

**CONSTRAINTS VERSUS ADAPTATIONS AS CONTENDING
EVOLUTIONARY EXPLANATIONS OF MORPHOLOGICAL STRUCTURE:
THE GIRAFFE (*GIRAFFA CAMELOPARDALIS*) HEAD AND NECK AS A
HEURISTIC MODEL**

Ludo Nlambiwa Badlangana (BSc. - *cum laude*, MSc.)

**A thesis submitted to the Faculty of Science, University of the Witwatersrand, in
fulfilment of the requirements for the degree of Doctor of Philosophy.**

Supervisor: Professor P.R. Manger

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DECLARATION

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

(Signature of candidate)

_____ day of _____ 2007

ABSTRACT

The current study uses the head and neck of giraffe (*Giraffa camelopardalis*) as a model for tracking the course of evolutionary change. Gould (2002) has argued that there are three main avenues of evolutionary change that result in the genesis of new morphologies. These are phylogenetic constraints, structural or allometric scaling laws of form, and specific unique adaptations. It is well known that the unique characteristic of the giraffe is its extremely long neck and yet, it only has seven cervical vertebrae. To study the neck the vertebral body lengths of different aged giraffes were measured to determine the contribution of the cervical vertebrae to the total vertebral column. The vertebrae of several extant ungulates as well as those of fossil giraffids were used as a comparison with the giraffe. CT scans were used on several giraffe skulls to study the extent of the frontal sinus in the giraffe in an attempt to explain why the giraffe evolved such a large frontal sinus. The vertebral columns and skulls of several ungulates, including the okapi (*Okapia johnstoni*) were also used to compare with the results obtained from the giraffe. Immunohistochemistry was used to study the medulla and spinal cord sections of the giraffe to determine if the location and size of the nuclei remained unchanged to the basic ungulate or mammalian plan in spite of the unusually long neck, or if this long neck led to changes in the nuclei found in those regions. The results of these stains were all compared to the published literature available. Although more studies need to be conducted on other ungulates to conclusively determine why giraffe have evolved a long neck, overall the results showed that the anatomy giraffe head and neck remained true to the basic mammalian plan, with very little changing in terms of its morphology. The giraffe brain and spinal cord also resembled that of a typical ungulate. This leads to the conclusion that constraints and allometric scaling laws of form play a greater role than previously thought in the evolution of extreme morphologies.

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1. CHAPTER 1: Introduction

The question as to why the giraffe (*Giraffa camelopardalis*) evolved its extraordinarily long neck is an old one. Today, the giraffe remains one of Africa's iconic mammals as it continues to intrigue people all over the world with questions about how its unusually long neck came to be and what is its possible advantage over a shorter one. Darwin (1872) proposed that giraffes with longer necks were favoured in terms of survival in periods when food was scarce as they were able to reach treetops and browse more productively than their short-necked conspecifics (even though extant giraffe, especially females, do not always stretch their neck when browsing, Mitchell and Skinner, 2003; Cameron and du Toit (2005) also observed that male giraffe “forage at lower neck angles” when there was a larger bull in the same area.) Consequently, Darwin surmised that the giraffe with slightly longer necks tended to “leave more surviving offspring that inherited their genetic propensity for greater height” (Gould, 1996). Hence, Darwin viewed the neck as an adaptation specifically for food acquisition, selected for over many generations.

Kodric-Brown and Brown (1984) on the other hand proposed that sexual selection could be a possible explanation of why the giraffe evolved a long neck. Sexual selection is described as, “that special form of natural selection which is responsible for the evolution of traits that promote success in competition for mates” Kodric-Brown and Brown (1984). Studies (Kodric-Brown and Brown, 1984) show that female giraffes prefer males with longer necks as mates, and in a herd the dominant males are usually those with the longest necks (they also happen to be the largest males). In the presence of oestrous females, males will engage in combat known as “necking” whereby two males stand side by side and exchange blows to the head by swinging their

heads towards their opponents (Simmons and Scheepers, 1996). The winner of these mating contests, usually the dominant male (i.e. the one with a longer neck), stands a better chance of siring offspring that bear his genes. This “necking” behaviour is also observed in the Okapi (*Okapia johnstoni*), the giraffe’s closest living relative, although the Okapi neck never reached the heights of that of its cousin. Thus, longer necks, sexually selected for in a microevolutionary scenario is another suggestion forwarded.

Cameron and du Toit (2003) suggest that the giraffe’s “elongated body form” has been selected for to gain browsing advantage over their smaller competitors as they can feed on more foliage that is out of the reach of the other browsers such as impala and kudu.

These suggestions still do not however, provide a clear rationale indicating why specifically the neck of the giraffe became so long. For example, why didn’t natural selection choose those giraffe with longer legs – surely longer legs would allow them to reach the tops of trees and browse food unavailable to others during lean periods? If sexual selection was the driving force in the giraffe evolving a long neck, it does not explain why the females also have long necks. There are many examples of clearly sex specific morphologies that arose through sexual selection, but these are specific to the male sex and in fact are often considered a hindrance to survival of the individual. This does not appear to be the case for the giraffe. Thus, we are left in the position where an understanding of giraffe neck length evolution is yet to be reached.

