the caudate with the fibres of the external capsule as its immediate lateral relation (Parent, 1986).

Nucleus Prepositus Hypoglossi (PRP)

The PRP is one part of the perihypoglossal complex (Marburg, 1931) and is considered a precerebellar nucleus by Brodal (1952) because it projects to the cerebellum. In the Baron et al (1996a) dataset the PRP also includes the nucleus supragenualis facialis to which it is functionally related (Brodal, 1952). The cytoarchitecture of the nucleus is said to be homogenous (Baron et al, 1996a). Based upon its connectivity the PRP is thought to be involved in the control of eye and head movements pertinent to gaze control (McCrea & Baker, 1985).

Cerebellum (CER)

The cerebellum lies dorsal to the pons and medulla and is separated from them ventrally by the 4th ventricle, occupying the posterior cranial fossa (Brodal, 1981). The internal structure of the cerebellum is stereotyped and homogenous compared to the cerebral cortex. It contains five principle cell types (Purkinje projection neurons and four classes of interneuron, the most common being the granule cells) and is arranged in four cell layers. Most superficial is a cell poor molecular layer, the purkinje layer consisting of rows of purkinje neurons, the densely packed granule cell layer and the deepest, medullary, layer (Baron et al, 1996a). It has been estimated that there are more neurons in the cerebellum than in the whole of the rest of the brain, its ratio of afferent to efferent fibres being 40:1 (Baron et al, 1996c).

The cerebellum consists of an outer cortex of grey matter with an inner core of white matter. The deep cerebellar nuclei are embedded in this white matter core (Voogd & Glickstein, 1998). Paired paramedian sulci separate the cerebellum into a central vermis medially and two hemispheres, laterally. The cortex is highly convoluted into folia and the folia are organised into ten lobules. In turn these lobules are divided into three lobes: the anterior (lobules I-V) and posterior (lobules VI-IX) lobes, which are separated by the primary fissure, and the flocculonodular lobe (lobule X) which is separated from the posterior lobe by the posterolateral fissure (Brodal, 1981). The anterior and posterior lobes are involved in the planning, execution and control of movement; the flocculonodular lobe is involved in the maintenance of balance and control of eye movements (Baron et al, 1996c).

The cerebellum may be divided functionally between the corpus cerebelli and flocculonodular lobe (Voogd & Glickstein, 1998). The corpus cerebelli may be divided into longitudinal zones on the basis of their inputs, either spinal or pontine. The anterior lobe, simple lobule, pyramis and gracile lobule receive mostly spinal and trigeminal input. The folium, tuber vermis, uvula and entire hemisphere are dominated by pontine inputs (though the hemispheres receive some spinal projections). Alternatively the cerebellum may be divided mediolaterally on the basis of its outputs into three functional

subdivisions consisting of part of the cerebellar cortex with one or more deep cerebellar nuclei (Brodal, 1981).

The cerebellum receives two types of input. Climbing Fibres come only from the inferior olivary nuclear complex in the form of olivocerebellar fibres. Mossy fibres come from the spinal cord, pontine (which receives projections from the cerebral cortex), vestibular and reticular formation nuclei and traverse the white matter before forming branches which enter several folia

The spinocerebellum receives somatosensory afferents from the spinal cord and some projections from other areas. It is composed of the vermis and medial hemisphere of both anterior and posterior lobes. These areas project to the fastigial (vermis) and interposed (hemisphere) nuclei. Due to it being considered phylogenetically "old" (Baron et al, 1996a) the spinocerebellum is sometimes called the palaeocerebellum.

The spinocerebellum is important in the control of body musculature and displays a somatotopic organisation. The vermis corresponds to the axial muscles while the medial hemisphere corresponds to limb muscles. Spinocerebellar tracts transmit proprioceptive information from the limbs and trunk, whilst the trigeminocerebellar tracts do the same for the head (Brodal, 1981). These projections are somatotopic in that there are distinct

areas for head, neck and trunk on the vermis and arms and legs on the medial hemisphere. However within each area are more complex representations where a part may be represented at multiple sites or a site may correspond to several different body parts. This is termed "fractured somatotopy" and is thought to bring information on different body parts together for complex movements (Voogd & Glickstein, 1998).

The cerebrocerebellum takes its input from the cerebral cortex, via the pontine nuclei. It is composed of the lateral hemisphere. The cerebrocerebellum is involved in the planning of movement and is interconnected with the contralateral cerebral cortex, indirectly via the pontine nuclei. The decussation occurs in the pons and projects to the cerebellum via the middle peduncle. The cerebrocerebellum projects to the dentate nucleus. The dentate, in turn, projects to the ventral lateral thalamus and from there to the rest of the cerebral cortex. It also projects to the parvocellular red nucleus which projects back to the ipsilateral inferior olivary nuclear complex.

The vestibulocerebellum is crucial in maintaining balance and controlling eye movements. It corresponds to the flocculonodular lobe and receives primary afferents from the vestibular apparatus and secondary afferents from the vestibular nuclei (VC). The vestibulocerebellum projects to the vestibular nuclei (medial, inferior and posterior) via the MCN.

