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ARE THERE ORDER SPECIFIC PATTERNS OF CORTICAL GYRIFICATION AND IF SO WHY?

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'A thesis submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Masters of Science.'

Johannesburg, 2007

I declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

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On the 4th day of May 2007

Abstract (for Chapter 2)

Objective: The aim was to test the hypothesis that the order is a significant phylogenetic grouping in terms of quantifiable gyrification indices. **Method:** The gyrification index (GI) was measured from serial sections of the brain of twenty five different mammalian species, representing the different orders i.e. primates, carnivores, artiodactyls and rodents. Image J analysis was used to measure the contours of the cerebral cortex and the GI was calculated using three different methods of analysis i.e. complete *vs* outer; gyral *vs* sulcal and outer *vs* inner surface contours. The measurements were then computed against the brain weights of each species within the order. **Results:** An increasing GI correlates with an increasing brain weight in all the mammalian orders. Each order has its own specific allometric patterns that are significantly different from the other orders examined. The artiodactyls were the mammals with the most gyrencephalic brains, these species being significantly more gyrencephalic than all other mammals when species of similar brain weights are compared. The North American beaver has an atypically lissencephalic brain for its size, differing from the trend for increased gyrencephaly found in the other rodent species examined. **Conclusions:** Our results show definite trends and patterns specific to each order. So it would seem that the order is a significant phylogenetic grouping in terms of this neural parameter, from which we can predict with a reasonable degree of certainty, the GI of any species of a particular order, if we know the brain weight.

Abstract (for Chapter 3)

The mammalian order has proven to be a significant phylogenetic grouping in terms of gyrification from which we can predict with a reasonable degree of certainty, the GI of any species of a particular order, if we know the brain weight. We have attempted in the present study to identify potential causes for gyrification at the class level by investigating relationships at the level of the order. It appears that clues to the extent and pattern of gyrification in the different mammalian orders might be related to the bones that constitute the braincase. The external surface areas of the bones of the cranial vault of seventeen different mammalian species were measured using a microscribe digitiser. These values were plotted against brain weight from which we could then calculate residual values, determining if there was more or less external cranial vault area than expected for the size of the brain. These residuals were then plotted against the gyrification indices determined in a previous study for the species examined. Results indicated that for the primates and artiodactyls the skull may potentially be considered as a limiting factor on the expansion of the cerebral cortex; however, the carnivore and rodent orders show conflicting results which suggest that the relative surface area of the skull appears to have no effect on the quantitative extent of gyrencephaly. These inconclusive findings suggest that causes contributing to the quantitative extent of gyrification across mammals may be multifactorial, and more parameters may need to be included in the analysis to arrive at an answer.

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C H A P T E R 1

1.1 General Introduction

One of the most salient features of the gross anatomy of the human brain is the degree of fissuring and folding of the outermost structure of the brain, the cerebral cortex. These cortical convolutions are called gyri, while the folds or fissures are called sulci (Welker, 1990). The technical term for this folding of the cerebral cortex is gyrification – the process of creating gyri. Why the cerebral cortex undergoes this gyrification has not yet been established, although a variety of concepts and experiments have led to a number of different proposals. Some view the process of gyrification (development of gyri) as a means to increase the surface area of the brain and results from mechanical forces creating buckling and folding during growth of the cortical mantle (Clark, 1945). By increasing the surface area of the brain, additional cortical circuits are integrated as required by the mammalian species, thereby saving space, but increasing the processing power of the brain. The most common reasons forwarded to explain why gyrification occurs are: (1) that it is due to mechanical pressures associated with the developing skull (Clark, 1945); or (2) that it is for more economical wiring of functionally related areas of the cerebral cortex (Welker, 1990).

There have been few evaluations undertaken in an attempt to determine the precise underlying mechanisms involved in the development and evolution of gyri and sulci across mammalian species (see Welker, 1990 for review). In contrast, much has been written about the homologies of cortical gyri across the mammalian orders as well as about the evolution of the cerebral cortex in general. By applying a special terminology to sulci and gyri occupying relatively the same position in the brains of

different species, it is possible to create an artificial impression of morphological identity where no such identity is likely to exist (Clark, 1945). It has been found that within mammalian orders sulcal and gyral homologies are clear, for example the sulcus lunatus in apes is homologous to the lunate sulcus in man and the primate sylvian furrow is the most stable amongst primates (Black, 1915a; Smith 1902b; Welker, 1990). Homologies between the orders are however, not as easy to establish.

The reasons as to why larger mammals with larger brains have a more convoluted cortex than small mammals in the same order (e.g. primates, Zilles et al., 1989), is not clearly understood. Welker, (1990) suggests that an examination of their perceptual, cognitive, and behavioural repertoires might reveal a greater behavioural complexity in the larger animals, although this has not been conclusively shown, and it is unclear if the degree of gyrification in itself is directly related to cognitive abilities (Manger, 2006). Some studies seem to be in favour of a differing approach, demonstrating a quantitatively measurable positive relationship between brain size and the degree of covolutedness, thereby indicating that the degree of cortical convolutedness is not an adaptive feature related to cognitive abilities, but rather an allometric imperative of absolute brain size. Based on a survey of 22 anthropoid brains, Zilles et al. (1989) quantitatively demonstrated an allometric increase in the gyrification index (GI) with increasing brain weight within the primate order (an intraordinal analysis). The mean rhesus monkey GI or degree of cortical folding was that expected for its brain size of 90 grams. At the same time the variation of GI within the species *Macaca mulatta* did not correlate with brain weight (an intraspecific analysis) thus, it seems that increasing GI does not correlate with brain weight within a primate species, such as the rhesus monkey (Armstrong et al.,

1991) and the human (Zilles et al., 1988), but the species average GI and brain weight are strongly correlated. In contrast to this, the intraspecific analysis undertaken for a range of dogs of differing breeds and brain size does show a strong relationship between GI and brain size (Wosinski et al., 1996).

Welker (1990) has also noted convolutional differences in brains of similar sizes. The figure below (taken from Welker, 1990, figure 63) shows a comparison of the brain from the least weasel (a carnivore) and the muskrat (a rodent) of approximately the same brain and body size. It is clear that the least weasel has a far more gyrencephalic cortex than the muskrat.

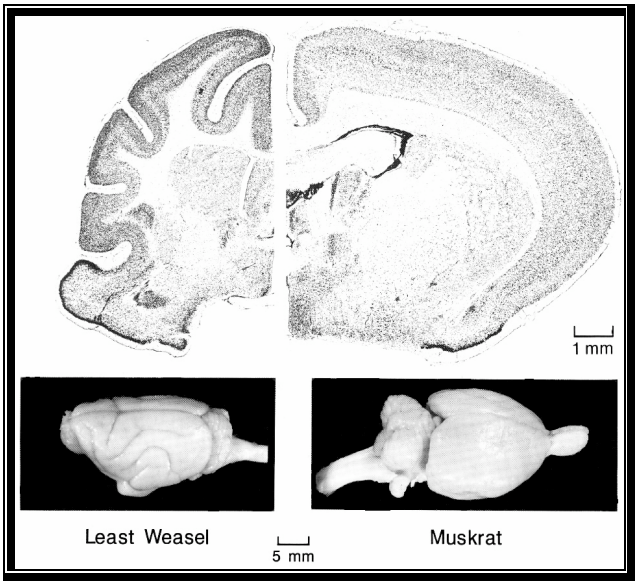


Fig. 1.1 Comparison of the brain from the least weasel (a carnivore) and the muskrat (a rodent) of approximately the same brain and body size reveal distinct differences in the amount of gyrification.

The differences in the expression of both the patterns of gyrification and the quantitative extent of gyrification appear to follow a pattern that is related to the various orders of

mammals, but this pattern has not been explicitly tested in a quantifiable manner. Thus, the following hypotheses can be created:

(1) *Within a mammalian order the larger the brain, the greater the quantitative extent of the gyrification of the cerebral cortex.*

(2) *This relationship between brain mass and GI will follow an allometric form of scaling in each order.*

(3) *This proposed explicit relationship will not apply between orders, indicating that quantitative measures of cortical gyrification will show predictable scaling within orders, but not across the mammalian class.*

To test these hypotheses, the first phase of the current study aims to calculate a gyrification index for a number of species in a range of mammalian orders (at least 5 species per order). The degree of folding measured will then enable us to establish whether a specific pattern of gyrification is found within each order or whether this is an index that relates to the mammalian class as a whole.

Once the hypotheses of the first phase of the study are either supported or rejected, a second phase can be started, this phase relating more specifically to the reasons why the varying degrees of cortical gyrification are seen across mammals. The skull and the brain are clearly interrelated, organismal components in both mammalian development and evolution, and it may be that one influences the degree of expression of parts of the other. Many studies have looked at intrinsic forces, (such as gyrogenesis) within the cerebral cortex for explanations of gyrification and fissuration. The processes of neuronal differentiation, orientation, and afferent penetration and efferent connectivity, among others, are believed to generate numerous miniature mechanical forces which,

together, constitute the primary, active, or intrinsic determinants of gyrus building, or gyrogenesis (Welker, 1990; Harrison et al., 2002). By examining structural differences within the cortex of gyral crowns, sulcal walls and fundi, it was observed that during the folding of the surface of the hemisphere, the floors of the sulci remain relatively fixed in relation to the deep surface of the cortex and that a gyrus is produced by the expansion of the inter-sulcal tissue (Smart and McSherry, 1986a). Structural changes during subsequent growth showed that there was tangential spreading of the more mature tissue at the gyral crown while at the site of future sulci the cortical tissue remained immature and retarded (Smart and McSherry, 1986b). Smart and McSherry (1986b) propose that these examples of the finer structural features of cortical development reflect the influence of passive mechanical forces secondary to primary intrinsic forces involved in gyrogenesis.

Determinants of gyral and sulcal form and pattern can be assessed to some extent experimentally by producing selective partial removals or destruction of specific neural structures and connections early in development while cerebral cortex is still relatively smooth (Welker, 1990). Goldman – Rakic and Rakic (1979), found from there experiments on macaque monkeys, that gyrus formation persisted after the removal of parts of the developing cortex, even though the remaining cortex did not entirely fill the cranium. According to Harrison et al., (2002) this rules out the possibility that pressure from the cranium is needed as a force in fold formation. Harrison et al., (2002), also suggests that additional intrinsic forces necessary for developing tissue account for the buckling and folding of the cortical sheet. They propose that the axons growing towards their targets by epigenetically regulated mechanisms are filled with incompressible fluid

that exerts forces both laterally and axially. The tension generated from these forces might therefore be able to draw different parts of the cortical sheet together to form folds.

Further investigations on the brain have similarly inferred that the forces primarily responsible for cortical folding are resident within the cortex, however, these primary forces may be modified by the growth of cells outside the cortex, for example fibre projection systems that enter the cortex (Barron, 1950). Rakic (1988) in his studies of thalamo-cortical connections, has provided evidence showing that lesions to axonal tracts with inputs to specific regions of the primate brain affects specific gyral patterns. It has also been found that axonal tracts begin to develop before cortical folding (Rakic, 2000), and that the appearance of functional areas of the cerebral cortex and their circuits occur rather late in development, well after connections from the thalamus are established. These findings have suggested that axonal connections are integral to cortical folding (Harris et al., 2004). One major difficulty with these proposals is the lack of a link between the microscopic features and forces of gyrogenesis, and the observed patterns of sulci and gyri, both qualitative and quantitative, across and within mammalian orders. While these forces may explain the development of gyri and sulci, they fail to logically explain observations made across the various mammalian taxa.

Apart from these microscopic studies on the brain, others have employed mechanical, geometric and mathematical models to account for the phenomena of cortical convolutions. Clarke (1945) suggests that the sulcal pattern of the brain might be determined by extrinsic mechanical factors operating during development, such as the shape of the skull, i.e. longitudinal fissuring predominates for brains housed in dolichocephalic skulls (e.g. artiodactyl brains) and transverse fissures predominate for

brains housed in brachycephalic skulls (e.g. the brain of the echidna). Welker (1990) suggests that the sulcal and gyral features of the brain of large and small species have greater similarities within mammalian orders than between orders, and as mentioned earlier we have hypothesized that order specific quantitative patterns of gyrification may exist. Thus we can ask: is it possible that the order specific patterns in the morphological parameters of the bones of the cranial vault are related to the order specific qualitative and quantitative patterns of GI?

Despite this potential interactivity, Welker (1990) argues that the skull is probably not a limiting factor on cortical expansion, and thus causing gyrification, by pointing out: (1) that the calvarium grows and is shaped in response to forces generated by the growing convolutions and expanding opercula and lobes; (2) that the impressions made by the gyri and sulci on the endocranium is only useful in evaluating the patterns of gyrification of mammals and does not serve as evidence that the skull is a restraining factor on the cerebrum; and (3) that skull sutures do not ossify until the brain has ceased growing, and if any portion of the cerebrum fails to develop or grow, due to pathological causes, the skull conforms to the size and shape of the cerebral remnant.

In addition to the reasoning forwarded by Welker (1990), Hofman (1984) found evidence in several cases of micrencephaly that a decline in brain weight during development is not accompanied by a similar reduction in head circumference (or skull growth). This finding suggests that the brain and skull develop as two separate entities where in normal development the skull may only have a passive mechanical influence on the expansion of the cerebral cortex.

The second phase of this project details investigations into the possible influence of the skull on the growth of the brain. If, as suspected, there is an order specific pattern to the extent of gyrification, it is possible that an order specific pattern in the morphological parameters of the bones of the cranial vault may exist and that these may relate to gyrification. Thus, we may be able to propose that to account for degree of gyrification in the different orders we would need to establish an order specific pattern in the structure of the bones of the cranial vault. Such a finding may reveal an underlying relationship between the extent of gyrification in the different mammalian brains and the morphology of the bones of the cranial vault. Thus, the specific hypotheses forwarded for testing are:

(1) *The bones of the cranial vault may act as a passive limiting factor on the expansion of the cerebral cortex.*

(2) *These passive effects will be manifested in an order specific pattern, as the relative occupation of the cranial vault by the various bones differs between orders.*

The second phase of this project will be an attempt to collate data that may help to answer any differences in order specific gyrification patterns I believe I will observe. By measuring the various cranial features making up the braincase we would be able to examine them for correlations to the order specific patterns of gyrification. For, example the total area of the cranial vault may show a strong positive correlation with the extent of gyrification.

In all mammalian orders there are some species that have gyrencephalic brains, some that don't, and some species that range in between the two. When we compare two species from different mammalian orders of similar brain weights it is clear that there are

differences in amount of gyrification in the cerebral cortex exhibited. Thus, it appears that the order might be a significant grouping in terms of quantifying gyrification indices. Making comparisons across mammals at the class level may not reveal significant relationships that are needed to determine the underlying mechanisms involved in the development and evolution of gyri and sulci of the cerebral cortex. What we are attempting to do is to uncover a causal relationship for gyrification by investigating relationships at an order specific level. Many of the theories that look at intrinsic factors within the cortex – e.g. gyrogenesis, as the causal factor of gyrification do so without specifically adding anything to phylogenetic variance. It may be that the intrinsic processes taking place within the brain of a macaque monkey to develop fissures and folds may not be the same for a species in another mammalian order and so may not be defining a general solution for all mammals. Thus, this study addresses a problem that is as yet unresolved, and revisits the concept of the skull acting as a passive factor that may account for the phylogenetic variance seen in gyrification across mammals.