1.1 Definition of evolutionary constraints

Traits that are non-changing or remain constant across numerous species may be thought of as constrained in their evolution. A change in such a

trait might result in some form of “handicap” through additional actions of the genes that mutate to provide the platform for the development of the novel trait. These differences in gene expression and effect (i.e. pleiotropy – the production by a single gene of unrelated effects) may limit an animal’s ability to survive in its natural environment and may even lead to lethal phenotypes (Galis, 1999). The constrained feature forming the central platform of the current study is the seven cervical vertebrae in the giraffe neck. All mammals, with the exception of three genera have seven cervical vertebrae. The exceptions are: manatees (*Trichechus*), which have six cervical vertebrae, two-toed sloth (*Bradypus*), which have nine or ten cervical vertebrae and the three-toed sloth (*Choloepus*), which possess only six cervical vertebrae. This variation in cervical vertebrae number is associated with neurologic and metabolic anomalies in these animals compared to other mammals. Galis (1999) avers that *Hox* genes play a vital role in axial skeletal and nervous system development, involving thousands of genes. A change in the developmental patterning of these genes in mammals results in “an increased risk for neural problems, neonatal cancer and still births” (Galis, 1999). Thus, it can be concluded that seven cervical vertebrae, as seen in the giraffe, is a constraining feature of mammalian evolution.

1.2 Definition of adaptation

An adaptation is a morphological or physiological change in a particular structure that has occurred in order to allow a specific species to survive successfully in a given environment. These adaptations are unique to a species. Dorland’s Illustrated Medical Dictionary (30th Edition) describes adaptation as, “the adjustment of an organism to its environment, or the process by which it enhances such fitness”. Thus, if the giraffe neck is to be considered

as an adaptation, the current study must lead to the conclusion that the long neck is unique to the giraffe. But overall length of the neck is but one level of organization in the morphology of the giraffe. The length of the neck is achieved under the constraint of possessing the standard mammalian feature of seven cervical vertebrae. Are there other features that can be considered morphological adaptations in the anatomy of the giraffe neck, or do all the features, at all levels of organization represent secondarily elongated forms of what is seen in a typical ungulate? Most simply put, is the giraffe a version of a radically stretched sheep?

Currently it is unclear what has led to the evolution of the long neck in the giraffe or how it occurred – was it a microevolutionary response to a specific evolutionary pressure, or was it some random genetic mutation that was passed rapidly through a small population of giraffe forebears that were not eliminated through natural selection? We do not know which features in the giraffe head and neck conform to a generalized mammalian or ungulate pattern, and what are unique. For example, we know the giraffe has seven cervical vertebrae, a typical mammalian feature, so can we conclude that the cervical skeletal system of the giraffe is a constrained feature, or do we classify the lengthening of the cervical vertebral bodies as an adaptation unique to the giraffe? It is this paradox of evolutionary biology that forms the platform for this study.

1.3 Aims

The two main aims of this thesis are: (1) to better understand and further our knowledge on the evolutionary processes that result in extreme morphologies in mammals using the giraffe head and neck as a model; and (2) to determine if morphological systems within the head and neck, such as the

osteology and neuroanatomy, stay true to the basic mammalian or ungulate morphotype (constrained) or show features unique and specifically related to the giraffe (adapted).

1.4 Hypothesis

We hypothesize that much of what will be found in the giraffe head and neck will be elongated typically ungulate (1st level of morpho-constraint) or mammalian structures (2nd level of morpho-constraint). There may, however, be some anatomical features that are unique to the giraffe and be the result of it evolving an unusually long neck (evolutionary adaptation).

1.5 Individual Chapters

1.5.1 Chapter 2: The Giraffe Cervical Vertebral Column: a Heuristic Example in Understanding Evolutionary Processes?

Chapter 2 is a study of the giraffe vertebral column with particular focus on the total contribution of the cervical vertebrae to the entire vertebral column in comparison to several other ungulates as a heuristic example in understanding evolutionary processes. The cervical vertebrae are the bones responsible for providing the length of the giraffe's neck. While most authors (e.g. Colbert, 1938; Mitchell and Skinner, 2003; Narita and Kuratani, 2005) agree that the giraffe has seven cervical vertebrae, has Solounias (1999) argued that the giraffe have eight cervical vertebrae and that the first thoracic vertebrae (T1) should be characterised as cervical (C8, which Solounias (1999) names V8) because of its morphology and that an "extra" cervical vertebra has been inserted somewhere between C2 and C6. The aim is to determine which osteological features are

constrained and what are adaptations in the giraffe vertebral column, in comparison to other ungulates. Vertebral columns of the giraffes varying in age from newborn to adult will be studied in order to understand potential evolutionary scenarios leading to the present phenotype. These are compared against similar measurements in several other ungulate species including two representative of the Camelidae which are also seen to have longer than “normal” necks.

1.5.2 Chapter 3: The Frontal Sinus of the Giraffe Skull

Chapter 3 examines the atypically large frontal sinus of the giraffe skull with the aim of proposing possible reasons why the giraffe evolved such an extensive frontal sinus. Mitchell and Skinner (2003) suggest that a large frontal sinus is essential in long necked animal as it lightens the mass of the skull. Thus, according to Mitchell and Skinner (2003), it is likely that the extensive frontal sinus in the giraffe came about as a result of the animal elongating its neck. Another suggestion is that the sinus aids in cooling the brain (Ganey, *et al*, 1990; Mitchell and Skinner, 2003). Several authors (Ganey, *et al*, 1990; Mitchell and Skinner, 2003; Colbert, 1938; Churcher, 1976) have examined the anatomy of the giraffe frontal sinuses from as early as 1938 where Colbert mentions that they are “extremely large... [and are found] above the brain and [extend] into the occipit.” (Colbert, 1938). Churcher (1976) also mentions that the giraffe has a “highly developed sinus”.