Connectivity of the Sensorimotor System

The sensorimotor system is a good example of a distributed system – it consists of quite separate parts that are closely integrated by afferent and efferent connections (Porter & Lemon, 1993). Almost the whole of the cerebral cortex projects to the corpus striatum and then to the globus pallidus (Parent, 1986). Likewise the cerebellum receives massive projections from the sensory areas of the cerebral cortex, but also from the ascending spinal sensory pathways involved in somatosensation and proprioception (Brodal, 1981). Both of these subcortical structures project back to the motor cortex forming the corticocerebellar and corticostriatal loops (Schieber, 1999). These loops link the striatum and cerebellum with the primary motor cortex. It is through these pathways that the striatum and cerebellum are able to influence spinal motoneurons (Porter & Lemon, 1993).

The TRP and TRS, subdivisions of the trigeminal sensory nerve (TR) receive somatotopically organised primary afferents from the trigeminal nerve (Kerr, 1963). In rodents and cats the vibrissae form a large part of the projection to TRP and the caudalis and interpolaris subnuclei of the TRS (Arvidson, 1982). The oralis subnucleus does not receive a vibrissal input, rather it receives projections relating to the oral and nasal

cavities. The caudalis subnucleus, in addition, receives nociceptive and thermal inputs, while the supratrigeminal nucleus receives a proprioceptive input. Other afferents include dorsal root fibres from the upper cervical spinal segments, cranial nerves (e.g. glossopharyngeal and vagus) as well as the cerebral cortex (Kerr, 1963). Clearly in animals that do not possess prominent vibrissae this plan may be considerably altered, but studies in primates are few and none have been attempted for bats (Baron et al, 1996a).

Efferent projections primarily go to the medial ventroposterior nucleus of the thalamus (VPM), as well as to other thalamic regions. Some fibres also pass to the superior colliculus, brain stem motor nuclei, zona incerta and inferior olive (Carpenter & Hanna, 1963). In the rodent, up to 70% of fibres from interpolaris subnucleus of TRS pass to the cerebellar vermis (Watson & Switzer, 1978).

FGR receives primary sensory afferents from the sacral and lumbar spinal segments by way of the fasciculus gracilis. Likewise the FCM and FCE receive primary afferents from the cervical and thoracic segments via the fasciculus cuneatus (Brodal, 1981).

The adjunct medial lemniscus projects to the pontine nuclei, parabrachial, dorsal reticular nuclei, as well as the external and ventromedial part of the central nucleus of the inferior olive (Baron et al, 1996a). In addition it sends axons to the medial part of the medial

geniculate body and the suprageniculate, pretectal and mesencephalic reticular nuclei (Brodal, 1981).

The main medial lemniscus projects primarily to the lateral ventroposterior nucleus of the thalamus, the gracile axons terminating laterally and the cuneate axons medially. The whole of the FCE projects to the ipsilateral cerebellum and inferior olive (Massopust et al, 1985).

Afferents of the REL arise from the cerebral cortex, red nucleus and the cerebellar and vestibular nuclei (Martin et al, 1977). The principle efferent projection of REL is to the cerebellum – it is a significant source of mossy fibres. Almost the whole of the cerebellum receives REL input, but the projections are concentrated on the anterior lobe vermis and pyramis of the posterior lobe (i.e. lobule 8) (Brodal, 1975). The REL has been divided into two zones on the basis of its axonal projections. The lateral REL that receives projections from the lumbar segments projects to the rostral anterior lobe and caudal paramedian lobe of the cerebellum (the hindlimb areas) (Baron et al, 1996a). The medial REL in turn receives a projection from the cervical segments, then projecting to the caudal anterior lobe and rostral paramedian lobe (the forelimb area) (Brodal, 1975).

Projections to the INO originate from many areas of the brain from the cerebral cortex to the lumbar spinal segments. Efferents pass solely to the cerebellum as climbing fibres. Three functional zones have been identified in the rat (Azizi & Woodward, 1987). The dorsal accessory olive receives peripheral somatosensory inputs from the spinal cord, dorsal column nuclei and spinal trigeminal nucleus. It projects to the mid-vermal and intermediate zone of the anterior lobe and to the intermediate zone of the posterior lobe of the cerebellum. The medial part of the medial accessory olive receives axons from the superior colliculus, pretectal and vestibular nuclei and nucleus of the optic tract (Brodal, 1981). It projects to the uvula and flocculus. The principle olive and rostral part of the medial accessory olive receives projections from the red nucleus, dentate nucleus and motor cortex. In turn it projects to the lateral cerebellum. This zone appears to integrate the inputs from these higher brain centres (Azizi & Woodward, 1987).

VM receives primary afferents from the vestibular apparatus (Wilson & Jones, 1979). A significant projection arises from the cerebellum (from the flocculus, nodulus and anterior lobe vermis), also from the spinal cord as spinovestibullar fibres and the mesencephalic occulomotor complex (Brodal, 1974). Efferents pass to the occulomotor nuclei, the cerebellar cortex (vermis and flocculus) and the intermediate cerebellar nuclei and lateral cerebellar nucleus and motoneurons in the cervical spinal cord (Carleton & Carpenter, 1983).