C H A P T E R 2
ORDER SPECIFIC QUANTITATIVE PATTERNS OF CORTICAL GYRIFICATION

2.1 Introduction

One of the most obvious features of the gross anatomy of the human brain is the degree of fissuring and folding of the cerebral cortex. The technical term for this folding is gyrification – the process of creating gyri, but why the cerebral cortex undergoes gyrification has not yet been established (Welker, 1990). The current axiom underlying many comparative studies is "bigger brains are more gyrencephalic" (Welker, 1990; Mayhew *et al.,* 1996). While this has a strong element of truth, even a brief examination of the surface of the cerebral cortex of various mammals of the same brain size, but from different orders, reveals distinct differences in the visible amount of gyrification. The images in Fig 3.C and Fig 5.C show a comparison between species (Spotted hyena and white-tailed deer) from different mammalian orders of approximately the same brain size. Previous quantitative analysis of gyrification has shown increases in the gyrification index (GI) with increasing brain weights. Zilles *et al.* (1989) quantitatively demonstrated an allometric increase in the GI with increasing brain weight within the primate order (an intraordinal analysis), but increasing GI does not correlate with brain weight within a primate species (an intraspecific analysis), such as the rhesus monkey (Armstrong *et al*., 1991) and the human (Zilles *et al*., 1988); however, this appears to occur for dogs of differing breeds and brain size (Wosinski *et al*., 1996).

Most early studies of the cerebral cortex examined the patterns of sulci and gyri in an attempt to determine homologies across species. It has been found that within the orders sulcal and gyral homologies are clear, for example, the sulcus lunatus in apes is

homologous to the lunate sulcus in man and the primate sylvian furrow is the most stable amongst primates (e.g. Black, 1915; Smith, 1902). Homologies between the orders were however, not as easy to establish. Thus, to date we are left with three observations regarding the evolution of sulci and gyri. First, the pattern of sulci and gyri within an order is quite coherent and predictable. Second, within the primates, the degree of gyrification is allometrically related to brain weight. Lastly, there appears to be no clear consistency across mammalian orders in terms of either patterns or quantification of the gyri and sulci. Thus, we can ask: is the order a significant phylogenetic grouping (Manger, 2005) in terms of quantifiable gyrification indices? By using freely available images of serial sections of the brain of various mammalian species within the primates, carnivores, artiodactyls and rodents, we aimed to determine if there are quantifiable order specific patterns of gyrification. All images used in this study were downloaded from the website of the Comparative Mammalian Brain Collections of the University of Wisconsin, and Michigan State University (http://www.brainmuseum.org).

2.2 Materials and Methods

2.2.1 Brain Collection

Twenty five species of mammals, for which we could calculate gyrification indices, were used in this study. The mammals were chosen from four different orders, which included primates, carnivores, artiodactyls and rodents (Table 1). Images of sectioned brains of these mammals (1 representative of each species) were downloaded from the website of the Comparative Mammalian Brain Collections of the University of Wisconsin, and Michigan State University (http://www.brainmuseum.org). The images used were coronal sections, and depending on the size of the brain approximately 30-40 evenly spaced sections through an entire cerebral hemisphere were analyzed for each species. Once the images were downloaded, it was possible to then measure the gyrification indices for the cerebral neocortex of each species.

2.2.2 Gyrification Index (GI) Measurement

The gyrification index of each species was measured using three different methods. The first method was the published method of calculating the GI, this measures cortical folding by comparing the lengths of complete (pial surface) and outer cortical contours of the cerebral neocortex (Zilles, 1988; Fig. 8, herein referred to as method 1) i.e. the perimeter of the complete contour divided by the perimeter of the outer contour. For the second method a ratio between sulcal vs. gyral cortex (Fig. 8, herein referred to as method 2) was calculated. The sulcal measurement was calculated by subtracting the results for the outer contour from the complete contour as measured in method 1, while measurement of the gyral cortex was calculated by summing the gyral crown lengths

from each section. The third method involved calculating the ratio between the complete contour and inner contour (inner surface of layer 6) of the cerebral neocortex (Fig. 8, herein referred to as method 3), i.e. the measured distance of the complete contour divided by the measured distance of the inner contour. In all three methods the entorhinal cortex, which is part of the hippocampal formation, was eliminated from measurements (Hevner & Wong-Riley, 1992). The entorhinal cortex was easily recognized in the images due to the distinct neuronal clusters in layer two.

The lengths of these contours as well as the gyral and sulcal lengths were determined by means of an image analysis system (Image J software). First the scale of each brain section was set and then using the polygon tool the distances of each contour was measured and recorded in centimetres. For example, in method one the sum of all the measurements of the complete contour was divided by the sum of all the measurements for the outer contour. These measurements were taken for each slice of the brain to calculate the final GI.

2.2.3 Statistical Analysis

Once the GIs for all the species were calculated, they were plotted against the brain weights of each of the species (the exact brain weights of the individuals measured was used). Using Microsoft Excel, graphs were generated with specific trend lines for each method. Least squared regression (LSR) statistics was used to calculate the P-value of correlation and statistical differences between the slopes.

2.3 Results

All three methods that were used showed an increase in the GI with increasing brain weight. Each order exhibited its own specific relationship between brain weight and GI. Allometric plots of all three methods show that the slopes for the carnivores and primates cross at the lower brain weights, and artiodactyls are the most gyrencephalic of all species. Observations on the rodents were unusual as the North American beaver has a large but lissencephalic brain; whereas other rodents of similar brain size were gyrencephalic. The results depicted two trends that might be specific to the Rodentia. (Fig. 9, 10 & 11).

2.3.1 Method One –Zilles' method

Using the method described by Zilles (1998) we found that in primates the GI values increased with increasing brain weight (Fig. 9). Comparisons between these two variables reveal a strong correlation coefficient (r^2), with 96% of the variability in GI of primates being accounted for by changes in brain weight. It was also shown that for every doubling in brain weight, there was a 113% increase in the GI. Thus, brain weight scales faster than the GI – a negative allometry.

The equation calculated for primates was:

$$GI = 0.8002 W_{br}^{0.1822} (r^2 = 0.96; P = 3.2 \times 10^{-4}).$$

The regression slope calculated for the primates is significantly different to that of the slopes calculated for the carnivores, rodents 1 and rodents 2, but not significantly

different to that of the slope calculated for artiodactyls using the mean squares between and within slopes: primates *vs* carnivores, $P < 0.05$; primates *vs* artiodactyls, $P > 0.05$; primates *vs* rodent 1, $P < 0.05$; primates *vs* rodent 2, $P < 0.05$.

For the carnivores there was also an increase in GI with increasing brain weight. Again the r^2 is high, with 99% of the variability in GI of the carnivores being accounted for by changes in brain weight. For every doubling in brain weight, there was a 108% increase in the GI. As in the primates, brain weight scales faster than the GI.
The equation calculated for carnivores was:

$$GI = 0.9875 W_{br}^{0.115} \; (r^2 = 0.99; P = 2.82 \times 10^{-4})$$

The regression slope of the carnivores is significantly less steep than the primates ($P = 0.0054$, using the mean squares between and within slopes). At smaller brain weights the carnivore and primate regression slopes cross so that smaller carnivores are more gyrencephalic than smaller primates, but due to the shallow slope of the carnivore regression, larger brained carnivores have a lower GI than larger brained primates. The calculated regression slope for the carnivores is significantly different to that of the slopes calculated for the artiodactyls, rodent 1 and rodent 2 groupings using the mean squares between and within slopes: carnivores *vs* artiodactyls, $P < 0.05$; carnivores *vs* rodent 1, $P < 0.05$, carnivores *vs* rodent 2, $P < 0.05$.

Artiodactyls were found to have the most gyrencephalic cerebral cortex compared to the other mammalian orders with the GI increasing by 110% with every doubling in brain weight. As in primates and carnivores, brain weight scales faster than GI and the *P*-value (0.0014) indicates a significant relationship between the two variables.

The equation calculated for the artiodactyls was:

$$GI = 1.4069 W_{br}^{0.1017} (r^2 = 0.45; P = 0.0014)$$

The slope of the line (0.1017) as calculated by the regression analysis is statistically significant different to the slopes of rodent group 1 and group 2 using the mean squares between and within slopes: artiodactyls *vs* rodent 1, $P < 0.05$; artiodactyls *vs* rodent 2, $P < 0.05$.

Comparisons between brain weight and GI for rodent group 1 (rat, mouse, hamster, agouti and capybara) revealed a strong correlation ($r^2 = 0.92$) between the two variables and showed that for every doubling in body weight there was a 104% increase in the GI. As in the primates, carnivores and artiodactyls, brain weight scales faster than GI.

The equation calculated for rodent group 1 was:

$$GI = 1.0194 W_{br}^{0.0605} (r^2 = 0.92; P = 0.0023)$$

As mentioned earlier the North American beaver had a large, but lissencephalic brain compared to other rodents of similar brain weights, thus we suggest that there might be two trends that are specific to this order. The trend depicted by the equation calculated for rodent group 1 (Table 1) indicates that the GI increases with increasing brain weight.

A second equation (rodent group 2) was calculated for rodents that included the rat, mouse, hamster and the North American beaver (Table 1):

$$GI = 1.0203 W_{br}^{-0.0003} (r^2 = 0; P = 45.55)$$

No correlation (P = 45.55) was found between the brain weight and GI for this set of rodents. The trend revealed in this group indicates that as the brain size of some rodents increases there is little or no change in the development of gyri and sulci in the cerebral cortex. This might suggest that some rodents maintain lissencephalic brains with increasing brain weight. Comparisons using the mean squares between and within slopes: rodent group 1 *vs* rodent group 2, P > 1, shows a significant difference between the slopes.

2.3.2 Method Two-Gyral *vs* Sulcal

Using the second method (Fig. 9) we found that the GI increases with increasing brain weight indicating that as the brain gets larger there is more sulcal cortex (Fig. 10). This method was intended to strengthen and confirm results from Zilles' method (Zilles, 1988, method 1) and we have found that again each order follows its own specific pattern and has similar trends to those found using Zilles' method.

The primate and carnivore regression slopes exhibit a similar intersection at smaller brain weights, indicating that smaller brained carnivores have more sulcal cortex than smaller brained primates, but again due to the shallowness of the carnivore regression slope, large carnivores have less sulcal cortex than larger brained primates.

The equation calculated for the primates is as follows:

$$GI = 8.394 W_{br}^{0.4352} \ (r^2 = 0.91, P = 0.042)$$

Again r^2 is high, with 91% of the variability in the GI of primates being accounted for by changes in brain weight, and for every doubling in brain weight there is a 135% increase in the ratio of sulcal to gyral cortex. The correlation between the two variables is statistically significant ($P = 0.042$). The calculated regression slope for primates is not significantly different from the slopes calculated for carnivores and artiodactyls, but significantly different to that of the slopes calculated for rodent group 1 and rodent group 2 using the mean squares between and within slopes: primates *vs* carnivores, $P > 0.05$; primate *vs* artiodactyls, $P > 0.05$; primate *vs* rodent 1, $P < 0.05$; primates *vs* rodent 2, $P < 0.05$.

There is a strong correlation ($r^2 = 0.98$) between GI and brain weight amongst the carnivores, with every doubling of brain weight, leading to a 126% increase in the GI. Thus, brain weight scales at a far more rapid rate than GI, indicating that sulcal cortex in carnivores increases at a slower rate than brain weight. The equation calculated for carnivores is:

$$GI = 0.1609 W_{\mathrm{br}}^{0.337} \ (r^2 = 0.98, P = 0.0085)$$

The calculated regression slope for carnivores is almost parallel to that of the artiodactyl slope, indicating that GI increases with increasing brain weight at a similar rate for both the orders. This graph shows there is no significant difference between the two calculated slopes using the mean squares between and within slopes: carnivores *vs* artiodactyls, $P > 0.05$. The calculated regression slope is however; significantly different from the regression slopes calculated for rodent group 1 and rodent group 2 using the mean

squares between and within slopes: carnivores *vs* rodent group 1, $P < 0.05$; carnivores *vs* rodent group 2, $P < 0.05$.

A very weak correlation ($r^2 = 0.28$) was revealed between the *GI* and brain weight amongst the artiodactyls, possibly as a result of the small sample size used. For every doubling in brain weight there was found to be a 117% increase in the *GI*. Despite this the artiodactyls evince a higher *GI* than the other orders, suggesting that they have more sulcal than gyral cortex compared to the other mammalian species examined. The equation calculated for artiodactyls was:

$$GI = 0.9654 W_{br}^{\ 0.1557} \ (r^2 = 0.28, P = 0.25)$$

Comparisons using the mean squares between and within the slopes show no statistical significance: artiodactyls vs rodent 1, $P > 0.05$; artiodactyls vs rodent 2, $P > 0.05$.

Rodent group 1 reveals a strong correlation ($r^2 = 0.84$) between the two variables and shows that for every doubling in brain weight, there was a 162% increase in GI. The slope is significantly steeper than that of the primates, carnivores and rodent group 2, suggesting that for some species of rodents the sulcal to gyral cortex ratio increases more rapidly than other mammalian species with increases in brain weight. The equation for rodent group 1 is:

$$GI = 0.0274 W_{br}^{0.6965} \ (r^2 = 0.84, P = 0.028)$$

Regression slopes for rodent group 1 and rodent group 2 are shown to be significantly different using the mean squares between and within slopes: rodent group 1 *vs* rodent group 2, $P < 0.05$. The equation for rodent group 2 is:

$$GI = 0.0234 W_{br}^{0.0476} \ (r^2 = 0.14, P = 0.20)$$

The correlation coefficient ($r^2 = 0.14$) indicates a very weak relationship between the two variables and no significant correlation ($P = 0.20$) between the variables exist. As mentioned before, two trends might exist within the Rodentia; again this trend suggests that the lissencephalic feature of the brain remains relatively stable as the brain weight of some rodent species increases.

2.3.3 Method Three-Complete *vs* Inner

The GI for this method was calculated by the measured complete contour (at the pial surface of the neocortex) divided by the measured inner contour (at the base of layer 6). The thickness of the cortex was taken into account using this method and the results indicate that the thinner the cortex the more extensive the gyrification.

All the slopes in this graph (Fig. 11) appear to asymptote with increasing brain size, which indicates a balance between increases in cortical thickness with increasing brain size and increasing gyrification. The results using this method reflect the patterns and trends found with the other two methods, for example the regressions of the primates and carnivores intersect. The artiodactyls have the thinnest cortex and thus the most extensive gyrification. The primates show a strong correlation ($r^2 = 0.86$) between the

two variables and the *P*- value (0.031) indicates a significant correlation. The equation calculated for the primates is:

$$GI = 1.643 W_{br}^{-0.0555} \ (r^2 = 0.86, P = 0.031)$$

The regression slopes for the primates and carnivores intersects at smaller brain weights indicating that small brained carnivores have a thinner cortex, and thus more extensive gyrification than small brained primates. As the brain gets larger the larger brained primates have a thinner cortex than the larger brained carnivores, and thus more extensive gyrification. The slope calculated for primates was not significantly different from the other mammalian orders using the mean squares between and within slopes: primate *vs* carnivore, $P > 0.05$; primate *vs* artiodactyl, $P > 0.05$, primate *vs* rodent group 1, $P > 0.05$; primate *vs* rodent group 2, $P > 0.05$.

The equation calculated for carnivores was:

$$GI = 1.4523 W_{br}^{-0.0321} \ (r^2 = 0.79, P = 7.9 \times 10^{-5})$$

Carnivores show a strong correlation between the two variables (*GI* and brain weight) and the calculated regression slope for the carnivores is significantly different from the slopes calculated for the artiodactyls using the mean squares between and within slopes: carnivores vs artiodactyls, $P < 0.05$; carnivores *vs* rodent group 1. However, there is no statistical difference between the slopes calculated for the carnivores and rodents using the mean squares between and within slopes: $P > 0.05$; carnivores *vs* rodent group 2, $P > 0.05$.