Despite these observations, no quantification of this expansive sinus in the adult or developing giraffe has been undertaken. Moreover, the extent to which this sinus in the adult giraffe is “extremely large” has not been quantified in comparison to other ungulate species. Skulls of giraffe of varying age (from

newborn to adult) are studied and compared to several other ungulates in order to understand potential evolutionary scenarios leading to the present phenotype and what the function of this phenotype might be in the giraffe.

1.5.3 Chapter 4: The Catecholaminergic and Serotonergic Systems of the Medulla of the Giraffe

Chapter 4 provides an immunohistochemical study of the catecholaminergic and serotonergic systems in the giraffe medulla oblongata in an attempt to see whether they resemble those of other ungulates previously investigated. The specific nuclei forming the catecholaminergic systems normally observed in the medulla of mammals are the rostral ventrolateral tegmental group (C1), the caudal ventrolateral tegmental group (A1), the rostral dorsomedial group (C2), the caudal dorsomedial group (A2), and the area postrema (Smeets and Gonzalez, 2000). All serotonergic neurons of the medulla, the caudal serotonergic cluster, project to the spinal cord (Tork, 1990). There are normally five serotonergic nuclei found in the medulla which are: the raphe magnus nucleus (RMg), raphe pallidus nucleus (RPa), raphe obscurus nucleus (ROb) and the rostral ventrolateral (RVL) and caudal ventrolateral (CVL) groups (Tork, 1990; Bjarkam, 1997), all of which are found in sheep (Tillet, 1987).

The aim of this study is to establish if there are any structural changes in the nuclei of the medulla, as a result of the giraffe possessing a long neck. The giraffe phenotype may pose a challenge to neurons that must project over a large distance in order to maintain their specific functions in the spinal cord and this may cause changes in the morphology and organization of these neurons. For example, all the serotonergic neurons project to the spinal cord. In the

catecholaminergic system, the C1 neurons also project to the spinal cord (Smeets and Gonzalez, 2000), and the neurons of area postrema are involved in blood pressure regulation. It is of interest to examine the medullary nuclei of these systems to reveal if they demonstrate structural differences specific to the giraffe and whether they resemble those found in other ungulates.

1.5.4 Chapter 5: The Corticospinal Tract of the Giraffe: an Immunohistochemical Study

Chapter 5 is an examination of the cortico-spinal tract of the giraffe. This tract contains motor axons that travel from the cerebral cortex to the spinal cord and whose main function is thought to be the mediation of voluntary movement. This study aims to determine the gross structure and anatomical relations of the spinal cord and vertebral canal in relation to gross and micro-anatomical structures that comprise the cortico-spinal tract of the giraffe. This information will be used to determine such features as the maximal axonal length of the cortico-spinal axons of giraffes. In the primary motor cortex of primates, the corticospinal tract originates at the Betz cells, which are gigantopyramidal cells found in layer 5 (Sherwood, *et al*, 2003). It has been shown that the Betz cells that project to a greater distance (e.g. to the lumbar spinal cord) are larger than those that project to say the cervical spinal cord (Murray and Coulter, 1981; Sherwood, *et al*, 2003). One might predict that the morphology of the giraffe neck may lead to potential alterations of the form of the corticospinal tract. For example, it may be possible that the giraffe will have enormous giant pyramidal cells, akin to Betz cells, correlating with the long axons required to reach the end of the spinal cord as is seen in primate cortex (Sherwood *et al.*, 2003). Alternatively, the giraffe may exhibit a

morphology of this neural pathway that is consistent with that seen in other ungulates. For example, within the primary motor cortex of the sheep (Ebinger, 1975) no Betz cells are present, but clusters of large pyramidal neurons, presumably the origin of the corticospinal pathway are seen. Does the giraffe follow order specific patterns of morphology (Manger, 2005), or does it exhibit a unique morphology?

1.6 Summary

The current study uses the head and neck of giraffe as a model for tracking the course of evolutionary change. Gould (2002) has argued that there are three main avenues of evolutionary change that result in the genesis of new morphologies. These include the phylogenetic constraints, structural or allometric scaling laws of form, and specific unique adaptations. Gould further argues that the first two avenues have the highest relative frequency of occurrence in terms of evolutionary change and that adaptation occurs the least often, i.e. new structures are most often built within the boundaries of phylogenetic history and according to changes that scale with such parameters as body size. In Gould's view, adaptation, or the evolution of a completely new morphology, occurs relatively rarely in evolution. Added to this, changes can occur at various levels of organization within a particular organism, or part of an organism. For example, it might be said that the overall length of the giraffe neck is an adaptation, but when one looks at the osteological level of organization, the class level phylogenetic constraint of seven cervical vertebrae prevails. Thus, in order to fully understand the evolution and current morphology of any organism or part thereof, all three potential avenues of evolutionary change, at all levels of morphological organization, must be

examined. These changes, or lack of changes, must then be related to phylogenetic levels of relatedness to see whether they correspond to species-specific features (adaptations), or whether they are common to a group of species that form for example, an order or a class (constraints) (Manger, 2005). Viewed in this sense, the neck of the giraffe becomes an incredibly interesting model in terms of understanding morphological evolution. While the current studies only approach some aspects related to the long neck of the giraffe, these are aspects where enough comparative data is available, or can be generated, to make the types of comparisons within the proposed evolutionary framework. It is thought that these types of studies will ultimately lead to a detailed understanding of the evolution of the giraffe neck, something that will help us understand the life history of the extant species and also, in a broader sense, aid in the understanding of evolutionary processes in general.

Chapter 2: The Giraffe (*Giraffa camelopardalis*) Cervical Vertebral Column: a Heuristic Example in Understanding Evolutionary Processes?