VI receives primary afferents from the vestibular apparatus, each receptor in the apparatus projecting to a specific part of the nucleus (Stein & Carpenter, 1967). VI also receives afferents from the spinal cord and cerebellum, especially the ipsilateral anterior lobe vermis, nodulus and uvula. There may also be a projection from the MCN (Carleton & Carpenter, 1984). Efferent targets include the spinal cord and cerebellum, with different parts of the nucleus projecting to distinct areas of cerebellum. The cell groups 'f' and 'x' receive afferents from the cerebellum (flocculus, nodulus and uvula) and MCN. The red nucleus also projects to these groups. Both cell groups project to the cerebellum (Brodal, 1974; Carleton & Carpenter, 1983). In addition 'x' gives rise to spinal fibres that travel in the dorsolateral funiculus. Group 'z' afferents arise chiefly from the lower part of the spinal cord but also from the red nucleus. Most efferents pass to the thalamus (Brodal, 1984).

The two divisions of VL exhibit different patterns of connectivity. The rostroventral part receives projections from the vestibular apparatus and sends efferent fibres to spinal segments corresponding to the neck and forelimb (Brodal, 1974). The dorsocaudal part receives spinal afferents and fibres from the cerebellar anterior lobe vermis (Carleton & Carpenter, 1983). Efferents pass to the hind limb spinal segments. Both parts receive afferents from the medial cerebellar nucleus (Brodal, 1984).

VS receives primary afferents from the vestibular apparatus as well as projections from the flocculonodular lobe of the cerebellum (Brodal, 1974, 1984). Efferents pass to the occulomotor nucleus, flocculonodular lobe and other parts of the cerebellum (Stein & Carpenter, 1984).

The striatum receives topographically organised projections from the cerebral cortex, the association areas projecting to the caudate (Goldman & Nauta, 1977) and the primary cortical areas projecting to the putamen (Liles & Updyke, 1985). The AMY is also a significant source of striatal afferents. In addition to projections to the olfactory ventral striatum (Kretek & Price, 1978) AMY projects widely to the caudate and putamen, its fibres overlapping limbic and sensory afferents (Kite & Kitai, 1990). Thalamic projections are known to exhibit significant species differences (Beckstead, 1984; Paat et al, 1986), however notable sources of afferents include the MGB and pulvinar (Roye, 1978).

In addition to the above, the STR receives projections from the pallidum, subthalamic nucleus, substantia nigra, midbrain raphe nuclei, locus coeruleus, peribrachical nuclei and pontine reticular formation (Parent, 1986).

There are only two targets of striatal efferents: the pallidum, which receives a projection mostly from the putamen, and the substantia nigra which derives its projection mostly from the caudate. Ultimately most striatal output is destined for the motor cortex.

The PRP receives a wide variety of afferents, including the pretectum (nucleus of the optic tract and olivary pretectal nucleus), other perihypoglossal nuclei, vestibular and extraocular motor nuclei, medullary and pontine reticular formation, cerebellar cortex and superior colliculus. Efferents pass mostly to the cerebellum, but also to the vestibular nuclei, extraocular nuclei, INO, SUC, reticular formation and the ventral division of the lateral geniculate nucleus (Brodal, 1952).

Most sensory systems project strongly to the cerebellum. Most notable are the extremely large spinocerebellar and cuneocerebellar tracts which relay somatosensory and proprioceptive information. The visual and auditory systems (especially the latter in echolocating species) also project strongly to the cerebellum (Eccles, 1967).

The cerebellum attaches to the brainstem via 3 cerebellar peduncles. The superior peduncle carries mostly efferent fibres from the dentate (LCN), emboliform and globose (i.e. the interposed) nuclei (ICN). It also carries a small fascicle from the fastigial nucleus (MCN). These fibres decussate in the caudal mesencephalon. Some spinocerebellar fibres

enter via the superior peduncle: these join with spinocerebellar fibres entering via the restiform body. The middle peduncle is entirely afferent and is by far the largest of the three, receiving mostly basal pontine fibres and some from the pontine tegmentum. The inferior carries both afferent and efferent fibres and may be divided into the restiform and juxtarestiform bodies. The restiform body is a compact fibre tract on the dorsolateral aspect of the medulla. It is purely afferent and contains tracts from the spinal cord and medulla (spinocerebellar, trigeminocerebellar, cuneocerebellar, reticulocerebellar & olivocerebellar). The juxtarestifrom body lies medial to the restiform body. It consists of scattered fibre bundles that pass through the vestibular nuclei to reach the cerebellum (primary and secondary vestibulocerebellar fibres) but it is mostly efferent and carries purkinje cell axons from the vermis and vestibulocerebellum, plus uncrossed efferent fibres from the fastigial nucleus. The crossed fibres of the fastigial nucleus enter the brainstem as the uncinate tract at the border of the restiform and juxtarestiform bodies after passing dorsal to the superior peduncle. The flocculonodular lobe and medial nucleus receive afferents from the vestibular canals and nuclei via the inferior cerebellar peduncle.