The regression of the artiodactyls falls below all the other mammalian orders examined, which as mentioned, is indicative of the thinnest of the cortex and thus, results in the most extensive gyrification with increasing brain weight. The equation calculated for artiodactyls was:

$$GI = 1.3361 W_{br}^{-0.0318} \ (r^2 = 0.96, P = 4.99 \times 10^{-4})$$

A strong correlation exists between the two variables (GI and brain weight) and the regression calculated for the artiodactyls is not significantly different to that calculated for rodent group 1 and rodent group 2 using the mean squares between and within slopes: artiodactyls *vs* rodent group 1, $P > 0.05$; artiodactyls *vs* rodent group 2, $P > 0.05$.

The slopes of rodent group 1 and rodent group 2 fall higher on the graph compared to other slopes indicating relatively thick cortex. Rodent group 1 has a strongly significant correlation ($r^2 = 0.78$) and the equation calculated for the regression slope of rodent group 1 was:

$$GI = 1.8172 W_{br}^{-0.0724} \ (r^2 = 0.78, P = 0.0038)$$

The equation calculated for rodent group 2 was:

$$GI = 1.8131 W_{br}^{-0.0322} \ (r^2 = 0.49, P = 0.026)$$

There was a significant correlation between GI and brain weight however, the correlation is relatively weak (0.49). There was no significant difference between the slopes calculated for rodent group 1 and rodent group 2 using the mean squares between and within slopes: rodent group 1 *vs* rodent group 2, $P > 0.05$.

Table 2.1: Brain weight and gyrification indices of all the mammalian species used in the analyses included in the present study. Note the gyrification indices were calculated using three different methods (see fig.8).

Table 2.1

Common name	*Species name*	*Brain weight (g)*	*GI (Zilles')*	*GI (gyral vs. sulcal)*	*GI (outer vs. inner)*
Primates					
Slow lorris	*Nycticebus coucang*	13.35	1.31	0.41	1.51
Owl monkey	*Aotus trivirgatus*	18	1.26	0.3	1.38
Mongoose lemur	*Eulemur mongoz*	21.8	1.33	0.35	1.42
Squirrel monkey	*Saimiri sciureus*	22.68	1.56	0.66	1.34
Rhesus monkey	*Macaca mulatta*	90	1.75	0.91	1.22
Mandrill	*Mandrillus sphinx*	155.9	2.18	1.37	1.23
Chimpanzee	*Pan troglodytes*	405.5	2.3	1.55	1.15
Human	*Homo sapiens*	1400	2.99	2.51	1.15
Carnivores					
Mierkat	*Cynictis penicillata*	14.53	1.35	0.39	1.32
Domestic cat	*Felis catus*	36.9	1.5	0.57	1.29
Hyena	*Crocuta crocuta*	162.5	1.74	0.88	1.28
African lion	*Panthera leo*	258	1.85	0.96	1.22
Polar bear	*Ursus maritimus*	458.6	2.04	1.36	1.16
Artiodactyls					
Domestic pig	*Sus scrofa domesticus*	95.3	2.16	1.87	1.16
White-tailed deer	*Odocoileus virginianus*	160	2.27	1.88	1.13
Llama	*Lama glama domesticus*	200.3	2.7	2.82	1.13
Zebu	*Bos taurus indicus*	474	2.53	2.34	1.1
Rodent 1					
Mouse	*Mus musculus*	0.65	1.03	0.03	1.72
Hamster	*Mesocricetus auratus*	0.9	1.01	0.02	1.85
Rat	*Rattus norvegicus*	2.48	1.02	0.02	1.9
Agouti	*Dasyprocta leporina*	17.2	1.23	0.31	1.48
Capybara	*Hydrochaeris hydrochaeris*	51	1.3	0.4	1.32
Rodent 2					
Mouse	*Mus musculus*	0.65	1.03	0.03	1.72
Hamster	*Mesocricetus auratus*	0.9	1.01	0.02	1.85
Rat	*Rattus norvegicus*	2.48	1.02	0.02	1.9
North American beaver	*Castor canadensis*	38.5	1.02	0.03	1.57

Figure 2.1: Lateral brain images from the primate species indicate an increase in gyrencephaly from the smallest to the largest brain (A-F). A = Mongoose lemur, B = Squirrel monkey, C = Rhesus monkey, D = Mandrill, E = Chimpanzee, F = Human. Scale = 1cm.

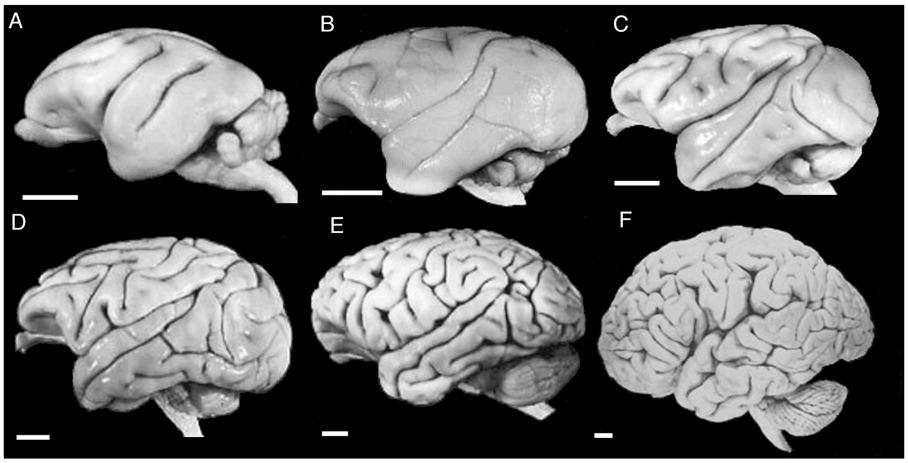

A
B
C
D
E
F

Figure 2.2: Histological, coronal sections at the level of the lateral geniculate nucleus indicate an increase in gyrencephaly from the smallest to the largest primate brain (A-F). A = Mongoose lemur, B = Squirrel monkey, C = Rhesus monkey, D = Mandrill, E = Chimpanzee, F = Human. Scale = 1cm.

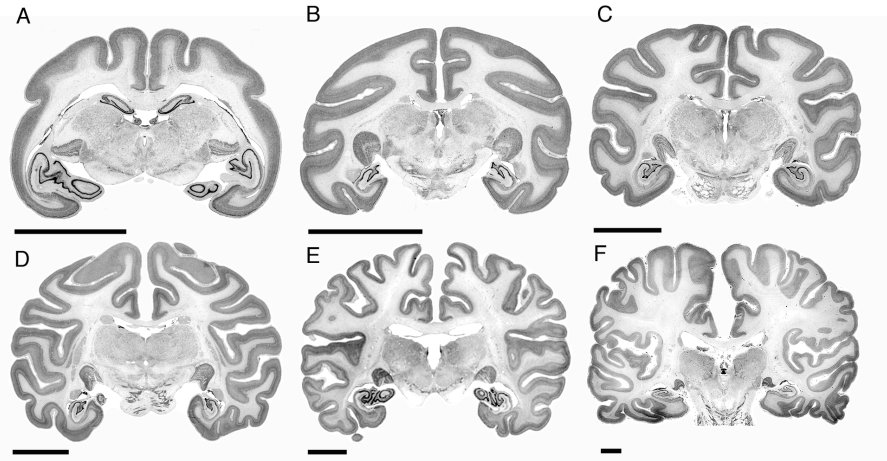

A
B
C
D
E
F

Figure 2.3: Lateral brain images from the carnivore species indicate an increase in gyrencephaly from the smallest to the largest brain (A-E). A = Yellow mongoose, B = Domestic cat, C = Spotted hyena, D = African lion, E = Polar bear. Scale = 1cm.

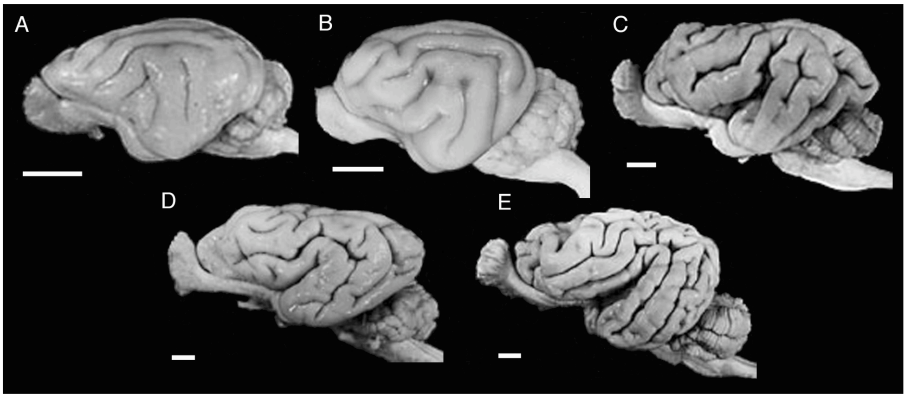

A
B
C
D
E

Figure 2.4: Histological, coronal images at the level of the lateral geniculate indicate an increase in gyrencephaly from the smallest to the largest carnivore brain (A-E). A = Yellow mongoose, B = Domestic cat, C = Spotted hyena, D = African lion, E = Polar bear. Scale = 1cm.

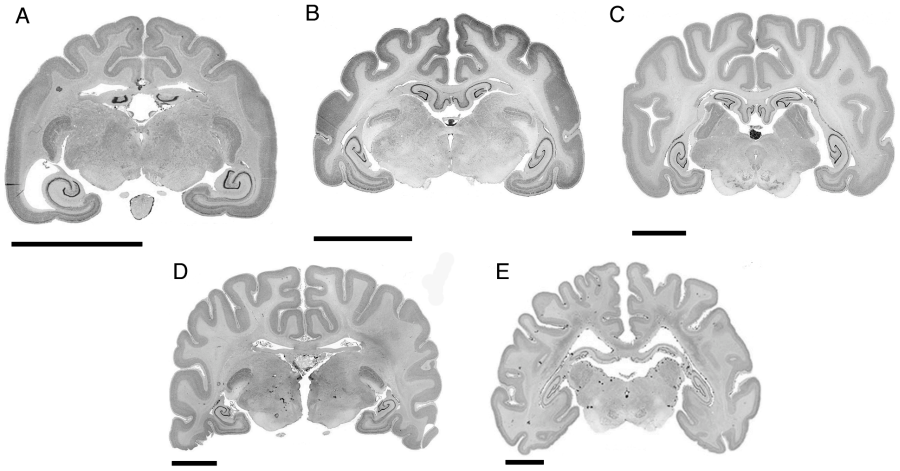

A
B
C
D
E

Figure 2.5: Lateral brain images from the artiodactyl species indicate an increase in gyrencephaly from the smallest to the largest brain (A-F). A = Rock hyrax, B = Domestic Pig, C = White-tailed deer, D = Llama, E = Zebu. Scale = 1cm.

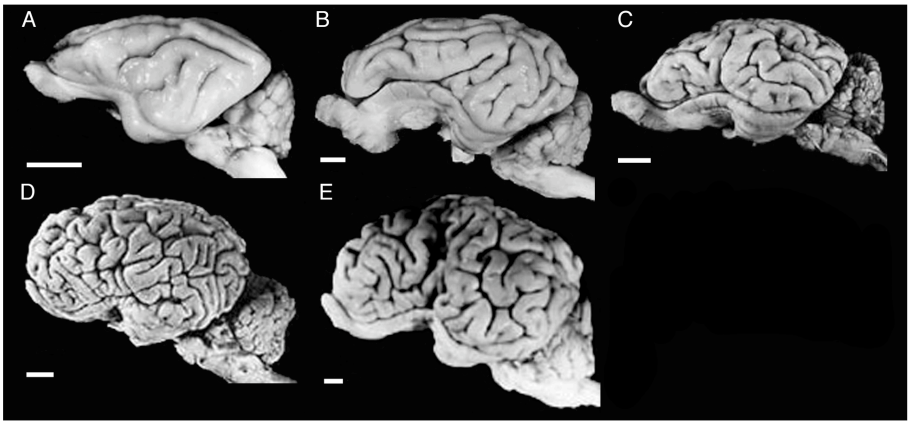

A
B
C
D
E

Figure 2.6: Histological, coronal images at the level of the lateral geniculate nucleus indicate an increase in gyrencephaly from the largest artiodactyl brain (A-F). A = Rock hyrax, B = Domestic pig, C = White-tailed deer, D = Llama, E = Zebu. Scale = 1cm.

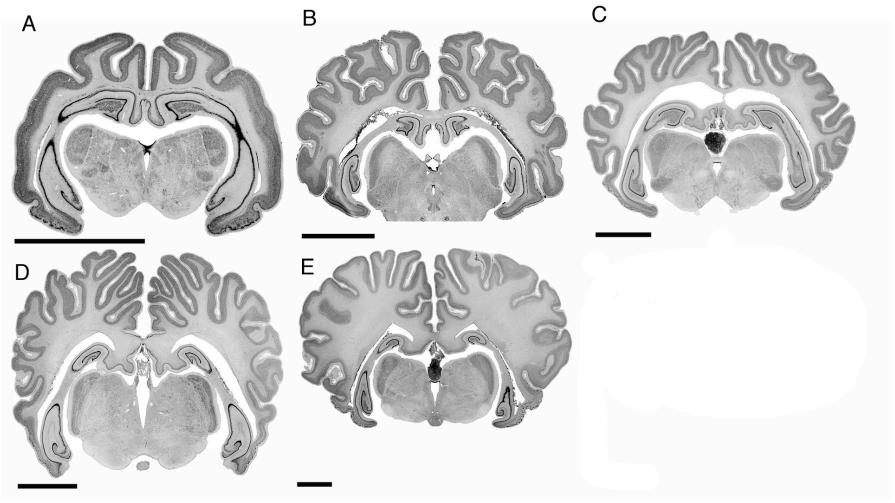

A
B
C
D
E

Figure 2.7: Lateral images from the rodent species indicate an increase in gyrencephaly from the smallest to the largest brain species (A-D). A = Mouse, B = Rat, C = North American beaver, D = Capybara. Scale = 1cm.

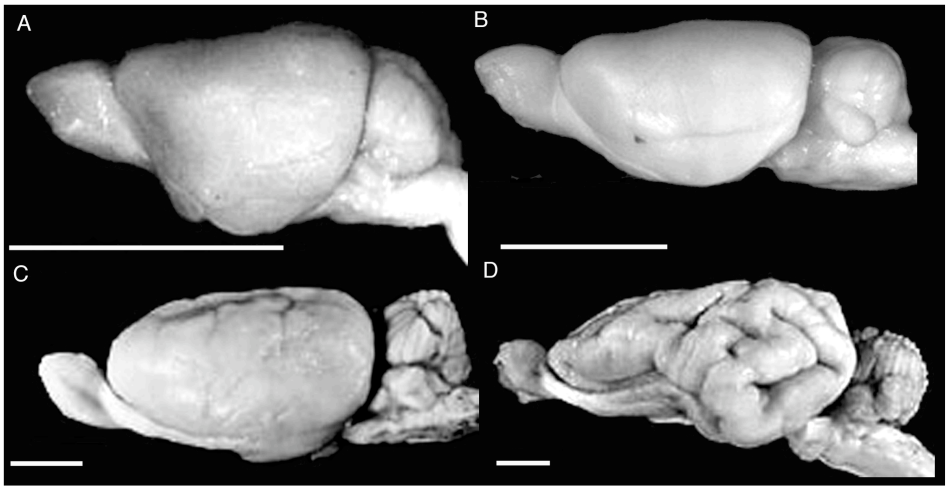
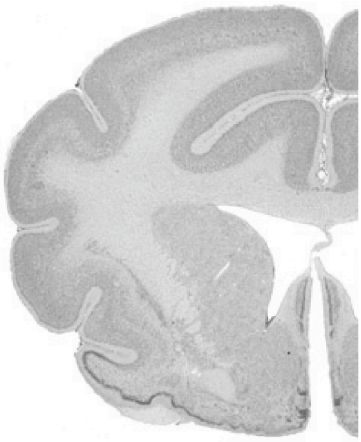
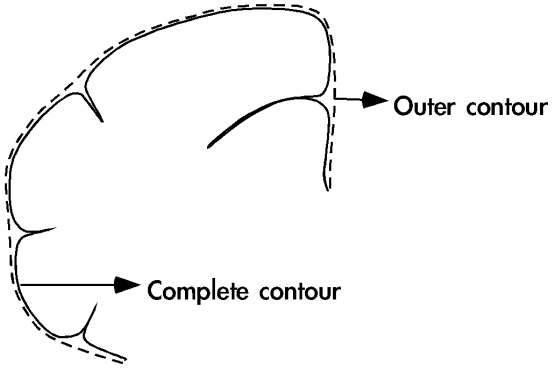

A
B
C
D

Figure 2.8: These images show the three methods used to calculate the gyrification indices for the different mammalian species. The first method involves calculating a ratio between the outer vs. the complete contour (Zilles' method), the second involves calculating a ratio between the sulcal vs. the gyral cortex and the third is a ratio of the complete vs. the inner contour.