2.1 Introduction:

The length of the neck of the giraffe is an example often used in evolutionary theory, with explanations ranging from Lamarckism to microevolutionary adaptation to sexual selection being proposed to account for the origin and persistence of this unique structure. Darwin (1872) proposed that giraffes with longer necks were favoured in terms of survival in periods when food was scarce as they were able to reach treetops and browse more productively than their shorter necked conspecifics. Consequently, they tended to “leave more surviving offspring that inherited their genetic propensity for greater height” (Gould, 1996). Hence, Darwin viewed the neck *in toto* as an adaptation, selected for over many generations within a microevolutionary framework.

In recent years, another microevolutionary proposal arguing for sexual selection has been developed in an attempt to explain why a long neck evolved in the giraffe (Kodric-Brown and Brown, 1984). Kodric-Brown and Brown (1984) describe sexual selection as, “that special form of natural selection which is responsible for the evolution of traits that promote success in competition for mates.” Studies show that female giraffe prefer males with longer necks as potential mates, and in a giraffe herd the dominant males are usually those with the longest necks (which also happen to be the largest animals overall). In the presence of oestrous females, males will engage in combat known as “necking” whereby two males stand side by side and exchange blows by swinging their heads towards their opponent (Simmons and Scheepers, 1996). The winner of

these contests, usually the dominant male (the one with a longer neck), stands a greater chance of siring offspring that bear his genes. This “necking” behaviour has also been observed in the okapi (*Okapia johnstoni*) (Simmons and Scheepers, 1996), the giraffe’s closest living relative, although the neck of the okapi has not reached a length comparable to that of the giraffe.

Despite these proposals, there is no “ultimate evolutionary selection pressure” indicating why the neck of the giraffe is the length currently observed. Cameron and du Toit (2007) have argued that the giraffe’s body been selected for it to gain a better advantage over its smaller browsing competitors. It is well known that females tend to browse at lower tree levels, but males have been observed browsing at lower levels if there is a larger bull in the area (Cameron and du Toit, 2005).

Hox gene patterning in the development of the axial skeleton as proposed by Galis (1999) may help to better explain this elongation in the cervical vertebrae of the giraffe. It is clear that the seven cervical vertebrae, as seen in the giraffe, is a constraining feature of mammalian evolution. However, Solounias (1999) argues that the giraffe have eight cervical vertebrae and that the first thoracic vertebrae (T1) should be characterised as cervical (C8) because of its morphology and that an “extra” cervical vertebra has been inserted somewhere between C2 and C6.

Comparative anatomical observation is one of the original cornerstones of evolutionary explanation. In the current study we use developmental and comparative anatomical observation, aligned with the concepts of phylogenetic and developmental constraints, allometric scaling laws of form, and adaptation (Gould, 2002) as the basis for deriving information of relevance to the evolution of the length of the giraffe neck. We believe that a structure such as the neck of the giraffe could prove to be heuristically valuable in our understanding of

evolutionary processes and play an important role in the development of evolutionary theory.

2.2 Materials and Methods:

Specimens

Vertebral columns of 15 ungulates representing 11 species were used (Table 1). Specimens were obtained from the Comparative Osteological Collection of the School of Anatomical Sciences, University of the Witwatersrand, Johannesburg, South Africa and the Transvaal Museum, Pretoria, South Africa. The specimens included four giraffe, ranging from a newborn to a large adult, two adult okapi, and nine other species representing a reasonable range of body mass and neck length across ungulates. The cervical vertebrae body lengths of five fossil giraffids of *Giraffa sp.*, *Paleotragus primaveus* and *Samotherium africanum* obtained from published data were also used in this study.

Length of Vertebral bodies

The vertebral skeleton of each extant specimen was articulated in order to establish the identity of individual vertebra. The length of each vertebral body, except for the atlas (C1, does not have a body) was measured using GPM Swiss made spreading callipers and the measurements were recorded in millimeters. The dens (of the axis, C2) was included in the measurements because it is the vertebral body of the atlas (Moore, 2006). The length measured was the distance between the highest point on the proximal end and the deepest point on the distal aspect of each vertebra. Each measurement was repeated three times over

several hours to eliminate intra-observer error. The average of the three measurements was used as the vertebral body length. The values were recorded on a Microsoft Excel spreadsheet for later analysis.

We used statistical analysis to determine the correlation and significance of the data collected. Using Microsoft Excel, we plotted the graphs of total vertebral length against individual cervical vertebral length (Fig. 5); total vertebral column length vs. body length (Fig. 6) and normalized vertebral column length vs. body length (Fig. 7) for all the extant specimens studied. Normalized vertebral column length (NVL) was calculated as the total vertebral column length minus the cervical vertebral length. We further plotted the graphs of total vertebral column length vs. body length (Fig. 8) and normalized vertebral column length vs. body length for all the specimens studied including the fossil giraffids (Fig. 8). Linear regression lines were drawn and r^2 values were obtained from the plots. P values (the probability of the data being uncorrelated) were calculated using a Rel8 Java program. For each graph, the data used to plot that graph is entered into a spread sheet within the program which calculates a P value.

2.3 RESULTS

The giraffe typically has seven cervical vertebrae each of which is greatly elongated in length. The cervical vertebrae comprise approximately 50% of the total length of the vertebral column in juvenile and adult giraffes, which is substantially greater than the other extant ungulate species examined, including the okapi, the giraffe's closest living relative. The lengths of the remaining vertebrae (i.e. thoracic, lumbar and sacrum) appear to be

proportioned in a fashion comparable to other ungulates. When the giraffe cervical vertebrae are taken in isolation (i.e. when looking at individual vertebrae), they scale appropriately for cervical vertebrae, in line with other ungulates; however the individual cervical vertebrae of the giraffe scale well above that seen for other ungulates, including the okapi, when compared to the total vertebral column length. A similar situation is seen when the individual cervical vertebrae of the giraffe are compared against a Normalized Vertebral Length (NVL, total vertebral length minus cervical vertebral length) again underscoring the extended length of the giraffe cervical vertebrae. Comparisons of the cervical vertebral lengths of extant giraffes and ungulates with those of fossil Giraffids show a distinction between short and long necked fossil forms.