Palaeocerebellum (which might also be termed spinocerebellum) receives afferents from the spinal cord: the anterior spinocerebellar tract via the superior peduncle and the

posterior spinocerebellar tract via the inferior peduncle. It also receives the cuneocerebellar tract from the external cuneate nucleus (FCE).

The neocerebellum, corresponding to the hemispheres receive fibres from the cerebral cortex via the pontine nuclei (pontocerebellar tract). The olivocerebellar tract projects to the whole of the cerebellar cortex.

The cerebellar nuclei are key elements in the circuitry of the cerebellum. They receive projections from the purkinje cells in the cerebellar cortex and constitute the sole target of output from the cerebellar cortex. MCN projects to the vestibular nuclei, while the ICN projects to the magnocellular red nucleus and the LCN to the parvocellular red nucleus. Both the ICN and LCN project via the ventral thalamus to the cerebral cortex (Brodal, 1981).

Relationship to Behaviour and Ecology

In order to properly discuss the issues involved in the control and sensory guidance of flight the basic physical mechanisms involved are discussed below. Only the detail necessary for an understanding of the subsequent analysis is presented. For a more

detailed treatment the reader is referred to excellent summaries by Altringham (1996) and Neuweiler (2000). For a detailed treatment see Norberg (1990).

Bats are the only mammals to have achieved sustained, powered flight. This highly advantageous adaptation is only possible due to the radical modification of the forelimb to form a wing (or patagium).

All wings, ranging from aeroplanes to birds and insects, are modelled on a fundamental shape and principle. They share an asymmetric tapered cross section with a convex upper surface, which causes air flowing under the wing to travel faster than air passing above it. Hence there is lower pressure above the wing and higher pressure below, leading to the generation of a net aerodynamic force (Neuweiler, 2000). This force has two components: lift and drag. Lift opposes the continuous pull of gravity while thrust moves the animal forward, countering drag forces as it does so which are exerted on the animal due to collision with air. Despite the similarities in the cross-sectional shape, bat wings are different to conventional aircraft in that the wing moves up and down in order to generate lift and thrust (Altringham, 1996). The shape of the wing may also be changed to alter its aerodynamic properties. Even compared with birds, bats are capable of greater changes of wing shape, allowing rapid acceleration and braking and an overall high level

of manoeuvrability (Neuweiler, 2000). All of this is dependent on the action of muscles and these in turn are dependent on sensorimotor control.

The wing membrane is suspended primarily from the forelimb and is supported by the humerus, radius, elongate metacarpals and 2^{nd}-5^{th} digits. The ulna is highly reduced and remains as a vestige. The 1st digit is free for use in climbing, grooming and terrestrial locomotion. It follows that the contraction of the forelimb or hindlimb muscles projecting into the wing may alter its shape and therefore is aerodynamic properties.

The wing is extended and flexed by two muscle chains. Contraction of the supraspinatus muscle produces a pull on the triceps which passively extends the elbow. The elbow extension in turn produces a passive pull on the extensor carpi radialis muscle which leads to an extension of all digits enclosed in the patagium. During flexion of the wing, only teres major contracts actively, producing a passive pull on biceps to flex the elbow, in turn pulling on the extensor carpi ulnaris to flex the fingers. It is desirable for a bat wing to be light so that the minimum muscle forces are needed to move it and so that stress is kept to a minimum. Since both supraspinatus and teres major are shoulder muscles their large relative mass, needed to generate a forceful contraction, does not adversely affect wing movement. All of the major muscles that contract actively to generate the wing beat are located in the shoulder (Altringham, 1996). Since the other

muscles of the wing contract very little, their weight is low. A series of intrinsic muscles maintain tension in the wing membrane during flight.

Bat flight can be divided into two phases. During the downstroke the wing is extended to maximise the surface area in order to generate lift. Drag is minimised during the upstroke by partly flexing the wing. The net result is forward momentum (Neuweiler, 2000). A flying bat must alter the shape of its wing and the angle of attack in order to generate forward thrust as well as lift. As the angle of attack is increased so the risk of stalling also increases. This effect can be countered by cambering the wing, which can be achieved by contraction of the occipitopollicalis muscle to move the thumb, or by the adductor digiti quinti muscle to move the 5^{th} digit. In both cases the effect is to raise the leading edge of the wing that increases the lift that can be generated during slow flight or tight manoeuvres. The bones of the forearm that protrude at a level above the patagium act as microturbulance generators, helping to prevent the separation of air moving along the surface of the wing which causes a stall.

From the above description of the mechanics of bat flight it is obvious that tremendous demands are placed on the sensorimotor system. The bat must be able to control its muscles in a precise fashion in order to shape the wing to the optimum aerodynamic configuration whatever the circumstances (Neuweiler, 2000). While the wing beat itself is

fairly stereotyped and may rely on spinal pattern generators (ten Donkelaar, 2001), the small modifications needed to manoeuvre or prevent stalls may require cortical input via the corticospinal or other descending motor pathways (Porter & Lemon, 1993).