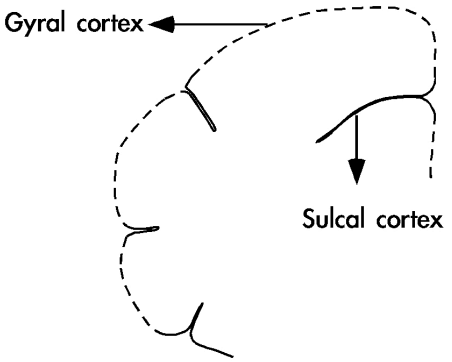
Original Section



Method 1(Zilles,1988)



Method 2 (Sulcal vs. Gyral cortex)



Method 3 (Complete vs. Inner contour)

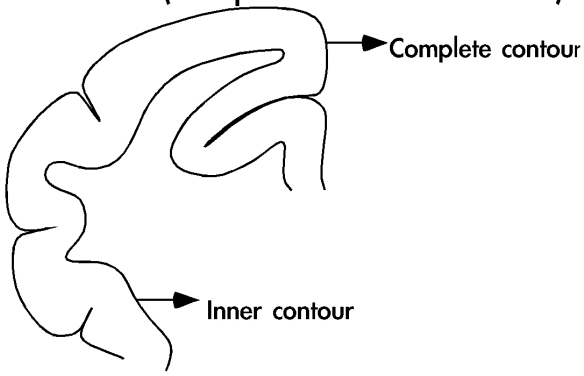


Figure 2.9: Regression lines and allometric equations of the various mammalian orders examined in the present analysis. Note that for each order the GI increases with increasing brain weight using Zilles' method.

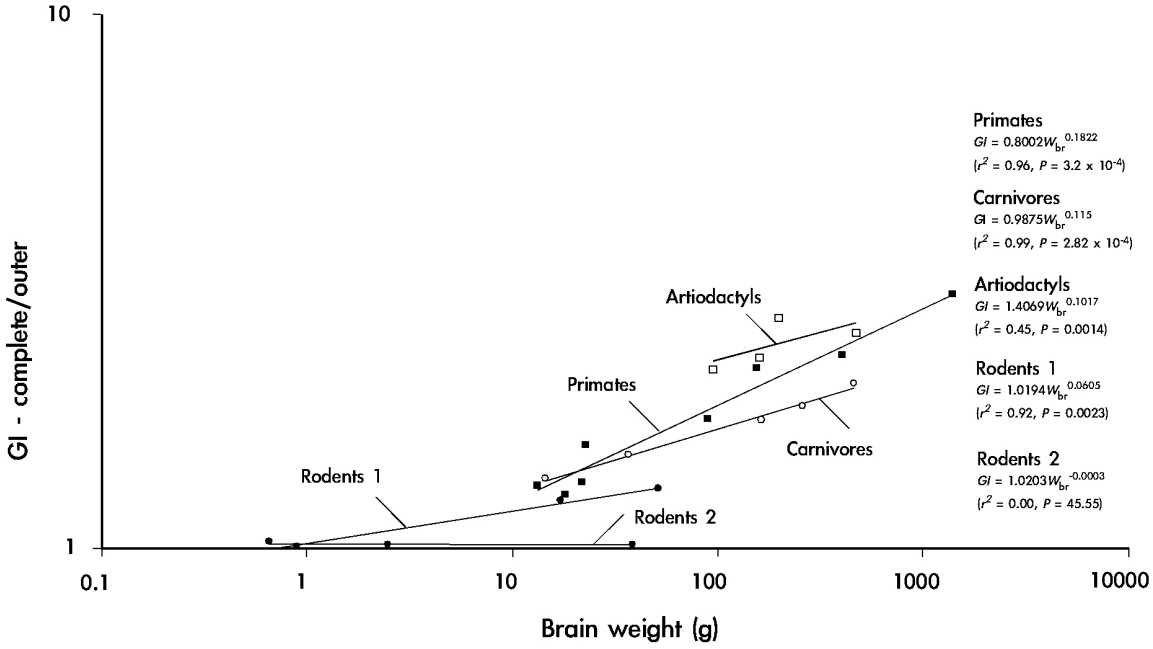

10
1
GI - complete/outer
0.1
1
10
100
1000
10000
Brain weight (g)
Artiodactyls
Primates
Carnivores
Rodents 1
Rodents 2
Primates
GI = 0.8002W_br^0.1822
(r^2 = 0.96, P = 3.2 x 10^-4)
Carnivores
GI = 0.9875W_br^0.115
(r^2 = 0.99, P = 2.82 x 10^-4)
Artiodactyls
GI = 1.4069W_br^0.1017
(r^2 = 0.45, P = 0.0014)
Rodents 1
GI = 1.0194W_br^0.0605
(r^2 = 0.92, P = 0.0023)
Rodents 2
GI = 1.0203W_br^-0.0003
(r^2 = 0.00, P = 45.55)

Figure 2.10: Regression lines and allometric equations calculated for each mammalian order. Results from method two (sulcal *vs* gyral) also indicate an increase in GI with increasing brain weight within mammalian orders.

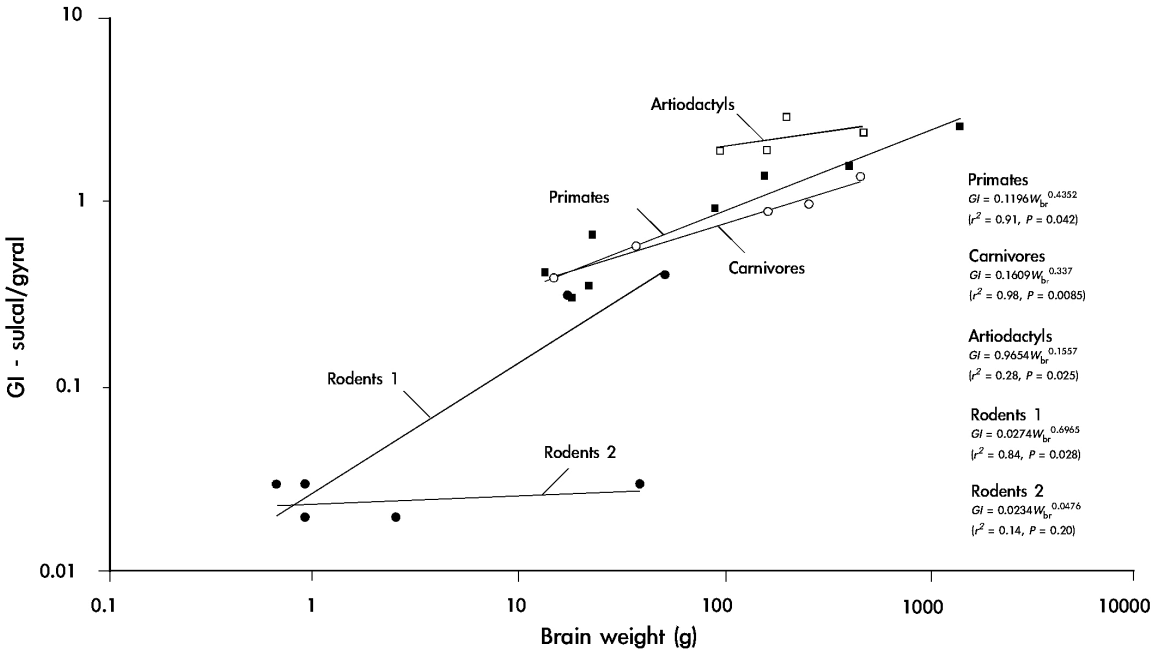
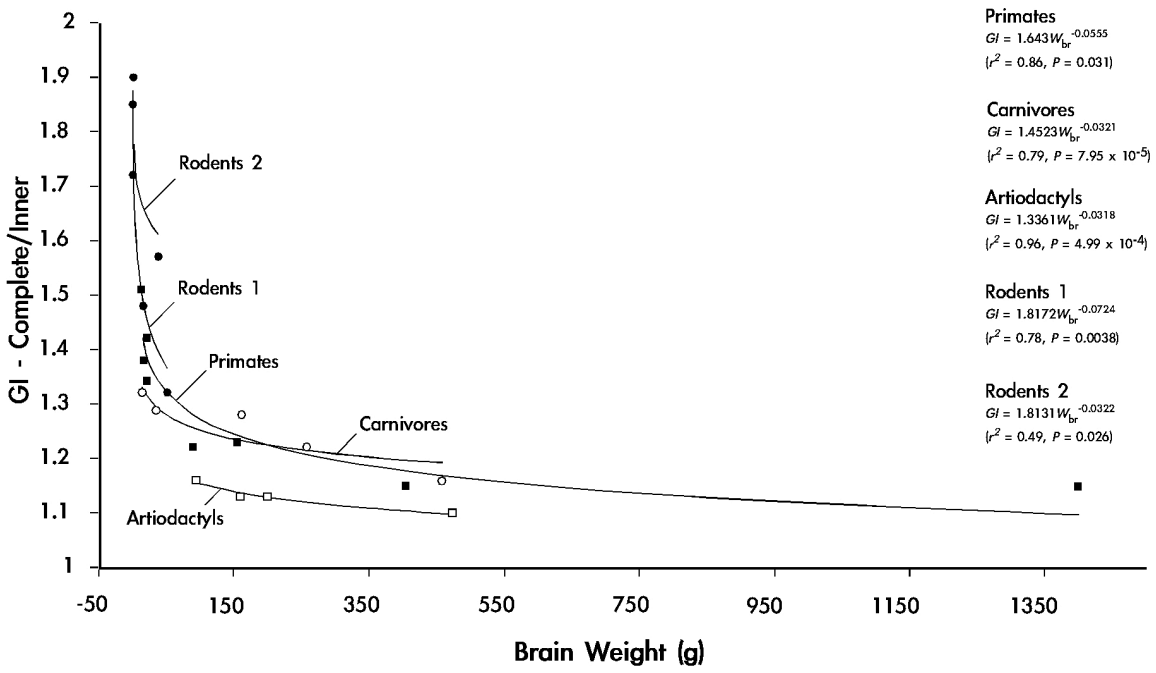

10
1
0.1
0.01
GI - sulcal/gyral
0.1
1
10
100
1000
10000
Brain weight (g)
Artiodactyls
Primates
Carnivores
Rodents 1
Rodents 2
Primates
GI = 0.1196W_br^0.4352
(r^2 = 0.91, P = 0.042)
Carnivores
GI = 0.1609W_br^0.337
(r^2 = 0.98, P = 0.0085)
Artiodactyls
GI = 0.9654W_br^0.1557
(r^2 = 0.28, P = 0.025)
Rodents 1
GI = 0.0274W_br^0.6965
(r^2 = 0.84, P = 0.028)
Rodents 2
GI = 0.0234W_br^0.0476
(r^2 = 0.14, P = 0.20)

Figure 2.11: Regression lines and allometric equations were calculated for each mammalian order. Method three examines the thickness of the cortex. Results show that thinner cortices are more gyrencephalic than thicker cortices which are relatively lissencephalic like the rodents.


GI - Complete/Inner
Brain Weight (g)
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
-50
150
350
550
750
950
1150
1350
Rodents 2
Rodents 1
Primates
Carnivores
Artiodactyls
Primates
GI = 1.643W_br^-0.0555
(r^2 = 0.86, P = 0.031)
Carnivores
GI = 1.4523W_br^-0.0321
(r^2 = 0.79, P = 7.95 x 10^-5)
Artiodactyls
GI = 1.3361W_br^-0.0318
(r^2 = 0.96, P = 4.99 x 10^-4)
Rodents 1
GI = 1.8172W_br^-0.0724
(r^2 = 0.78, P = 0.0038)
Rodents 2
GI = 1.8131W_br^-0.0322
(r^2 = 0.49, P = 0.026)

2.4 Discussion

The main observation made in the present study was that with increasing brain size in the mammals, there was an increasing gyrencephaly. This previously qualitative assessment Welker (1990) is confirmed quantitatively across several mammalian species in the present study. However, even though there is this general trend, the quantitative measures made indicate that within each mammalian order there is a specific allometric relationship between brain size and the extent of gyrification that is significantly different from the other orders examined. We found that the artiodactyls are the mammals with the most gyrencephalic brains, these species being significantly more gyrencephalic than all other mammals when species of similar brain weights are compared. Lastly, the North American beaver appears to be a rodent with an atypically lissencephalic brain for its size, differing from the trend for increased gyrencephaly found in the other rodent species examined.

2.4.1 Methodological Considerations

In the present study we used three measures of gyrencephaly to assist in determining whether specific patterns could be found. Using the previously published method of Zilles and co-workers (Fig. 8, method 1) (Zilles *et al.,* 1988, 1989; Armstrong *et al.,* 1991) and a method comparing the amount of cortex in the sulci compared to the gyral crowns (Fig. 8, method 2), similar results were found. This similarity indicates that the previously published method of Zilles and co-workers is a reliable and robust method for calculating the GI across all mammalian species. The third method (outer cortical

contour *vs* inner cortical contour, fig. 8, method 3) provided data that enabled us to relate the thickness of the cerebral cortex to the extent of gyrification. Using this method we were able to deduce that a thinner cortex could buckle and fold more easily than a thicker cerebral cortex, thus resulting in a more gyrencephalic brain.

2.4.2 Order Specific Patterns of Increased Gyrencephaly With Increased Brain Size

In his extensive review of the literature Welker (1990) indicates that most comparative neuroanatomical studies have concluded that larger brains are more gyrencephalic than smaller brains. Welker indicates however that there are certain exceptions to this general trend, and cites the examples of the large but lissencephalic brain of the Florida manatee and North American beaver, and compares the highly gyrencephalic brain of the least weasel with the lissencephalic but similar sized brain of the muskrat. He concludes that these examples have not been adequately studied to understand why they are exceptions to the general trend. Welker (1990) also indicates that within each order of mammals, there is a specific overall pattern to the gyri and sulci that is specific to that order. While certain sulci and gyri can be compared between orders, they are more easily compared within an order, as with previous examples of order specific patterns of evolution within mammalian brains (Manger, 2005). Taxonomic specific allometric patterns of gyrencephaly and brain weight have been previously shown for primates Zilles *et al.* (1989). Based on a survey of 22 anthropoid brains, the mean rhesus monkey GI or degree of cortical folding is that expected for its brain size of 90 grams Zilles *et al.* (1989). At the same time the variation of GI within the species *Macaca mulatta* does not correlate with brain weight. When different species of

primates are compared, mean GI's of entire brains increase as a function of brain weight Zilles *et al.* (1989). This result is in accordance with the results compiled in this study as the data indicates that for each mammalian order examined the GI increases with increasing brain weight.