2.3.1 The giraffe has seven cervical vertebrae

Despite a report by Solounias (1999) indicating that the giraffe has eight cervical vertebrae, our observations, which are corroborated by others (e.g. Mitchell and Skinner, 2003), show that the giraffe indeed has seven cervical vertebrae a feature found in all ungulates examined in the present study, and for all mammals in general except for three genera (Galis, 1999). We compared the osteological features of C2 to C7 of the giraffe to 10 other ungulate species. Our observations showed that their features were typical for cervical vertebrae, the most distinguishing one being the presence of transverse foramina in the transverse processes. Other typical cervical vertebrae observed features were an elongated vertebral body (except C1, which lacks a vertebral body) and short spinous and transverse processes (compared to the thoracic and lumbar vertebrae).

The seventh cervical vertebra, C7, however, is atypical in all vertebrates because its spinous process is longer than that of the other cervical vertebrae (Figs. 1, 2). All the C7 vertebrae of the species studied were morphologically similar except that the giraffe's C7 was longer and more robust. The one unusual feature of the osteology of the giraffe C7 vertebra was that it had very large transverse foramina through which the vertebral arteries pass (Solounias, 1999), and this feature was not seen in the other species studied. Interestingly, the variation of the anatomical location of the transverse foramina is not unique to the giraffe. The transverse foramina of the camel run within the spinal canal of the C6-C2 vertebrae and those of the llama run in the spinal canal of the C5 to C3 vertebrae.

The thoracic vertebrae of the giraffe all show evidence of typical mammalian thoracic vertebrae, the most significant features are the long spinous processes and articulating facets for the ribs (Figs. 1 and 2). The first thoracic vertebra, T1, of the giraffe, however, is atypical in that it has a shorter spinous process than that seen in the other thoracic vertebrae (T2 – T10) examined, and it appears to be a transitional vertebra from the cervical into the thoracic region. The giraffe T1, in spite of this, remains similar to that of the other ungulates in that it exhibits a longer transverse process than that of the cervical vertebrae and lacks the transverse foramina.

2.3.2 Half of the giraffe vertebral column length is comprised of the cervical vertebrae

Our study examined a developmental series of four giraffes ranging in age from newborn (n=1) to juvenile (n=1) to adult (n=2) (exact ages not determined from museum records, so approximate developmental stages given).

The cervical vertebrae of the newborn giraffe occupied approximately 45% of the total vertebral length (Fig. 3, Table 1). In the other three giraffe, the cervical vertebrae comprised 52 to 54% of the total vertebral length (Fig. 3). The total vertebral column length of the giraffe was substantially longer than that recorded in the other ungulates examined in the current study. The llama had the second largest proportion of cervical vertebrae to vertebral column length with the cervical vertebrae occupying 44% of the total vertebral length, followed by the camel with 40% (Fig. 3, Table 1). The cervical vertebrae of the okapi comprised 35% of the total vertebral length and the cervical vertebrae contribution from the remainder of the ungulates examined ranged from 33% to 27%.

2.3.3 Proportions of the remainder of the giraffe vertebral column

It is clear that the length of the giraffe cervical vertebrae is unusual (Figure 3). We therefore examined whether the proportions of the remainder of the vertebral column were similar to the other ungulates examined to allow us to determine whether the giraffe is just a “typical” ungulate with an unusually long neck. To investigate this, we created a Normalized Vertebral Length (NVL). This is the Total Vertebral Length minus the Total Cervical Vertebral Length (Tables 1, 2).

The results show that for the adult giraffe, the thoracic vertebrae occupy, on average, 62% of the NVL. The lumbar vertebrae occupy, on average, 21% of the NVL, whereas the sacrum occupies, on average, 17% of the NVL (Fig. 4, Table 2). Interestingly the thoracic vertebrae of the okapi occupy, on average, 62% of the NVL, the lumbar vertebrae occupy, on average, 21% of the NVL, and the sacrum on average, 18% of the NVL (Fig. 4, Table

2). In the camel, the thoracic vertebrae occupy 55% of the NVL, the lumbar vertebrae 31% and the sacrum 15% (Fig. 4, Table 2) while the thoracic vertebrae of the llama occupy 48% of the NVL, the lumbar vertebrae 38% and the sacrum 13% (Fig. 4, Table 2). The range for the other ungulates is 55-46% for the thoracic vertebrae, 36-26% for the lumbar vertebrae and 20-13% for the sacrum of the NVL (Fig. 4, Table 2).

Thus, apart from the cervical vertebrae, the proportions of the vertebral column in the giraffe and okapi are very similar. In comparison to the other ungulates examined the giraffe is within the normal range of variation observed, although both the giraffe and okapi appear to have a greater proportion of the thoracic vertebrae occupying the NVL. This finding highlights the unusual length of the giraffe cervical vertebrae and also shows the constraint the giraffe non-cervical vertebrae are under as they appear to increase their length in accordance to a specific ungulate pattern (Fig. 4, Table 2).

2.3.4 The relationship of individual cervical vertebrae lengths to total cervical vertebral length

We wanted to determine if the cervical vertebrae were longer than expected for the length of the “typical ungulate” neck and hence contravened a specific ungulate plan.