Perhaps more than any other form of locomotion, the maintenance of flight requires that large amounts of sensory feedback be used to control movement. Special sensory cutaneous receptors have been described in bats that are thought to detect the speed and direction of air movement along the surface of the wing (Zook & Fowler, 1985) and it is also thought that sinus hairs on the orofacial region also detect air flow via the trigeminal nerve (Neuweiler, 2000). It is likely that these are relayed along the ascending sensory pathways to higher brain centres such as the somatosensory cortex and cerebellum. Movements will additionally be registered by the vestibular system which is known to be fundamental for flight control in birds (Bilo, 1992) especially since these bring about reflex movements of the head and eyes (Wilson & Jones, 1979). Since the head is the heaviest part of the bat body and partly suspends the wing, movements of the head contribute much to the control of flight (Altringham, 1996).

Bats make use of the most suitable flight speed for their activity. Migrating bats fly fast to minimise power requirements, while foraging bats minimise travel costs per unit time by

flying slowly (Altringham, 1996). There is a strong relationship between the foraging niche of a bat and its wing morphology. This mostly reflects the tight relationship between the need for a mode of flight which suits the prey that bats hunt (Neuweiler, 2000). Flight is advantageous to frugivorous species because it allows them to cover large distances quickly whilst foraging for a geographically and temporally distributed food resource (Neuweiler, 2000). Because most fruit is found in the terminal branches of trees and bushes it is usually not possible for large bodied bats (such as the Megachiroptera) to land directly adjacent to fruit; rather they must clamber from an open part of the tree to the region where the fruit is located (Dietz, 1973). Arboreal locomotion places significant demands on movement control centres and might be expected to be reflected in the development of motor brain structures.

Species of bats vary both in the amount of time foraging on the ground and their dexterity once there (Dietz, 1973). The vampire bat has a surprising level of agility whilst moving along the ground (Schutt et al, 1997), as do some insect eaters that forage in the leaf litter; some species rarely move terrestrially, if at all (Neuweiler, 2000).

Results

Multiple regressions were performed for twenty-six pairs of structures within the sensorimotor system that are known to possess strong anatomical connections, whilst controlling for variation in the volume of the rest of the brain. Separate analyses were performed for Megachiroptera and Microchiroptera. Significant partial correlations were found in both clades (thirteen in Megachiroptera and twenty in Microchiroptera); all those found in Megachiroptera were also found in Microchiroptera. In five out of thirteen significant partial correlations the predicted relationship was the strongest, compared with five out of twenty for Microchiroptera.

The trigeminal sensory nucleus showed a strong correlation with the inferior olive in both Megachiroptera (R^2_{adj}=0.986, df=2,13, p=0.009) and Microchiroptera (R^2_{adj}=0.905, df=2,57, p=0.0001). In both clades this predicted partial correlation was stronger than for that with the rest of the brain. In Microchiroptera a significant partial correlation was also observed between TR and the superior colliculus (R^2_{adj}=0.876, df=2,57, p=0.023) but this was not found in Megachiroptera (R^2_{adj}=0.904, df=2,13, p=0.252). This pathway is important since it is involved in reflexes that compensate for effect of masticatory

Figure 8:
Multiple regression of functionally connected sensorimotor brain structures and rest of brain volume for Megachiroptera

Dependent Variable	Independent Variables	R^2_{adj}	d.f.	F	F Sig.	Beta	t	Sig.
NEO	CER	0.997	2,18	2955.90	0.0001	-0.765	4.141	**0.001**
	R. of Brain					-1.761	-9.526	**0.0001**
NEO	STR	0.990	2,18	900.66	0.0001	-0.302	-0.724	0.480
	R. of Brain					-1.297	-3.107	**0.007**
TR	SUC	0.904	2,13	62.50	0.0001	0.556	1.209	0.252
	R. of Brain					-0.407	0.886	0.394
TR	INO	0.986	2,13	445.53	0.0001	0.689	3.141	**0.009**
	R. of Brain					-0.307	1.399	0.189
TR	CER	0.988	2,13	527.80	0.0001	0.95	0.420	0.682
	R. of Brain					-0.901	3.982	**0.002**
FGR	INO	0.983	2,13	387.53	0.0001	0.159	1.004	0.337
	R. of Brain					-0.838	5.308	**0.0001**
FCE	CER	0.990	2,13	660.02	0.0001	0.022	0.171	0.867
	R. of Brain					-0.974	7.496	**0.0001**
FCE	INO	0.983	2,13	384.18	0.0001	0.300	1.812	0.097
	R. of Brain					-0.698	4.216	**0.001**
REL	CER	0.990	2,13	626.25	0.0001	0.174	0.758	0.464
	R. of Brain					-0.823	3.584	**0.004**
REL	VS	0.946	2,13	115.69	0.0001	0.589	1.603	0.137
	R. of Brain					-0.392	1.067	0.309
REL	VM	0.974	2,13	242.48	0.0001	0.974	3.955	**0.002**
	R. of Brain					-0.015	0.062	0.952
REL	VL	0.973	2,13	235.09	0.0001	0.881	3.085	**0.010**
	R. of Brain					-0.109	0.382	0.710