Our central finding in the current study is that each mammalian order exhibited its own individual and allometrically predictable pattern of cortical gyrification (as found for primates by Zilles *et al.*, 1989). Moreover, each order specific relationship was statistically significantly different from the other orders. In each order the largest brains were the most gyrencephalic, however, as previously suggested by Welker (1990), the most "differentiated" brains were not the most gyrencephalic when compared across mammalian orders. The present finding of quantitative order specific patterns of gyrencephaly have direct implications regarding the possible reasons as to why sulci and gyri develop and are similar in pattern in the various mammalian orders. In particular this may relate to the mechanical hypothesis of gyral formation, whereby due to limited devotion of space within the skull to the brain, the cortex becomes gyrencephalic to attain a larger surface area in a relatively smaller portion of the skull. If the manner in which the portion of the skull devoted to housing the brain is different in each mammalian order, then one would predict the order specific patterns found in the present study. Thus, what we have demonstrated appears to support in some sense the mechanical hypothesis of cortical folding, however, further work needs to be done regarding the proportions of the skull, especially the cranial vault, and how this relates to gyrification. This further work may explain why the manner in which GI scales with brain weight in for example the artiodactyls and primates differs, and why in the non-beaver rodent series

the rate of gyrencephaly is significantly faster than other mammalian orders when brain weight increases.

2.4.3 Why are Artiodactyls More Gyrencephalic than Primates?

In the present study we found that for species of similar brain weights, the artiodactyls had far larger GIs than the primates. This is an unusual finding, but the three methods used allow us to forward a possible explanation for this. Our results comparing the outer and inner cortical surfaces show that the artiodactyls have the thinnest cerebral cortex (Fig. 11). Having a thinner cerebral cortex will allow for easier mechanical buckling and will also reduce in size the "gyral window" needed for the cortical afferents and efferents as indicated in the earlier study of Prothero and Sundsten (1984). Both these features will therefore result in a more gyrencephalic cortex. This combination of features may explain the extensive gyrencephaly seen in cetaceans (Welker, 1990) as the cetaceans have a very thin cortex allowing for easier buckling and less cortical neurons indicating that there will be less afferents and efferents allowing for a smaller gyral window (Manger, 2006).

This combination of features may also come into play in specific human abnormalities of gyrencephaly. For example in schizophrenia a thinner cerebral cortex and a reduced volume of the superficial layers of the cortex, is coupled with more extensive cortical folding (Sallet *et al.,* 2003; Harrison, 1999). Previous pathological findings have also found fewer neurons and thinner cortices to be characteristic of polymicrogyric brains. In his study of cortical malformations Rakic (1988) found that polymicrogyric brains had a characteristic diminished production of neurons in the cortex

and this resulted in fewer neurons and a thinner cortex. Based on a study of patients diagnosed with bilateral polymicrogyria, a pathological examination in one case revealed a diffusely thin and excessively folded cerebral cortex lacking a normal six-layered architecture (Chang *et al.*, 2004). The increased gyrencephaly in these pathological cases may be explained by the thinness of the cerebral cortex.

2.4.4 The Abnormally Lissencephalic Brain of the North American Beaver

We have suggested that there could be two trends within the Rodentia; however the second trend has been produced solely as a result of the lack of gyrification of the cerebral cortex of the North American beaver. The beaver exhibited insignificant cortical folding, which is not what would be expected for its brain weight in comparison to other rodents, and indeed other mammals (Figs. 9,10). Welker (1990) has pointed out this unusual feature of the beaver brain previously. The beaver does not appear to have an unusually thick cerebral cortex that may prevent cortical folding mechanically, nor does it have an unusually high number of neurons indicating a need for a larger gyral window (see Fig. 63 of Welker, 1990). But, the beaver does appear to have relatively large lateral ventricles (although this is a qualitative impression that has not been quantified). This feature is common to the Florida manatee which also shows a mostly lissencephalic cerebral cortex (Welker, 1990). It is possible that the enlarged lateral ventricles are related in some way to the lack of cortical folding in these two species.

In answer to the question posed earlier on: is the order a significant phylogenetic grouping in terms of quantifiable gyrification indices? Our results show definite trends

and patterns specific to each order. So it would seem that the order is a significant phylogenetic grouping in terms of this neural parameter, from which we can predict with a reasonable degree of certainty the GI of any species of a particular order if we know the brain weight. Unusual exceptions to this type of order specific pattern, such as the North American beaver and the Florida manatee require further observation and quantification to provide explanations for these exceptions.

C H A P T E R 3
DOES THE SKULL CONTRIBUTE TO THE QUANTITATIVE EXTENT OF CORTICAL GYRIFICATION: RELATIONSHIP TO EVOLUTIONARY PATTERNS

3.1 Introduction

In his extensive review of the literature on comparative cortical gyrification, Welker (1990) indicates that most studies have concluded that larger brains are more gyrencephalic than smaller brains. Welker indicates however that there are certain exceptions to this general trend, and cites the examples of the large but lissencephalic brain of the Florida manatee and North American beaver, and compares the highly gyrencephalic brain of the least weasel with the lissencephalic but similar sized brain of the muskrat. He concludes that these examples have not been adequately studied to understand why they are exceptions to the general trend.

A taxonomic specific allometric pattern of gyrencephaly and brain weight was demonstrated for primates by Zilles *et al.* (1989). Further quantification of cortical gyrification of mammalian species representing the primate, carnivore, artiodactyl and rodent orders allowed us to reveal order specific patterns of gyrification (Pillay & Manger, 2007). For each order we found that with increasing brain size there was an increase in gyrencephaly (expressed as the gyrencephalic index, GI), with the above mentioned exception of the North American beaver.

The skull and brain are clearly interrelated organismal components in both development and evolution, and it may be that one influences the degree of expression of parts of the other. From our previous study identifying order specific patterns of cortical gyrification, and the clear order specific patterns of expression of the bones of the skull

(which is often used in taxonomy), it appears that clues to the quantitative extent of gyrification in the different mammalian orders might be related to the proportions of the bones that create the braincase. Clarke (1945) suggests that the sulcal pattern of the brain might be determined by extrinsic mechanical factors operating during development, such as the shape of the skull, i.e. longitudinal fissuring predominates for brains housed in dolichocephalic skulls and transverse fissures predominate for brains housed in brachycephalic skulls. Welker (1990) suggests that the sulcal and gyral features of the brain of large and small species have greater similarities within mammalian orders than between orders. Pillay & Manger (2007) have done further investigations and have shown that within each mammalian order there is a specific allometric relationship between GI and brain weight. Thus we can ask: is it possible that the order specific pattern in the morphological parameters of the bones of the cranial vault is related to the order specific quantitative pattern of GI?

Despite this possible relationship, Welker (1990) argues that the skull is probably not a limiting factor on cortical expansion, and thus causing gyrification, by pointing out: (1) that the calvarium grows and is shaped in response to forces generated by the growing convolutions and expanding opercula and lobes; (2) that the impressions made by the gyri and sulci on the endocranium is only useful in evaluating the patterns of gyrification of mammals and does not serve as evidence that the skull is a restraining factor on the cerebrum; and (3) that skull sutures do not ossify until the brain has ceased growing, and if any portion of the cerebrum fails to develop or grow, due to pathological causes, the skull conforms to the size and shape of the cerebral remnant.

In contrast, Hofman (1984) found evidence in several cases of micrencephaly that a decline in brain weight during development is not accompanied by a similar reduction in head circumference (or skull growth). This finding suggests that the brain and skull develop as two separate entities where in normal development the skull may only have a passive mechanical influence on the expansion of the cerebral cortex. While several ideas regarding the development and evolution of cortical gyrification have been proposed (e.g. Clarke, 1945; Rakic, 1985; Prothero and Sundsten, 1984) a satisfactory answer has not yet been reached (Welker, 1990).

We have already established that there are order specific patterns of gyrification (Pillay & Manger, 2007), and in the current study we investigate the possibility that these gyrification patterns are related to a passive restriction on cortical expansion due to order specific formations of the bones of the cranial vault. Such a finding may reveal an underlying factor in the extent of gyrification in different mammalian lineages, a problem that is as not yet understood.

3.2 Materials and Methods

3.2.1 Skull Specimens

All skulls used in the study were obtained from either the Hunterian museum situated in the School of Anatomical Sciences at the University of Witwatersrand, or the Transvaal Museum, Tshwane, South Africa. The skulls were selected to represent four mammalian orders that coincided with species for which we had previously determined a range of gyrencephalic indices (Pillay and Manger, 2007). Seventeen species were analysed in the present study and included members from: primates (n = 4), carnivores (n = 4), artiodactyls (n = 5), and rodents (n = 4) (Table 1).

3.2.2 Measurement of Surface Areas

The external surface areas of the bones that constitute the cranial vault were measured using a microscribe digitiser. The Immersion Microscribe 3DX digitiser works with physical objects of various shapes, sizes, and materials. By tracing over the contours of a physical object, e.g. a skull, a 3D computer model can be created. Software packages allow you to create complex 3D models using points, lines, polygons, splines, or other standard geometric entities. The skulls used in the present study were placed firmly in a defined workspace that had co-ordinates set up along the x and y axes.

The bones that were measured included the parietal, occipital, frontal, temporal and sphenoid bones. Using rhinoceros software (McNeel, North America) we were able to plot demarcation points along the sutures of the cranial vault in order to define each bone

(Fig 1). Where bony protuberances occurred, demarcation points were plotted around the base of the protuberance so as to exclude measurement of the surface area it occupied, to ensure a smooth outer surface was measured across the species. Any holes that were present in the selected cranial bone for example the foramen magnum in the occipital bone were also excluded as a surface area measurement. Once the bone was defined, several points (up to seven points per square centimetre) were plotted within the area of the bone to form a grid from which surface area could be measured. The total surface area was measured by the sum total of the selected areas based on the grid and plotted in squared centimetres.

The surface area of all the bones that made up the cranial vault was summed to give the total surface area for each species. Using Microsoft Excel the total area of the cranial vault as well as the area of the parietal bone of the various species was then plotted against the species brain weight to determine whether a relationship existed between these variables. The square root of the data for the total and parietal areas was first obtained and then cubed to create a measurement in cm^3, the brain weight was then divided by 1.036 to obtain a volumetric measurement in cm^3(Stephan et al., 1981). By normalising these values we were then able to plot them against each other to determine if a relationship existed (Table 1). From this plot we were able to obtain a positive result (see results later) from which we then went on to calculate residuals for both the total external skull surface area and the external surface area of the parietal bone. Given that both skull surface area and gyrencephalic indices correlate with brain mass across mammals and with each other (see fig 2), we could calculate skull area residuals in

comparison to brain mass. The residuals (residual of cranial vault area, CVA_{res}, or residual of parietal bone area, PA_{res}), of the area of the skull/parietal bone in relation to the size of the brain, were calculated using the allometric equation derived from the plot of brain weight (W_{br}) against surface area (total cranial vault surface area, CVA, and parietal area PA) (see results for exact calculations). If: (1) it was found that the residual was equal to 1, we concluded that this species has the expected amount of skull surface area for a mammal of its brain mass, or the mammalian norm; (2) it was found that the residual was greater than 1, then this species has more skull surface area than expected for a mammal with its brain mass; or (3) it was found that the residual was less than 1, then this species has less skull surface area than expected for its brain mass

By creating these specific residuals and comparing them with known gyrencephalic indices (Pillay and Manger, 2007), we could specifically test the possibility that skull surface area is a limiting factor on the expansion of cerebral cortex. If this possibility is correct, then residuals greater than 1, indicating more skull surface area than predicted for brain weight, should be associated with lower gyrencephalic indices, while those residuals less than 1, indicating less skull surface area than predicted for brain weight, should be associated with higher gyrencephalic indices. We could test these assumptions across mammalian species, and also specifically within mammalian orders. Least squared regression (LSR) was used to calculate the P- value of correlation and statistical differences between the slopes as described previously (Pillay and Manger, Chapter 2).

3.3 Results

The total surface area of the cranial vault (cm^2) was calculated by summing the calculated external surfaces areas of the parietal, occipital, frontal, temporal and sphenoid bones. These values were then analysed with brain weight and the gyrification indices to establish relationships and patterns that may exist. The surface area of the parietal bone was also analysed as a separate value to validate patterns established with total area, brain weight and the gyrification indices (*GI*). Our findings suggest a mixed interpretation of the effect of the skull on the expansion and thus gyrification of the cerebral cortex.

3.3.1 Cranial Vault Area vs Brain Weight

Plotting the cranial vault area (*CVA*) against brain weight yielded a strong correlation between the two variables. Data was determined for the species for which we had previously calculated a range of gyrencephalic indices (Pillay and Manger, 2007). In this study, it was shown that the three different methods of calculating *GI* had strong order specific correlations with brain weight suggesting that the order would be a significant phylogenetic grouping in terms of this neural parameter. By plotting those same three types of *GI* values across all the mammals we were also able to yield strong correlations with brain weight (Fig. 2) however, the r^2 values were not as strong as is within each order (Pillay & Manger, 2007). From these observations we could conclude that if brain weight correlates with *CVA* across mammals, and *GI* correlates with brain weight, then *GI* should correlate with cranial vault area. Thus, plots were set up between *CVA* and GI (see fig 2) to test this and results show reasonably strong relationships between these two variables for all three methods of calculating the *GI* value (Pillay & Manger, 2007). From

these plots we were able to establish that the residuals from the *CVA vs* brain weight plot were useful indices to determine whether there was more or less external cranial vault area than expected for the size of the brain.

A plot of the raw data of the *CVA* (cm^3) and brain weight (W_{br}) (cm^3) of all the mammalian species investigated was analysed (see fig 3.3A). The following allometric equation was calculated:

$$CVA = 7.6608 W_{br}^{1.2056} (r^2 = 0.94;\ P = 1.30 \times 10^{-11})$$

The r^2 value is high so that for the mammals investigated, 94% of the variability in cranial vault area can be accounted for by the variation in the mass of the brain. This regression also indicates a positive allometry, whereby for every doubling in brain weight, there is a 2.3 times increase (or $2^{\wedge 1.2056}$) in the external surface area of the bones of the cranial vault. Residual values from this plot were then calculated using the following equation (Table1):

$$CVA \text{ Residuals } (CVA_{res}) = \frac{CVA^3}{7.6608 * (W_{br}^{1.2056})}$$

These residuals indicate whether there is more or less external cranial vault surface area than expected for the size of the brain. A residual of one indicates the expected result for a given brain mass, those greater than one indicate "excess" external cranial vault surface area, and those less that one indicate a "lack" of external cranial vault surface area for a given brain mass.

3.3.2 Cranial Vault Residuals vs GI (Zilles' method only) in the Mammalian Orders

The CVA residual (CVA_{res})of each species was plotted against the gyrification index (*GI*) for each of the different mammalian orders i.e. primates, carnivores, artiodactyls and rodents (see fig 3.3B). In this following analysis, only the *GI* calculated using the method of Zilles' (1998) (Chapter 2) was used.

For the primates the regression equation calculated was:

$$log\ GI = -0.4024\ (CVA_{res}) + 0.6243\ (r^2 = 0.8042;\ P = 0.01)$$

The primate trend indicates a statistically significant relationship with a decreasing *GI* related to an increasing CVA_{res} (see fig 3.3B). This suggests that a skull with a surface area smaller than expected relative to brain weight would be a limiting factor on cortical expansion, thus increasing the folding of the cerebral cortex (i.e. an increased *GI*) (Pillay and Manger, 2007). The regression slope calculated for the primates is significantly different to that of the slopes calculated for the carnivores, artiodactyls and rodents using the mean squares between and within slopes: primates *vs* carnivores, $P < 0.05$; primates *vs* artiodactyls, $P < 0.05$; primates *vs* rodents , $P < 0.05$.