We compared individual cervical vertebrae lengths of C2 to C7 against the total cervical vertebrae lengths across all the extant species studied (Table 3). We found that all ungulate species examined in the present study, including giraffe, exhibited a linear regression specific for each of the cervical vertebrae (Fig. 5). The vertebral body length of C2 was the relatively longest in all the

ungulate species and C7 was the relatively shortest. The remaining vertebrae were very close in relative length, but the relative lengths decreased in order from C2 to C7. Slopes of the calculated regression lines indicate that C2 increases in length at a faster rate (slope = 0.18), with an increasing total cervical vertebral length, than all other cervical vertebrae. The slopes of the regressions calculated for C3 to C6 were similar, where the slope was approximately 0.17, and C7 showed the shallowest slope of 0.14 (Fig. 5). The conclusion that can be drawn from this comparison is that while the individual cervical vertebrae of the giraffe are long, in terms of the overall length of the neck they are in line with that expected for an ungulate with a neck of this length.

2.3.5 The relationship of individual cervical vertebral lengths to total vertebral length (TVL)

Our results show that the giraffe neck is quantitatively longer than expected as it occupies half of the entire vertebral column, but the individual cervical vertebrae are the length predicted for an ungulate with a neck the length seen in the giraffe, i.e. there is a predictable scaling to the length of the cervical vertebrae across ungulates. Given these two disparate findings, we investigated the relationship between the length of the individual cervical vertebrae and the total length of the vertebral column (TVL) by plotting the length of the individual cervical vertebrae against TVL in all the extant species studied (Fig. 6). Using a range of ungulates (to the exclusion of the giraffe, camel and llama) we found a baseline “ungulate” relationship showing that the lengths of the cervical vertebrae were strongly correlated to the TVL (Fig. 6, Table 3).

All the giraffe cervical vertebrae, although correlated to the TVL, were much longer than would be predicted based on the regression determined from other ungulates. Furthermore, the slopes were steeper, with a range of 0.13 to 0.08 from C2 to C7 compared with 0.07 to 0.05 for the other ungulates. This indicates that the cervical vertebrae in the giraffe elongate more rapidly with increased TVL compared to the “typical” ungulate baseline pattern which includes the okapi (Fig. 6).

Interestingly, the camel and llama show a scaling of the cervical vertebral length that is different to the other ungulates. The C2-C5 vertebrae of the llama fall on or are very close to the regression derived for the giraffe developmental series. For the llama C6, the vertebral length is midway between the giraffe and ungulate regression. For the llama C7, the vertebral length is coincident with the regression line determined for the other ungulates. For the camel, the C2-C6 vertebral lengths fall midway between those of the giraffe developmental series and the ungulate regression. However, the camel C7 vertebral length is found to be very close to the ungulate regression, but remains slightly higher than that of the other ungulates, including the llama (Figure 6).

The results for the camel and llama are concurrent and fall in line with observations of similar cervical vertebrae anatomy described earlier. Perhaps with investigations of other species in the *Camelidae* family, as well as looking at their developmental series, we might find a third type of cervical vertebrae length regression in the ungulates.

2.3.6 The relationship of individual cervical and thoracic vertebrae to the Normalized Vertebral Length (NVL)

We standardized the giraffe vertebral column with respect to the other ungulates by introducing an NVL as describe above (also see Table 2). By removing the elongated cervical vertebral lengths to obtain an NVL, plots of the proportions of the remaining vertebral lengths were found to be similar to the other ungulates (Fig. 4, Table 2). This perhaps may be a more precise way of examining the scaling of the cervical vertebrae in giraffe in comparison to the other ungulate species examined.

The “baseline” regressions determined for the “typical” ungulates (this being all species examined to the exclusion of giraffe, llama and camel) showed strong correlations across all vertebrae measured from C2 to T9. A close examination of the slopes found with these regressions show an interesting phenomenon. For C2, the slope is 0.1, for C3 is 0.08, for C4 and C5 the slope is 0.09, for C6 the slope is 0.08, and for C7 the slope is 0.07. Thus, there is a progressive decrease in the regression slopes as one moves from vertebra C2 to C7, indicating that the length of C2 scales more rapidly with increased NVL than the other vertebrae. For the thoracic vertebrae, T1 showed a slightly higher slope (0.05) than the remaining vertebrae, all of which had a similar slope (0.04) (Fig. 7).

All giraffe cervical vertebrae are longer than would be expected for an ungulate with the NVLs that they have (Fig. 7), but these exhibit strong correlations. When compared to the “baseline” regressions determined for the “typical” ungulate, the slopes of the regression determined for giraffe were steeper. Interestingly, these slopes, while steeper, show a progressive decrease from C2 to C7, where the slope at C2 was 0.31, C3 was 0.25, C4 was 0.23, C5

was 0.24, C6 was 0.23, and C7 was 0.19 (Fig. 7). This progressive reduction in the gradient of the slopes parallels that observed in the regressions of the “typical” ungulates.