REL	VI	0.954	2,13	135.61	0.0001	1.022	2.813	**0.017**
	R. of Brain					0.042	0.117	0.909
INO	CER	0.995	2,13	1279.47	0.0001	0.468	3.041	**0.011**
	R. of Brain					-0.532	3.463	**0.005**
CER	VM	0.972	2,13	230.47	0.0001	1.043	2.652	**0.022**
	R. of Brain					0.055	0.140	0.892
CER	VS	0.959	2,13	154.35	0.0001	0.874	1.853	0.091
	R. of Brain					-0.109	0.232	0.821
CER	VL	0.986	2,13	220.80	0.0001	0.837	1.866	0.089
	R. of Brain					-0.151	-0.337	0.742
CER	VI	0.955	2,13	137.89	0.0001	1.089	1.958	0.076
	R. of Brain					0.109	0.196	0.848
PRP	VM	0.955	2,13	138.38	0.0001	0.557	4.270	**0.001**
	R. of Brain					-0.451	3.459	**0.005**
PRP	VS	0.926	2,13	82.74	0.0001	0.434	2.577	**0.026**
	R. of Brain					-0.561	3.331	**0.007**
PRP	VL	0.967	2,13	194.33	0.0001	0.541	4.329	**0.001**
	R. of Brain					-0.467	3.734	**0.003**
PRP	VI	0.919	2,13	74.51	0.0001	0.443	2.222	**0.048**
	R. of Brain					-0.543	2.725	**0.020**
PRP	SUC	0.914	2,13	69.67	0.0001	0.605	3.281	**0.007**
	R. of Brain					-0.382	2.075	0.062
PRP	CER	0.989	2,13	566.25	0.0001	0.174	1.822	0.096
	R. of Brain					-0.829	8.692	**0.0001**
PRP	INO	0.980	2,13	322.15	0.0001	0.318	2.628	**0.023**
	R. of Brain					-0.685	5.668	**0.0001**

Figure 9:
Multiple regression of functionally connected brain structures and rest of brain volume for Microchiroptera

Dependent Variable	Independent Variables	R^2_{adj}	d.f.	F	F Sig.	Beta	t	Sig.
NEO	CER	0.964	2,95	1279.47	0.0001	-0.557	-5.022	**0.0001**
	R. of Brain					-1.526	-13.761	**0.0001**
NEO	STR	0.971	2,95	1602.01	0.0001	-0.134	1.257	0.212
	R. of Brain					-1.118	10.458	**0.0001**
TR	SUC	0.876	2,57	201.95	0.0001	0.314	2.345	**0.023**
	R. of Brain					-0.637	4.752	**0.0001**
TR	INO	0.905	2,57	271.86	0.0001	0.498	4.041	**0.0001**
	R. of Brain					-0.469	3.803	**0.0001**
TR	CER	0.932	2,57	394.41	0.0001	0.005	0.042	0.967
	R. of Brain					-0.962	8.080	**0.0001**
FGR	INO	0.921	2,57	334.6	0.0001	-0.029	0.349	0.728
	R. of Brain					-0.987	12.015	**0.0001**
FCE	CER	0.935	2,57	408.70	0.0001	0.085	0.873	0.386
	R. of Brain					-0.888	9.105	**0.0001**
FCE	INO	0.898	2,57	251.51	0.0001	0.369	3.449	**0.001**
	R. of Brain					-0.599	5.604	**0.0001**
REL	CER	0.940	2,57	448.98	0.0001	0.156	1.606	0.114
	R. of Brain					-0.822	8.480	**0.0001**
REL	VS	0.864	2,57	182.17	0.0001	0.264	2.217	**0.031**
	R. of Brain					-0.684	5.736	**0.0001**
REL	VM	0.938	2,57	430.72	0.0001	0.307	3.765	**0.0001**
	R. of Brain					-0.681	8.352	**0.0001**

REL	VL	0.902	2,57	263.40	0.0001	0.431	4.036	**0.0001**
	R. of Brain					-0.540	5.057	**0.0001**
REL	VI	0.891	2,57	234.05	0.0001	0.291	2.801	**0.007**
	R. of Brain					-0.674	6.499	**0.0001**
INO	CER	0.945	2,57	491.96	0.0001	0.365	3.707	**0.0001**
	R. of Brain					-0.620	6.305	**0.0001**
CER	VM	0.951	2,57	559.45	0.0001	0.476	4.486	**0.0001**
	R. of Brain					-0.510	4.805	**0.0001**
CER	VS	0.878	2,57	206.44	0.0001	0.080	0.493	0.624
	R. of Brain					-0.863	5.354	**0.0001**
CER	VL	0.903	2,57	263.84	0.0001	0.366	2.387	**0.020**
	R. of Brain					-0.594	3.867	**0.0001**
CER	VI	0.911	2,57	293.44	0.0001	0.457	3.303	**0.002**
	R. of Brain					-0.509	3.683	**0.001**
PRP	VM	0.957	2,57	635.75	0.0001	0.537	9.827	**0.0001**
	R. of Brain					-0.476	8.710	**0.0001**
PRP	VS	0.858	2,57	173.62	0.0001	0.502	5.098	**0.0001**
	R. of Brain					-0.491	4.682	**0.0001**
PRP	VL	0.850	2,57	163.13	0.0001	0.307	2.908	**0.005**
	R. of Brain					-0.645	6.110	**0.0001**
PRP	VI	0.928	2,57	365.97	0.0001	0.637	9.284	**0.0001**
	R. of Brain					-0.361	5.259	**0.0001**
PRP	SUC	0.859	2,57	175.13	0.0001	0.420	4.175	**0.0001**
	R. of Brain					-0.541	5.387	**0.0001**
PRP	CER	0.924	2,57	348.02	0.0001	0.190	2.108	**0.040**
	R. of Brain					-0.786	8.735	**0.0001**
PRP	INO	0.915	2,57	308.98	0.0001	0.565	6.930	**0.0001**
	R. of Brain					-0.422	5.179	**0.0001**