The regression equation calculated for the carnivores was:

$$log\ GI = 0.0979\ (CVA_{res}) + 0.0755\ (r^2 = 0.8074;\ P = 0.01)$$

In this case the *GI* increases with an increasing CVA_{res} and there is a strong ($r^2 = 0.81$) and statistically significant ($P = 0.01$) relationship between the two variables. However,

the skull and the brain appear to be acting independently as the brain gets larger; i.e. the external surface area of the skull gets larger and does not seem to act as a limiting factor on the increasing size of the cerebral cortex as seen in primates. The regression slope of the carnivores is significantly different to the slope calculated for the artiodactyls and rodents using the mean squares between and within slopes: carnivores *vs* artiodactyls, $P < 0.05$; carnivores *vs* rodents, $P < 0.05$.

The artiodactyls exhibit a similar trend to that of the primates in that both have a decreasing GI with increasing CVA_{res}. The equation calculated for the artiodactyls was:

$$log\ GI = -0.0474\ (CVA_{res}) + 0.4387\ (r^2 = 0.38;\ P = 0.15)$$

The correlation between the two variables is not strongly predictive ($r^2 = 0.38$), and not statistically significant ($P = 0.15$). The slope calculated for the artiodactyls is not significantly different to regression slope calculated for the rodents using the mean squares between and within slopes: artiodactyls *vs* rodents, $P > 0.05$.

Rodents and carnivores have similar trends in that both have an increasing GI with increasing CVA_{res}, the equation calculated for the rodents was:

$$log\ GI = 0.0196\ (CVA_{res}) + 0.0234\ (r^2 = 0.43;\ P = 0.12)$$

The relationship between the two variables (*GI* and CVA_{res}) is not strong for the rodents ($r^2 = 0.43$) and is not statistically significant ($P = 0.12$). The positive regression slope

calculated suggests that the skull does not have any mechanical influence on the fissuring and folding of the cerebral cortex in this mammalian order.

3.3.3 Cranial Vault Residuals vs GI across all Mammalian Species

To examine if the above variability was related to a small sample size from each order, we examined all species combined to determine if an overall pattern would emerge. The equation calculated for all the mammals was (see fig 3.3C):

$$log\ GI = -0.0307\ (CVA_{res}) + 0.2866\ (r^2 = 0.04;\ P = 0.37)$$

The relationship depicted between the two variables (*CVA* & *GI*) shows a very weak correlation ($r^2 = 0.04$) and no significant relationship between the two variables ($P = 0.37$). Thus, the order specificity detailed above appears to hold a more predictive value than a comparison across all species.

3.3.4 Parietal Bone External Surface Area *vs* Brain Weight

The parietal bone of the cranium occupies the largest proportion of the external surface area of the cranial vault in relation to the brain compared to the other bones that form part of the calvarium. We reasoned that, as this would be the bone with the largest surface area, the external surface area of the parietal should also be compared with the brain weight and *GI* as this bone would potentially have a greater interaction with possible expansion of the brain.

A plot of the raw data of the external parietal area (PA) (cm^3) and brain weight (W_{br}) (cm^3) of all the mammalian species (see fig 3.4A), yielded the following equation:

$$PA = 0.919 W_{br}^{1.2245} \ (r^2 = 0.97; P = 2.18 \times 10^{-13})$$

A strong ($r = 0.97$) and statistically significant ($P = 2.18 \times 10^{-13}$) correlation exists between the two variables indicating that 97% of the variability in brain weight can be accounted for by the total parietal bone area. This regression also indicates a positive allometry, whereby for every doubling in brain weight, there is a 2.3 times increase (or $2^{\wedge 1.2245}$) in the external surface area of the parietal bone, closely paralleling that seen for the entire external cranial vault area (see above). Residual values from this plot were calculated using the following equation:

$$PA \text{ Residuals } (PA_{res}) = \frac{PA^3}{0.919 * (W_{br}^{1.2245})}$$

These residuals indicate whether there is more or less external parietal bone surface area than expected for the size of the brain. A residual of one indicates the expected result for a given brain mass, those greater than one indicate "excess" external parietal bone surface area, and those less that one indicate a "lack" of external parietal bone surface area for a given brain mass.

3.3.5 Parietal Area Residuals vs GI in the Mammalian Orders

Again the *PA* residual (PA_{res}) of each species was plotted against the gyrification index (GI) for the different mammalian orders i.e. primates, carnivores, artiodactyls and rodents (Fig 3.4B). Also in this analysis, only the *GI* calculated using the method of Zilles' (1998) (Chapter 2) was used.

For the primates the regression equation derived was:

$$log\ GI = -0.3171\ (PA_{res}) + 0.6451\ (r^2 = 0.48;\ P = 0.10)$$

This trend is similar to the primate trend when the total area was calculated and provides support for the earlier results, but is not as strongly predictive as total external surface area ($r^2 = 0.48$, compared with $r^2 = 0.8042$), and is not statistically significant ($P = 0.10$). A decreasing *GI* with increasing parietal area suggests that the area of the parietal bone may act a limiting factor on the fissuring and folding of the cerebral cortex. The regression slope calculated for the primates is significantly different to that of the slopes calculated for the carnivores and rodents, but not significantly different to that of the slope calculated for artiodactyls using the mean squares between and within slopes: primates *vs* carnivores, $P < 0.05$; primates *vs* artiodactyls, $P > 0.05$; primates *vs* rodents , $P < 0.05$.

The regression equation derived for the carnivores was:

$$log\ GI = 0.1117\ (PA_{res}) + 0.0516\ (r^2 = 0.54;\ P = 0.07)$$

With increasing *GI* there is an increase in the residual *PA*, this suggests as before that the parietal surface area of the skull and brain size increase independently, and the skull may not be a factor contributing to the amount of fissuring and folding that takes place. This result is similar to and supports the results obtained when the entire volume of the skull was compared, although using just the parietal bone, the relationship is not as strongly predictive and is not statistically significant as was the case with the entire external surface area. The regression slope calculated for the carnivores is not significantly different to that of the slopes calculated for the artiodactyls and rodents using the mean squares between and within slopes: carnivores *vs* artiodactyls, $P > 0.05$; carnivores *vs* rodents, $P > 0.05$.

The regression equation calculated for the artiodactyls was:

$$log\ GI = -\ 0.0501\ (PA_{res}) + 0.4197\ (r^2 = 0.16;\ P = 0.44)$$

This trend is similar to that of the primates and suggests that the external surface area of the parietal bone may be a factor contributing to the amount of gyrification that the brain exhibits, however the correlation between the two variables is weaker than that seen for the total external surface area ($r^2 = 0.16$ vs 0.44), only 16% of the variation in the GI can be accounted for by the volume of the skull, and is not statistically significant. The regression slope calculated for the artiodactyls is not significantly different to that of the slope calculated for the rodents using the mean squares between and within the slopes: artiodactyls *vs* rodents, $P > 0.05$.

The regression equation calculated for the rodents was:

$$log\ GI = 0.0414\ (PA_{res}) + 0.0021\ (r^2 = 0.54;\ P = 0.07)$$

Again the pattern seen here is similar to that of the carnivores; the external surface area of parietal bone appears not be a limiting factor on the amount of fissuring and folding that the brain exhibits. The relationship between the two variables (*GI* and PA_{res}) is not strong for the rodents ($r^2 = 0.54$) and is not statistically significant (P = 0.07).

3.3.6 Parietal Bone Residuals vs GI across all Mammalian Species

Again, to determine if there was a general trend for mammals that overshadowed that seen in the individual orders, we compared the parietal bone residuals across all species irrespective of phylogenetic affinity. The regression equation calculated for all the mammals investigated was (Fig 3.4C):

$$log\ GI = -\ 0.0755\ (PA_{res}) + 0.3312\ (r^2 = 0.08;\ P = 0.18)$$

The general trend amongst the mammals reveals a weakly predictive ($r^2 = 0.08$) and not statistically significant ($P = 0.18$) relationship between the surface area of the parietal bones and the *GI*; however, there is a trend suggesting that the parietal bone may be a contributing factor to increased gyrencephaly of the cerebral cortex for all mammals. The result found using the total parietal area is similar to the result generated using the total area of the skull, but neither comparisons serve to lead to conclusive findings.

Table 3.1: Brain weight; gyrification indices (GI's); cranial vault surface area; parietal area and their residuals for all the species used in the present study. Note that in order to normalize data brain weight measurements were converted to (cm^3) and the GI values were logged before plotting the values against the residuals.

Table 3.1

Common name	*Species name*	*Brain weight/1.036 (cm^3)*	*Cranial vault area (CVA) (cm^3)*	*Gyrification index (GI)*	*CVA Residuals*	*log GI*	*Parietal area (PA) (cm^3)*	*PA Residuals*
Primates								
Rhesus monkey	*Macaca mulatta*	86.87	1568.03	1.75	0.95	0.24	271.61	1.23
Mandrill	*Mandrillus sphinx*	150.48	1812.28	2.18	0.57	0.34	337.03	0.78
Chimpanzee	*Pan troglodytes*	391.41	7307.83	2.3	0.73	0.36	1119.10	0.81
Human	*Homo sapiens*	1351.35	20087.96	2.99	0.45	0.48	5291.01	0.85
Carnivores								
Mierkat	*Cynictis penicillata*	14.03	173.94	1.35	0.94	0.13	30.35	1.26
Domestic cat	*Felis catus*	35.62	441.26	1.5	0.78	0.18	63.35	0.85
Hyena	*Crocuta crocuta*	156.85	5135.42	1.74	1.54	0.24	692.40	1.52
African lion	*Panthera leo*	249.03	11823.9	1.85	2.03	0.27	1440.39	1.81
Artiodactyls								
Domestic pig	*Sus scrofa domesticus*	91.99	3317.06	2.16	1.88	0.33	276.45	1.17
White-tailed deer	*Odocoileus virginianus*	154.44	2868.40	2.27	0.87	0.36	192.68	0.43
Llama	*Lama glama domesticus*	193.34	2651.19	2.7	0.62	0.43	302.50	0.52
Zebu	*Bos taurus indicus*	457.53	17845.49	2.53	1.48	0.40	1604.66	0.96
Rodents								
Mouse	*Mus musculus*	0.63	2.87	1.03	0.65	0.01	0.35	0.64
Rat	*Rattus norvegicus*	2.39	24.95	1.02	1.13	0.01	2.76	0.99
Agouti	*Dasyprocta leporina*	16.60	137.5	1.23	0.61	0.09	26.46	0.90
Capybara	*Hydrochaeris hydrochaeris*	49.23	3628.62	1.3	4.36	0.11	300.46	2.71

Figure 3.1: Pictures A, B, C, and D are examples of the various skulls that were measured with the microscribe equipment. Demarcation point were plotted along the sutures of each of the bones measured, e.g. frontal – F, parietal - P, temporal – T and sphenoid – S, as indicated by the dash line. A = Human (Primates), B = Hyena (Carnivores), C = Domestic pig (Artiodactyls), D = Capybara (Rodents).

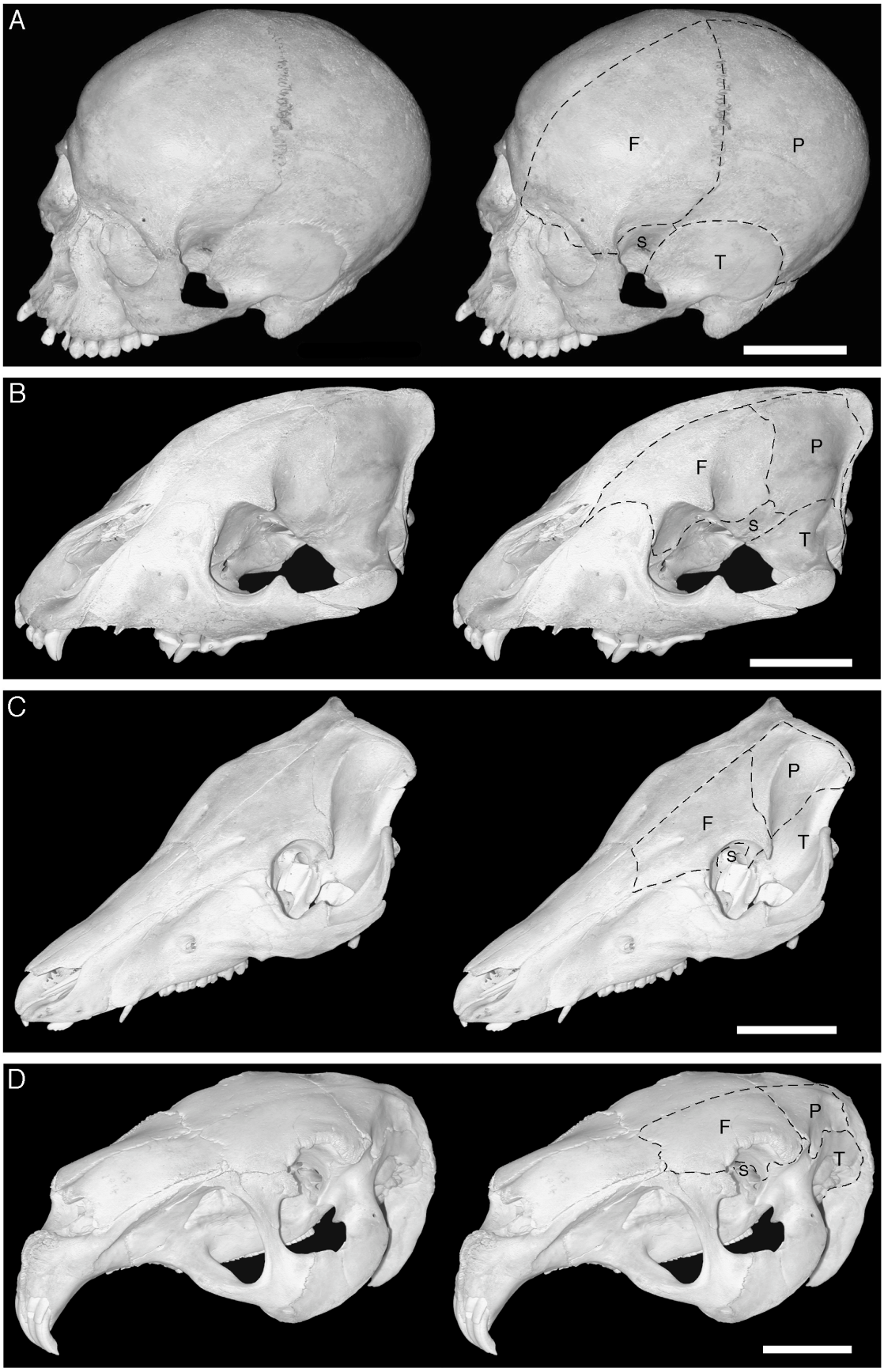

A
F
P
S
T
B
F
P
S
T
C
P
F
T
S
D
F
P
S
T

Figure 3.2: Regression lines and allometric equations calculated for mammals between *GI vs* brain weight (W_{br}) and cranial vault *vs GI* are (*CVA*) for Zilles' method for A) Zilles' method, B) gyral vs sulcal method and C) inner vs outer sulcal contours. Given that both skull surface area and gyrencephalic indices, calculated using all three methods (Pillay & Manger, 2007), correlate with brain mass across mammals and with each other, we could calculate skull area residuals in comparison to brain mass.