For the giraffe thoracic vertebrae, T1, T2 and T3 are longer than predicted for the other ungulates (Fig. 7, Table 3). T1 shows the greatest difference and this lessens through to T4, where the relative length of the giraffe T4 to NVL is indistinguishable from that observed for the other ungulates. The remaining thoracic vertebrae (T5-T9) also exhibit regressions that are indistinguishable from the other ungulates. For the giraffe T1, the slope of the regression (0.08) is far shallower than that seen for giraffe C7 (0.19). Although the slope of the regression for the giraffe T1 is not exactly parallel to that of the regression for the ungulate T1 ungulate, it is still much closer to the ungulate T1 than to the giraffe C7 (Fig. 7). By T2, the regression slope for the giraffe matches that of the “typical” ungulate (slope = 0.04) (Fig. 7). Thus, while the scaling of the cervical vertebral lengths of the giraffe is readily distinguishable from that seen for other ungulates, and there is a small transition zone mostly marked in T1 and T2 of the giraffe - where their slopes become nearly parallel to the ungulate line, at T4 the scaling of the vertebral lengths of the giraffe are indistinguishable to that observed for other ungulates. This once again underscores the difference between the cervical vertebrae of the giraffe and those of the “typical” ungulate.

In the camel and llama, C2-C5 scale higher than expected compared to the “typical” ungulate. Like the giraffe and the other ungulates, the gradient decreases from C2 to C7 (Fig. 7). At C6, there is some moderation in this scaling, where the lengths of C6 are closer to the ungulate regression, and at C7, they are much closer to the ungulates, but still higher than expected. At T1,

the lengths of this vertebra compared with NVL for the camel and llama are indistinguishable from the “typical” ungulate regression (see Figure 7).

2.3.7 Cervical vertebrae of fossil giraffe

We gathered data from the published literature on cervical vertebrae lengths of fossilized giraffids to compare with those determined empirically in the present study for extant giraffe and the range of ungulates examined (Table 4; Churcher, 1970; Harris, 1991). The total cervical and total vertebral lengths are unknown in these species. We assumed that they were adults and used the regressions determined for the “typical” ungulates and giraffes to calculate potential TVL and NVL (Table 4). We then plotted the fossil cervical vertebrae body lengths against the giraffe and ungulate baseline data to see where these calculated results would lie.

For the *Giraffa sp.* all the cervical vertebrae (C2, C5 and C7, see Table 4) came from the same individual. Using the giraffe regression to calculate both TVL and NVL for each of the respective vertebrae, we found that the *Giraffa sp.* scaled in a manner that could be considered to be a normal sized adult long-necked extant giraffe (see C2 plot in Fig. 8) to a slightly larger than expected long-necked adult extant giraffe (see C5 and C7 plots in Fig. 8). On the other hand, when the TVL and NVL were determined from the “typical” ungulate regression, we found that this animal would have to be considered an extremely large ungulate with a typical ungulate neck length (see C2, C5 and C7 plots in Fig. 8). Thus, this particular individual is either a long-necked giraffe or an extremely, and possibly abnormally, large normal-necked ungulate.

The *Paleotragus primaveus* cervical vertebrae analysed (C2 and C6, see Table 4) came from two individuals. Using the giraffe regression to determine possible TVL and NVL, we noted that both these individuals could be considered to be very small (smaller than a newborn extant giraffe) long-necked extant giraffe. Using the ungulate regression to determine possible TVL and NVL, we concluded that these two individuals could be considered to be small to medium sized normal-necked ungulates (see C2 and C6 plots in Fig. 8). Thus, individuals of this species are either very small long-necked giraffe or small to medium sized normal-necked ungulates.

The *Samotherium africanum* cervical vertebrae analysed (C3 and C6, see Table 4) also came from two individuals. Using the giraffe regressions to determine possible TVL and NVL it was decided that both of these individuals were probably around the same size as a newborn giraffe, assuming that they were long-necked (see C3 and C6 plots in Figure 8). Using the ungulate regression to determine possible TVL and NVL reveals that these animals could be medium to large sized ungulates with normal sized necks (see C3 and C6 plots in Fig. 8). Thus, *Samotherium africanum* can be considered to be either a very small long-necked giraffe, or a medium to large sized normal-necked ungulate.

Table 1: Vertebral body lengths of the extant ungulate species studied showing the percent contribution by the cervical, thoracic and lumbar vertebrae.

Species	Total Vertebrae Length	Total Cervical Vertebrae Length (C2-C7)	Cervical Vertebrae %	Total Thoracic Length	Thoracic Vertebrae %	Total Lumbar Length	Lumbar Vertebrae %	Total Sacral Length	Sacral vertebrae %
<i>Giraffa camelopardalis</i> – ZA1265	1126	511	45	391	35	130	12	94	8
<i>Giraffa camelopardalis</i> – ZA1253	1740	932	54	512	29	180	10	116	7
<i>Giraffa camelopardalis</i> – AZ635	2056	1074	52	590	29	168	8	224	11
<i>Giraffa camelopardalis</i> – AZ121	2638	1421	54	755	29	293	11	169	6
<i>Okapia johnstoni</i> - AZ2348	1227	431	35	513	42	144	12	139	11
<i>Okapia johnstoni</i> – AZ2440	1292	453	35	496	38	195	15	148	11
<i>Camelus</i>	2560	1030	40	836	33	472	18	222	9
<i>Lama glama</i>	1233	545	44	332	27	264	21	92	7
<i>Tragelaphus strepsiceros</i>	1358	398	29	505	37	297	22	158	12

<i>Kobus</i>									
<i>ellipsiprymnus</i>	1338	408	30	506	38	241	18	183	14
<i>Kobus leche</i>	1156	326	28	401	35	298	26	131	11
<i>Aepyceros</i>									
<i>melampus</i>	939	308	33	346	37	198	21	87	10
<i>Capra hircus</i>	828	222	27	280	34	215	26	111	13
<i>Ovis aries</i>	782	208	27	312	40	192	25	70	9
<i>Antidorcas</i>									
<i>marsupialis</i>	743	227	31	284	38	163	22	69	9

Table 2: Normalized vertebral body lengths of the ungulate species studied.