movements on vision (Hendry et al, 1999). Its absence in Megachiroptera may simply reflect an insufficient sample size.

As expected, cerebellum and inferior olive show a significant partial correlation in both Megachiroptera (R^2_{adj}=0.995, df=2,13, p=0.011) and Microchiroptera (R^2_{adj}=0.945, df=2,57, p=0.0001). The inferior olive is the sole (but significant) source of climbing fibre inputs to the cerebellum.

The vestibular system is very important in bats since it plays a major role in the control of flight. Vestibular structures show strong partial correlations with other structures in the sensorimotor system.

The cerebellum showed a significant partial correlation with medial vestibular nucleus in Megachiroptera (R^2_{adj}=0.972, df=2,13, p=0.022) and Microchiroptera (R^2_{adj}=0.951, df=2,57, p=0.0001). For Megachiroptera this predicted partial correlation was stronger than for the rest of the brain. In Microchiroptera the lateral (r2=0.903, df=2,57, p=0.02) and inferior (R^2_{adj}=0.911, df=2,57, p=0.002) vestibular nuclei also showed significant partial correlations. These were not present in Megachiroptera, but it is unclear to what extent this represents a lack of statistical power.

Precerebellar nuclei that in turn project to the cerebellum also showed significant partial correlations with the vestibular nuclei. The lateral reticular nucleus exhibits strong partial correlation with the medial (Megachiroptera: R^2_{adj}=0.974, df=2,13, p=0.002; Microchiroptera: R^2_{adj}=0.938, df=2,57, p=0.0001) lateral (Megachiroptera: R^2_{adj}=0.973, df=2,13, p=0.01; Microchiroptera: R^2_{adj}=0.902, df=2,57, p=0.0001) and inferior (Megachiroptera: R^2_{adj}=0.954, df=2,13, p=0.017; Microchiroptera R^2_{adj}=0.891, df=2,57, p=0.007) vestibular nuclei. The superior vestibular nucleus showed a significant correlation in Microchiroptera (R^2_{adj}=0.864, df=2,57, p=0.031) but not Megachiroptera (R^2_{adj}=0.946, df=2,13, p=0.137). For the Megachiroptera all of the predicted partial correlations were the strongest compared to the rest of the brain.

The nucleus prepositus hypoglossi is another precerebellar nucleus intimately connected with the vestibular system. Significant partial correlations were found between the PRP and all four vestibular nuclei in Megachiroptera and Microchiroptera: medial (Megachiroptera: R^2_{adj}=0.955, df=2,13, p=0.001; Microchiroptera: R^2_{adj}=0.957, df=2,57, p=0.0001), superior (Megachiroptera: R^2_{adj}=0.926, df=2,13, p=0.026; Microchiroptera: R^2_{adj}=0.858, df=2,57, p=0.0001), lateral (Megachiroptera: R^2_{adj}=0.967, df=2,13, p=0.001; Microchiroptera R^2_{adj}=0.850, df=2,57, p=0.005) and inferior (Megachiroptera: R^2_{adj}=0.919, df=2,13, p=0.048; Microchiroptera: R^2_{adj}=0.928, df=2,57, p=0.0001). Additionally the PRP showed a significant partial correlation with SUC in both

Megachiroptera (R^2_{adj}=0.914, df=2,13, p=0.007) and Microchiroptera (R^2_{adj}=0.859, df=2,57, p=0.0001). For both clades the predicted relationship was stronger than for rest of brain.

Since both the REL and PRP are classed as precerebellar nuclei they might be expected to show significant partial correlations with cerebellum. The lateral reticular nucleus did not show a significant partial correlation with cerebellum in either Megachiroptera (R^2_{adj}=0.990, df=2,13, p=0.464) or Microchiroptera (R^2_{adj}=0.940, df=2,57, p=0.114). However a significant partial correlation was found between PRP and cerebellum in Microchiroptera (R^2_{adj}=0.924, df=2,57, p=0.0001), but not Megachiroptera (R^2_{adj}=0.989, df=2,13, p=0.096) although this may be due to the low sample size. PRP was found to have a significant partial correlation with INO in both Megachiroptera (R^2_{adj}=0.980, df=2,13, p=0.023) and Microchiroptera (R^2_{adj}=0.915, df=2,57, p=0.0001). As stated previously the sole efferent pathway from the INO is to the cerebellum.