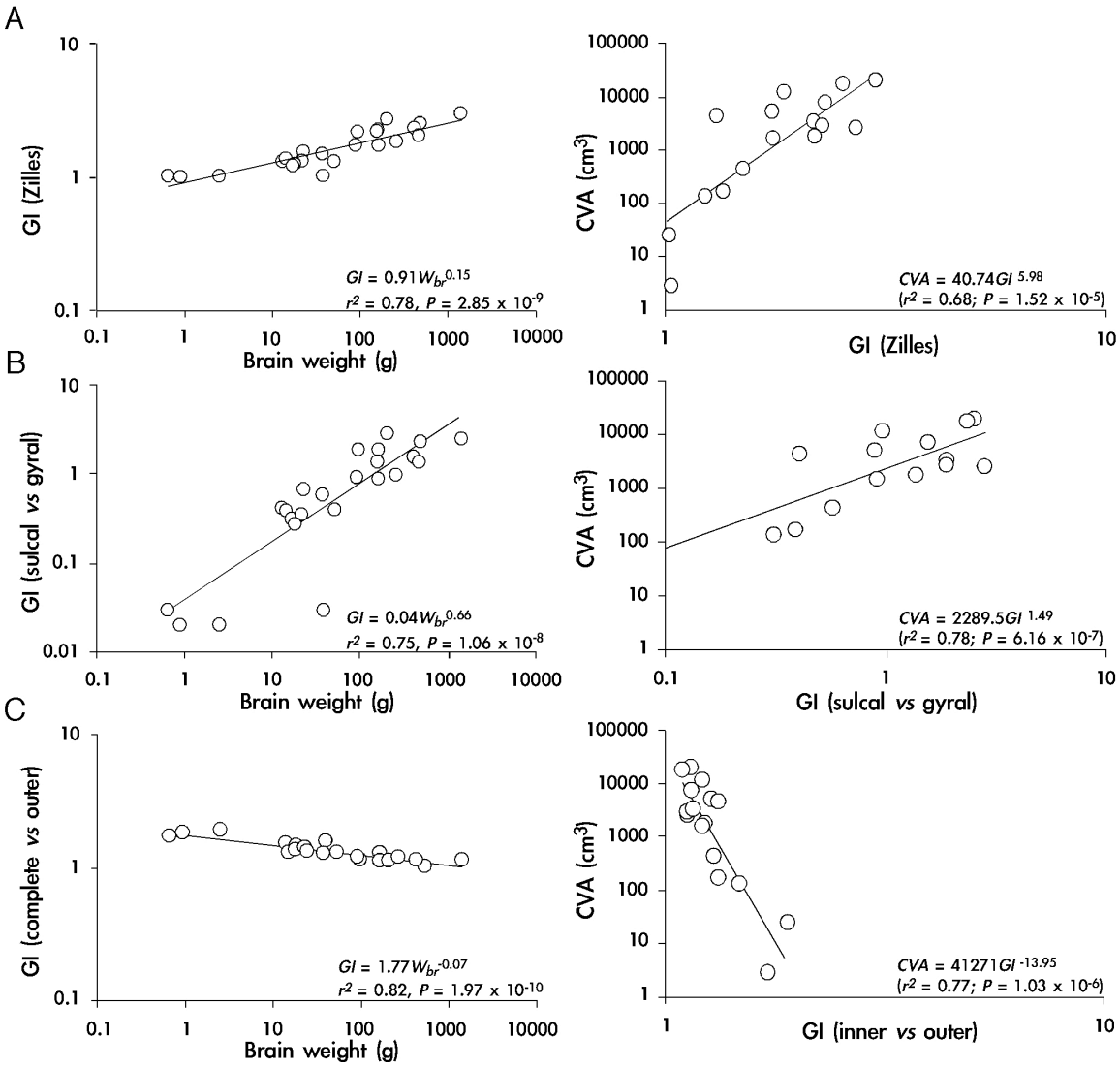
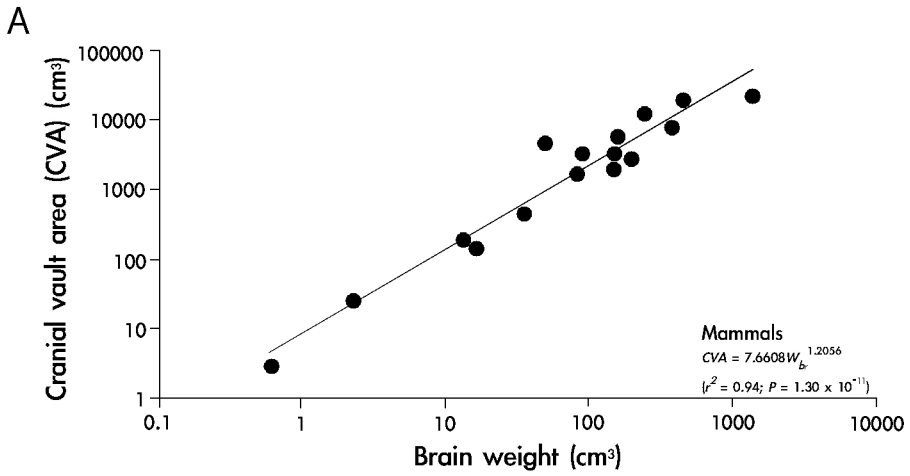
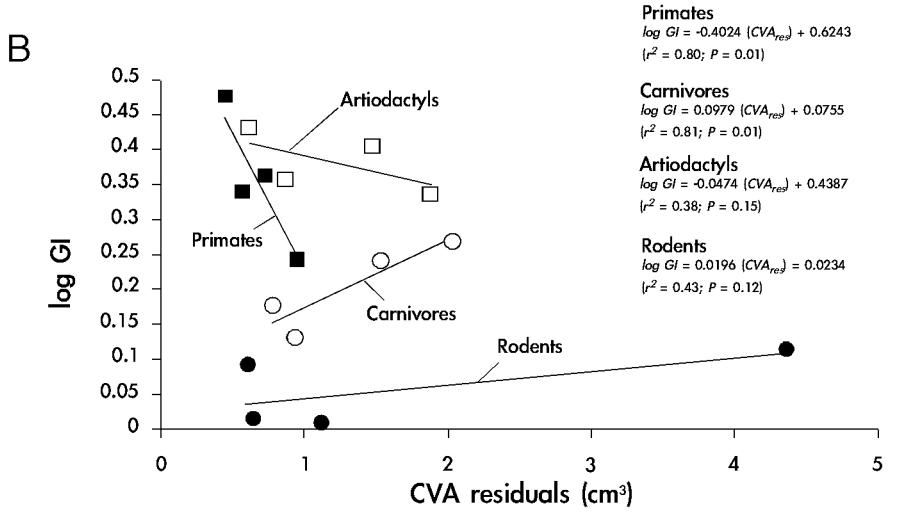

A
GI (Zilles)
Brain weight (g)
GI = 0.91W_br^0.15
r² = 0.78, P = 2.85 x 10^-9
CVA (cm³)
GI (Zilles)
CVA = 40.74GI^5.98
(r² = 0.68; P = 1.52 x 10^-5)
B
GI (sulcal vs gyral)
Brain weight (g)
GI = 0.04W_br^0.66
r² = 0.75, P = 1.06 x 10^-8
CVA (cm³)
GI (sulcal vs gyral)
CVA = 2289.5GI^1.49
(r² = 0.78; P = 6.16 x 10^-7)
C
GI (complete vs outer)
Brain weight (g)
GI = 1.77W_br^-0.07
r² = 0.82, P = 1.97 x 10^-10
CVA (cm³)
GI (inner vs outer)
CVA = 41271GI^-13.95
(r² = 0.77; P = 1.03 x 10^-6)

Figure 3.3: Regression lines and allometric equations calculated for A) all the mammalian species show an increase in the cranial vault surface area (*CVA*) of the skull with increasing brain weight (W_{br}), both measured in cm^3, B) various mammalian orders examined in the present analysis. Note that the primates and artiodactyls show that with increasing gyrencephaly there is a decrease in the cranial vault surface area of the skull. Carnivores and rodents show an increase in the total surface area with increasing gyrencephaly and C) all the mammalian species which indicates a general trend of decreasing cranial vault surface area with increasing gyrencephaly.


A
Cranial vault area (CVA) (cm³)
100000
10000
1000
100
10
1
0.1
1
10
100
1000
10000
Brain weight (cm³)
Mammals
CVA = 7.6608W_b^1.2056
(r² = 0.94; P = 1.30 x 10^-11)


B
log GI
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
1
2
3
4
5
CVA residuals (cm³)
Artiodactyls
Primates
Carnivores
Rodents
Primates
log GI = -0.4024 (CVA_res) + 0.6243
(r² = 0.80; P = 0.01)
Carnivores
log GI = 0.0979 (CVA_res) + 0.0755
(r² = 0.81; P = 0.01)
Artiodactyls
log GI = -0.0474 (CVA_res) + 0.4387
(r² = 0.38; P = 0.15)
Rodents
log GI = 0.0196 (CVA_res) + 0.0234
(r² = 0.43; P = 0.12)

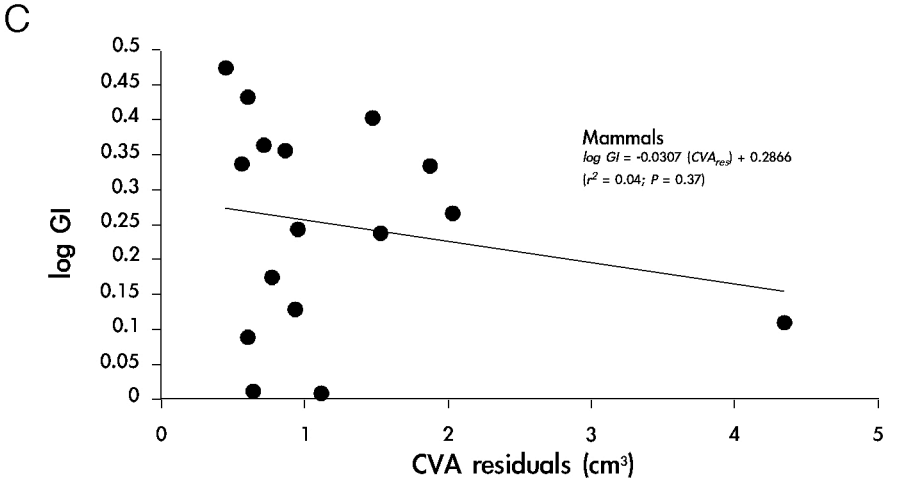
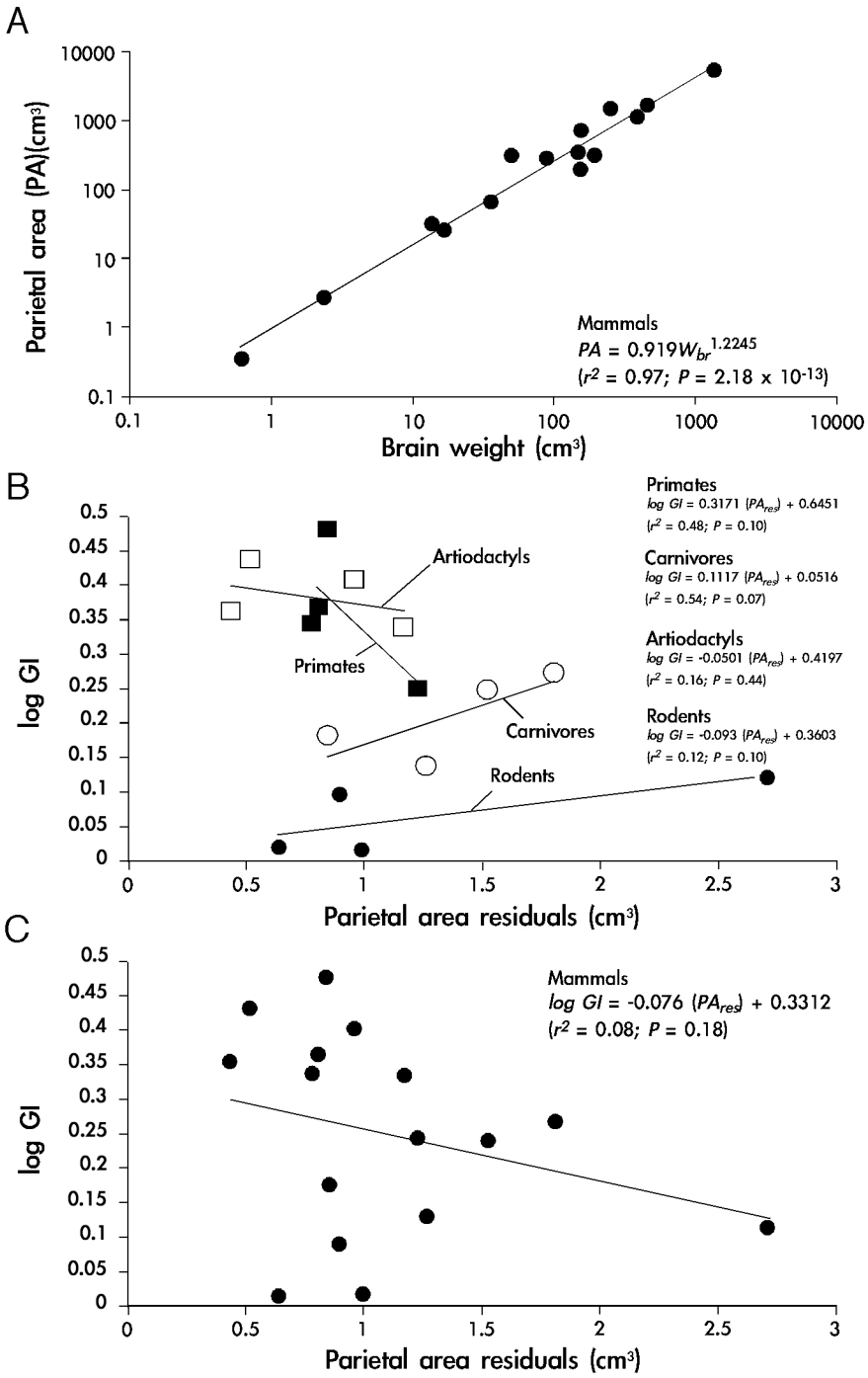

C
log GI
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
1
2
3
4
5
CVA residuals (cm³)
Mammals
log GI = -0.0307 (CVA_res) + 0.2866
(r² = 0.04; P = 0.37)

Figure 3.4: Regression lines and allometric equation calculated for A) all the mammalian species, indicates an increase in the parietal area of the skull with increasing brain weight (W_{br}), B) primates and artiodactyls, using only the parietal area, show an increasing gyrencephaly with decreasing surface area. Carnivores and rodents indicate an increase in the parietal surface area with increasing gyrencephaly as with cranial vault surface area, and C) all the mammalian species indicates a general trend of decreasing parietal surface area with increasing gyrencephaly.


A
Parietal area (PA)(cm³)
10000
1000
100
10
1
0.1
0.1
1
10
100
1000
10000
Brain weight (cm³)
Mammals
PA = 0.919W_br^1.2245
(r² = 0.97; P = 2.18 x 10^-13)
B
log GI
Parietal area residuals (cm³)
Artiodactyls
Primates
Carnivores
Rodents
Primates
log GI = 0.3171 (PA_res) + 0.6451
(r² = 0.48; P = 0.10)
Carnivores
log GI = 0.1117 (PA_res) + 0.0516
(r² = 0.54; P = 0.07)
Artiodactyls
log GI = -0.0501 (PA_res) + 0.4197
(r² = 0.16; P = 0.44)
Rodents
log GI = -0.093 (PA_res) + 0.3603
(r² = 0.12; P = 0.10)
C
log GI
Parietal area residuals (cm³)
Mammals
log GI = -0.076 (PA_res) + 0.3312
(r² = 0.08; P = 0.18)

3.3 Discussion

The present study was designed specifically to test the possibility that the bones of the cranial vault can act as a limiting factor on the expansion of the cerebral cortex in a phylogenetic context. The approach of the present study was to quantitatively determine order specific patterns in the morphological parameters of the bones that make up the cranial vault and investigate how these patterns relate to the index of gyrification (Zilles et al., 1988) determined previously in a range of mammalian species (Pillay and Manger, 2007). As mentioned earlier order specific qualitative and quantitative patterns of gyrification amongst mammals have already been established (Zilles et al., 1989; Welker, 1990; Pillay and Manger, 2007). The results of the present study are conflicted; the primates and artiodactyls appear to conform to the concept that the skull may be a passive limiting factor on cortical expansion, by showing that a smaller relative skull area is correlated to an increased gyrification index. On the other hand, our results for carnivores and rodents demonstrate the opposite trend, indicating that the skull and cerebral cortex are two independent morphological entities that do not interact, even passively.

3.3.1 Methodological Issues

The central methodological concern encountered with the approach used in the current study is the employment of the measurement of the outer skull surface area for comparison with gyrencephalic indices. Firstly by taking into account only the outer surface area of the skull we discount the role that the thickness of the bone may play in

the process of gyrification and the difference between the outer and inner surface areas (the inner of course should be smaller). Despite this, the relationship between the inner and outer surface areas of the skull should be in direct proportion, thus while our actual figures may be slightly skewed, the potential relationships demonstrated should be consistent. Further to this, the outer surface is not in direct contact with the brain as is the internal surface of the skull. It is clear from the impressions of the gyri and sulci apparent on the inner surface of the skull that there exists some sort of interaction, the inner surface of the skull and the cerebral cortex form the cranio-cerebral interface (Tobias, 1994). The major reason why we didn't use the inner surface area of the skull is that to measure it accurately would require invasive procedures, which would then lead to potential discrepancies in the results as part of the surface may be damaged. Moreover, invasive procedures were excluded from the study as the skulls used were museum specimens, and could not be damaged. Thus, we used the outer surface of the skull, which when compared to the weight of the brain showed a highly significant regression across all mammalian orders (see fig 3.3A). It may be that the inner surface area shows a stronger relationship to brain weight, but given the present results, our use of the outer skull surface area may not lead to major errors, but we do acknowledge this potential confounding factor.

A final potential error is the use of different individual animals in the study, i.e. we did not use the same animal for measurements of gyrencephalic indices (the GI's used being reported previously, Pillay and Manger, 2007) and skull surface areas. However, to obtain GI's from the same animals would require removal of the brain from the skull and thus destruction of the skull in order to obtain GI, thereby eliminating the possibility

to determine inner or outer skull surface areas. Of course imaging techniques such as MRI or CT scanning may overcome these problems, but the lower resolution obtained using these techniques brings about a differing suite of confounds to the study. Despite these potential problems with the study, we believe that the results obtained do provide useful information, which while they may not be considered definitive, are certainly instructive.

3.3.2 The Hypothesis Tested

We initially began the current study with the working hypothesis that in a phylogenetic context, the skull would act as a passive limiting factor on the expansion of the cerebral cortex, and as such would be a major contributing factor to the degree of cortical gyrification. Our results show that the total external surface area of both the skull and the parietal bone demonstrate strong correlations with brain mass. This finding allowed us to assume that the total external surface area is equivalent to the internal surface area, keeping in mind the limits that have been stated earlier. In previous studies of gyrencephalic indices in a phylogenetic context (Zilles et al., 1989; Pillay and Manger, 2007) we see that gyrencephaly is strongly correlated to brain mass, such that larger brains exhibit increased gyrification albeit in an order specific manner. Given that both skull surface area and gyrencephalic indices correlate with brain mass across mammals and with each other (Fig 2), we could calculate skull area residuals in comparison to brain mass to specifically test our working hypothesis. The residual values (R) from the plot of surface area *vs* brain mass across all mammals were calculated, and if indeed the skull surface area acted as a limiting factor on cortical expansion, and thus increasing the

amount of gyrification, then those species with skull surface area residuals less than one should show higher gyrencephalic indices, while those with residuals greater than one should show lower gyrencephalic indices. While previously the relationship between the skull and gyrencephaly has been discussed (Clark, 1945; Welker, 1990), this is the first study to attempt to quantitatively relate skull surface area to gyrencephaly.

3.3.3 Conflicting Findings: Primates and Artiodactyls Vs Carnivores and Rodents

The analysis undertaken here has shown that the skull may potentially be considered as a limiting factor on the expansion of the cerebral cortex for the primates and artiodactyls. As the relative size of the surface area of the cranial vault decreases the number of folds and fissures seem to get more complex, i.e. there is increased quantitative gyrencephaly in these two mammalian orders. However, we found conflicting results for the carnivore and rodent orders, such that the amount of gyrencephaly exhibited by the cerebral cortex and the relative size of the cranial vault may be considered to evolve as two separate entities, i.e. the relative surface area of the skull appears to have no effect on the quantitative extent of gyrencephaly. For both these mammalian groupings, these findings are systematic for the relative size of both the total surface area of the skull and parietal bone. Despite these contradictory results, we did find that there is a general trend amongst the mammals that suggests the relative surface area of the skull could be a limiting factor on cortical expansion and thus enhance, albeit passively, the quantitative development of the fissures and folds of the cerebral cortex. This trend however, displays a weak predictive capability between the two variables (GI and residual surface area) and is not statistically significant, thus no definitive

conclusions can be reached. However, the trends and patterns specific to each order are clearer, and this suggests that clues to an underlying relationship between the extent of gyrification in the different mammalian brains and the morphology of the bones of the cranial vault might be specifically related to the orders.

We have previously established that there are order specific patterns amongst mammals in terms of gyrification (Pillay & Manger, 2007). It would seem that the order is a significant phylogenetic grouping (Manger, 2005) in terms of this specific neural parameter, from which we can predict with a reasonable degree of certainty, the GI of any species of a particular order, if we know the brain weight. We proposed in the beginning of this study that to account for the degree of gyrification in the different orders we would need to establish that order specific patterns in the structure of the bones of the cranial vault correlated strongly with quantitative measures of gyrencephaly. It would seem, from the results obtained, that this proposal may hold true for certain orders, such as the primates and artiodactyls, but not for carnivores and rodents.

What is becoming clear from this study and our previous study (Pillay and Manger, 2007) is that any attempt to understand the evolutionary patterns and causes of gyrification in mammals must do so at an order specific level. Our current attempt to identify a causal factor for gyrification at the class level by investigating relationships at the level of the order, while ultimately not successful, does establish the appropriate phylogenetic level of relevance to revealing potential causes. Comprehensive comparisons across mammals at the class level, such as that by Welker (1990), would appear to have difficulty in extracting pertinent information. Moreover, the causes

contributing to the quantitative extent of gyrification across mammals may be multifactorial, and more parameters may need to be included in the analysis to arrive at an answer. For example, our previous study showed that the thickness of the cerebral cortex strongly influenced the extent of cortical gyrification, where thinner cortices were more gyrencephalic (Pillay and Manger, 2007). Inclusion of such factors as cortical thickness and bone surface areas in a multifactorial analysis at the order level may reveal an answer to this currently problematic class level phenomenon.

C H A P T E R 4

4.1 Discussion

The aim of the present study was to attempt to find clues leading toward an explanation as to why sulci and gyri form, how they form, and how and why they vary across species. This problem was investigated by looking within the orders in an attempt to uncover patterns that might help to understand the variation in gyrification patterns and quantities across mammalian species.

In the first phase of the present study it was proposed that within the various mammals studied we might observe order specific, quantitative patterns of gyrification. This proposal was clearly supported, in that it was seen that with increasing brain size in the differing mammalian orders, there was an increasing and predictable quantifiable gyrencephaly. Thus, the first part of the hypothesis in the first phase of the project can be confirmed, i.e. within a mammalian order the larger the brain, the greater the quantitative extent of the gyrification of the cerebral cortex. The second finding in the first phase of this project indicates that within each mammalian order there is a specific allometric relationship between brain size and the extent of gyrification that is significantly different from the other orders examined. In each order it can be seen that for each doubling of brain weight there is a correlating increase in the amount of gyrification although, at a varying rates. For example, in primates it was shown that for every doubling in brain weight, there was a 113% increase in the GI (calculated using Zilles' method), while for

carnivores, for every doubling in brain weight, there was a 108% increase in the GI. In all four orders, the GI scaled faster than brain weight.

Thus, the second part of the hypothesis can be confirmed, in that brain mass and GI do follow an allometric form of scaling in each order. Lastly the results show that quantitative measures of cortical gyrification have a strong predictable scaling within the orders which does not apply across the mammalian species. In each order the largest brains were the most gyrencephalic, however, as previously suggested by Welker (1990), the most "differentiated" brains were not the most gyrencephalic when compared across mammalian orders. In conclusion this part of the study established that there are predictable order specific quantitative patterns of gyrification in mammals.

Another interesting finding was that the artiodactyls were the mammals with the most gyrencephalic brains, these species being more significantly gyrencephalic than all other mammals when species of similar brain weights are compared. This was at first a surprising finding, but the three methods used to measure gyrification indices (GI) all revealed the same result, and have led to a possible explanation for this. The results comparing the outer and inner cortical surfaces show that the artiodactyls have the thinnest cerebral cortex. Having a thinner cerebral cortex will allow for easier mechanical buckling and will also reduce in size the "gyral window" needed for the cortical afferents and efferents as indicated in the earlier study of Prothero and Sundsten (1984). Both these features will therefore result in a more gyrencephalic cortex.

The findings from this part of the study 1) provide the first description of order specific patterns of GI across mammalian orders, in agreement with Zilles' (1989) study in primates, and in agreement with overall order specific patterns of gyri and sulci

(Welker, 1990); and 2) demonstrate that the methods used allow insights into extents of gyrification, i.e. thinner cerebral cortices show higher GI values than thicker cortices. These comparative observations may allow insights into specific alterations accompanying human neural deformations such as agyria and polymicrogyria (Welker, 1990).

In the second phase of the study the underlying mechanisms involved in the development of gyri and sulci in mammals were investigated. It was proposed that clues to the extent of gyrification might be related to the surface area of the bones that make up the cranial vault, such that to account for the degree of gyrification in the different orders, an order specific pattern in the structure of the bones of the cranial vault needed to be established. The major methodological problem encountered in this phase dealt with the thickness of the skull bone. By taking into account only the outer surface area of the skull the role that the thickness of the bone may play in the process of gyrification and the difference between the outer and inner surface areas (the inner of course should be smaller) was discounted. The results derived from measurements made on skulls from seventeen mammalian species show that the total external surface area of both the skull and the parietal bone demonstrate strong correlations with brain mass. This finding allowed the assumption that the total external surface area of the skull is equivalent to the internal skull area to be made.

Findings from phase one of the project indicate that gyrencephaly is strongly correlated to brain mass, such that larger brains exhibit increased gyrification albeit in an order specific manner. Given that both skull surface area and gyrencephalic indices

correlate with brain mass across mammals and with each other, allowed the calculation of skull area residuals (that amount of the external surface area of the skull in comparison to brain mass) to specifically test the working hypothesis that the degree of gyrencephaly is correlated to the amount of space available for expansion of the developing brain. The residual values (R) from the plot of surface area *vs* brain mass across all mammals were calculated, and any residuals that were greater than 1 indicated more surface area of skull (or parietal bone) than expected for brain mass in comparison to the other mammals. If the residual was equal to 1, then the surface area of skull (or parietal bone) was what would be expected for brain mass compared with the other mammals, and if the residuals were less than 1, then less surface area of skull (or the parietal bone) was found than expected for the brain mass in comparison to the other mammals studied. It then follows that those species with higher skull residuals should show lower GIs and those with smaller skull residuals should have higher GIs.

The results yielded from this analysis suggested that the cranial vault may act as a passive limiting factor on the expansion of the cerebral cortex for two of the four mammalian orders investigated: primates and artiodactyls, in that those primates and artiodactyls with smaller GIs had higher skull residuals, or more room available for the expansion of cerebral cortex, and those primates and artiodactyls with higher GIs had smaller skull residuals, indicating a limitation on the space available for expansion of the cerebral cortex. However, this scenario does not hold true for the carnivores and rodents. Despite these contradictory results, a general trend amongst the mammals that suggests the relative surface area of the skull could be a limiting factor on cortical expansion was found, but this trend displays a weak predictive capability and is not statistically

significant. Thus, no definitive conclusions could be reached regarding the possible influences of the skull on the growth of the brain. It is clear that there are trends and patterns specific to each order; this is in accordance with the second part of the second hypothesis which suggested that order specific patterns might emerge. It also suggests that clues to an underlying relationship between the extent of gyrification in the different mammalian brains and the morphology of the bones of the cranial vault might be specifically related to the orders.

From the findings of the current study it would seem reasonable to conclude that the order is a significant phylogenetic grouping in terms of quantifying cerebral cortical gyrification, and that the GI of any species of a particular order can be predicted with a reasonable degree of certainty, if the brain weight is known and the GI of other species of that order have been previously established and compared to brain weight. In the second phase of this study the possible influence of the skull on the growth of the brain was shown to be inconclusive. For the primates and the artiodactyls it seemed that the skull was indeed a limiting factor on the expansion of the cerebral cortex, i.e. as the relative size of the surface area of the cranial vault decreases the number of folds and fissures seem to get more complex. This was however, not the case for the carnivores and rodents. It therefore seems that other factors could be involved in determining the extent of gyrification across and within mammalian orders and it may be that more parameters, such as cortical thickness, may need to be included in the analysis to arrive at an answer. It is clear from both phases of this study that there are specific allometric trends and patterns specific to each order. From the first phase of the study each mammalian order

exhibited its own individual and allometrically predictable pattern of cortical gyrification; also each order specific relationship was statistically different from the other orders. In the second phase of the study each order also exhibited its own specific patterns and trends suggesting that clues to an underlying relationship between the extent of gyrification in the different mammalian brains and the morphology of the bones of the cranial vault might be specifically related to the orders. What is becoming clear from this study is that any attempt to understand the evolutionary patterns and causes of gyrification in mammals must do so at an order specific level (Manger, 2005). As mentioned before making comparisons across mammals at the class level may not reveal significant relationships that are needed to determine the underlying mechanisms involved in the phylogenetic variance and evolution of gyri and sulci of the cerebral cortex. The current attempt to identify a causal factor for gyrification by investigating relationships at the level of order, while not ultimately successful, does establish the appropriate phylogenetic level of relevance to revealing potential causes.

Some theories suggest that the bony braincase is responsive to the size of the growing brain, not a constraint on it. Ogle et al. (2004), from their experiments on rats suggest that tissue interactions with the underlying dura mater participate in suture patency and bone morphogenesis in the overlying neurocranium. Other intrinsic forces such as gyrogenesis within the cortex are also believed to be the primary forces in fold formation (Harrison et al, 2002; Welker, 1990; Barron, 1950). However, as mentioned before these suggestions for the causal factors of gyrification are done without adding any explanatory value to the problem of phylogenetic variance of gyrencephaly. It may be that the processes taking place in the brain of the rat or monkey might not apply across mammals in general. Thus,

the approach taken in this study has been to investigate potential causes of gyrification at an order specific level, to better understand evolutionary patterns and variation in gyrification amongst mammals and this has led to clues regarding the variance in gyrification across mammals that can't be derived from experimental studies specifically focused on just a few species. The central finding, of quantifiable order specific patterns of cortical gyrification, highlights the comparative approach as an appropriate avenue of exploration.

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