PRP is mediates a number of vestibulo-occular reflexes. A significant partial correlation between PRP and superior colliculus was found both for Megachiroptera (R^2_{adj}=0.914, df=2,13, p=0.007) and Microchiroptera (R^2_{adj}=0.859, df=2,57, p=0.0001). For Megachiroptera the correlation coefficient was larger than that for the rest of the brain.

The corticocerebellar and corticostriatal loops are important pathways returning information to the motor cortex in order to modify motor behaviours. Despite this, negative partial correlations were observed between the neocortex and cerebellum in both Megachiroptera (R^2_{adj}=0.997, df=2,18, p=0.001) and Microchiroptera (R^2_{adj}=0.964, df=2,95, p=0.0001). No significant partial correlation was found between the neocortex and striatum in either Megachiroptera (R^2_{adj}=0.990, df=2,18, p=0.480) or Microchiroptera (r2=0.964, df=2,95, p=0.212). As with the auditory and olfactory systems, many of the significant correlations with the rest of the brain were negative. This may be due to multicollinearity.

These results, especially those for the vestibulocerebellar structures and the trigeminal sensory complex provide strong evidence that correlated evolution has occurred in the sensorimotor systems of bats independent of changes in the rest of the brain.

7
Discussion

The presence of significant partial correlations, particularly those for which the predicted relationship was stronger, provide evidence for the correlated volumetric evolution of brain structures within functional systems. This has previously been shown for Primates and Insectivores (Barton & Harvey, 2000). Although it has been claimed that developmental constraints limit such mosaic evolution (Finlay & Darlington, 1995), these constraints are clearly not sufficient to prevent functionally structures evolving together independent of the rest of the brain.

Whilst such correlated evolution can be demonstrated for major brain subdivisions such as the neocortex, diencephalon, mesencephalon, cerebellum and medulla oblongata, the results were clearest among brain structures which share functional as well as anatomical connections. Modern tract tracing methods clearly demonstrate the course of fibre projections within the brain, but they are not necessarily able to demonstrate the level of synaptic activity that results between two brain structures (Zigmond et al, 1999). In order to demonstrate functional connections physiological investigations are required. It is notable that the structures which demonstrate correlated evolution most clearly are those for which functional connections can be demonstrated, for example the lower auditory

pathway (connections between the cochlear nuclei, superior olive and inferior colliculus) and the vestibulocerebellar structures (vestibular nuclei, cerebellum, lateral reticular nucleus and nucleus prepositus hypoglossi). Results were least clear among those limbic structures that share dispersed projections to numerous other brain structures.

Although there is the possibility that multicollinearity may have affected some of the results, similar patterns of correlated volumetric evolution can be demonstrated in the auditory and sensorimotor systems of both Megachiroptera and Microchiroptera. Likewise the patterns of evolution between bats, primates and insectivores (Barton & Harvey, 2000) suggests that the general pattern of correlated evolution may be similar across mammalian species. This could be investigated in the future by performing similar analyses on the auditory and vestibulocerebellar structures of insectivores and primates.

Most volumetric datasets represent relatively crude subdivisions. Structures such as the amygdala or striatum consist of numerous parts that have different patterns of connectivity or functions and these different parts may coevolve with other areas of the brain independent of other subdivisions of the same structure. For example the 'olfactory' ventral striatum may show correlated evolution with olfactory structures whilst the remainder of the striatum coevolves with motor structures. Such fine patterns cannot be detected by the methods used by this thesis, and they may serve to confound the results

obtained. However the only way to improve this resolution is for investigators to perform more detailed architectonic studies with separate volume measurements for structural subdivisions and including structures not included in the Baron et al (1996a) dataset, such as the nuclei of the thalamus.

Despite this, it has been shown that structures within functional systems to show correlated size changes when changes in the volume of the rest of the brain has been taken into account. It therefore appears that despite the presence of developmental constraints (Finlay & Darlington, 1995), the bat brain has evolved in a mosaic fashion, with specific functional systems being the targets of selection.

8
Conclusions

(1) Significant partial correlations were found between brain structures sharing anatomical connections when variation in the volume of the rest of the brain was taken into account.

(2) In many cases the correlations between these linked structures were stronger than any correlation with the rest of the brain.

(3) This trend was strongest in structures that have strong but topographically localised projections, for example the vestibulocerebellar structures or the lower auditory pathway.

(4) Limbic structures comprising part of the olfactory system showed the fewest significant partial correlations. This is likely due to the widely dispersed nature of projections from these structures within the brain.

(5) When Megachiroptera and Microchiroptera were tested separately there was generally good correspondance between these clades for the auditory and

sensorimotor systems. This was not true for the limbic olfactory structures. Where the pattern of correlations did not correspond between the two clades this was interpreted in relation to what is known of differences in function of the structures concerned in each clade. In many cases these disparities could be explained with reference to the function of the structures in each clade.

(6) These results are interpreted as evidence for mosaic evolution in the chiropteran brain.

9
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