Species	Normalized Vertebrae Length (mm)	Total Thoracic Length (mm)	Normalized Thoracic Vertebrae Length %	Total Lumbar Length (mm)	Normalized Lumbar Vertebrae Length %	Total Sacral Length (mm)	Normalized Sacral Vertebrae Length %
<i>Giraffa camelopardalis</i> – ZA1265	615	391	64	130	21	94	15
<i>Giraffa camelopardalis</i> – ZA1253	808	512	63	180	22	116	14
<i>Giraffa camelopardalis</i> – AZ635	982	590	60	168	17	224	23
<i>Giraffa camelopardalis</i> – AZ121	1217	755	62	293	24	169	14
<i>Okapia johnstoni</i> - AZ2348	796	513	64	144	18	139	17
<i>Okapia johnstoni</i> – AZ2440	839	496	59	195	23	148	18
<i>Camelus</i>	1530	836	55	472	31	222	15
<i>Lama glama</i>	688	332	48	264	38	92	13
<i>Tragelaphus strepsiceros</i>	960	505	53	297	31	158	16
<i>Kobus ellipsiprymnus</i>	930	506	54	241	26	183	20
<i>Kobus leche</i>	830	401	48	298	36	131	16
<i>Aepyceros melampus</i>	631	346	55	198	31	87	14
<i>Capra hircus</i>	606	280	46	215	35	111	18
<i>Ovis aries</i>	574	312	54	192	33	70	12
<i>Antidorcas marsupialis</i>	516	284	55	163	32	69	13

Table 3: Total, cervical and thoracic (T1 to T9) vertebrae body lengths of the extant ungulates studied. The measurements are in millimeters (mm).

Species	Total Vertebrae Length	Total Cervical Vertebrae Length (C2-C7)	C2	C3	C4	C5	C6	C7	T1	T2	T3	T4	T5	T6	T7	T8	T9
<i>Giraffa camelopardalis</i> – ZA1265	1126	511	73	92	102	90	85	69	45	38	33	30	30	25	27	27	22
<i>Giraffa camelopardalis</i> – ZA1253	1740	932	176	157	153	150	157	139	75	53	43	38	39	32	33	33	34
<i>Giraffa camelopardalis</i> – AZ635	2056	1074	210	186	180	177	171	150	74	52	43	39	38	39	39	38	39
<i>Giraffa camelopardalis</i> – AZ121	2638	1421	270	244	243	240	232	192	90	65	60	52	49	53	46	48	50
<i>Okapia johnstoni</i> - AZ2348	1227	431	91	71	72	71	68	68	39	35	34	34	34	34	33	33	34
<i>Okapia johnstoni</i> – AZ2440	1292	453	96	75	75	76	70	61	43	37	35	32	35	33	33	34	35
<i>Camelus</i>	2560	1030	217	186	180	169	156	122	77	66	74	71	67	68	68	68	70
<i>Lama glama</i>	1233	545	98	105	105	101	84	52	30	28	27	27	27	27	27	27	27
<i>Tragelaphus strepsiceros</i>	1358	398	91	71	69	64	57	46	38	38	37	38	38	38	38	38	37

<i>Kobus</i>																	
<i>ellipsiprymnus</i>	1338	408	92	75	70	65	58	48	43	40	39	37	38	37	36	36	36
<i>Kobus leche</i>	1156	326	79	59	55	49	45	39	33	33	33	33	32	32	32	32	33
<i>Aepyceros</i>																	
<i>melampus</i>	939	308	72	58	54	48	42	34	29	28	27	27	26	26	25	25	25
<i>Capra hircus</i>	828	222	54	41	38	34	30	25	22	22	23	23	23	22	22	22	24
<i>Ovis aries</i>	782	208	53	37	33	32	30	23	21	22	23	23	23	22	22	23	25
<i>Antidorcas</i>																	
<i>marsupialis</i>	743	227	54	42	39	35	31	26	22	22	22	22	21	21	21	21	20

Table 4: Cervical vertebral body lengths of the fossil giraffids studied.

Species	Fossil Number	Site	Million years before present	Cervical Vertebra	Vertebral Body Length	Calculated TVL, giraffe regression	Calculated TVL, ungulate regression	Calculated NVL, giraffe regression	Calculated NVL, ungulate regression
<i>Giraffa sp.</i>	KNM-ER 3205	Koobi Fora	3.36	C2	265	2593.4	3766.2	1168.9	2612.4
<i>Giraffa sp.</i>	KNM-ER 3205	Koobi Fora	3.36	C5	256	2821.1	4348.7	1285.6	3118.0
<i>Giraffa sp.</i>	KNM-ER 3205	Koobi Fora	3.36	C7	255	3365.9	5355.9	1519.1	3908.5
<i>Paleotragus primaveus</i>	74.64	Fort Ternan	~ 14	C2	78	729.0	1109.9	573.5	764.5
<i>Paleotragus primaveus</i>	2010.62	Fort Ternan	~ 14	C6	70.7	903.2	1468.5	512.5	1032.5
<i>Samotherium africanum</i>	353.64	Fort Ternan	~ 14	C3	99.2	1185.5	1757.9	617.6	1223.3
<i>Samotherium africanum</i>	3186.63	Fort Ternan	~ 14	C6	87.5	1082.5	1759.6	585.4	1245.9

Figure 1: Photographs of the left aspect of giraffe vertebrae C6 to T2, to show how they are articulated in a living individual and the differences between cervical and thoracic vertebrae. Note the size of the transverse foramen in C7 through which the vertebral artery passes. Also note the longer spinous process of T1 compared to C6 and C7.

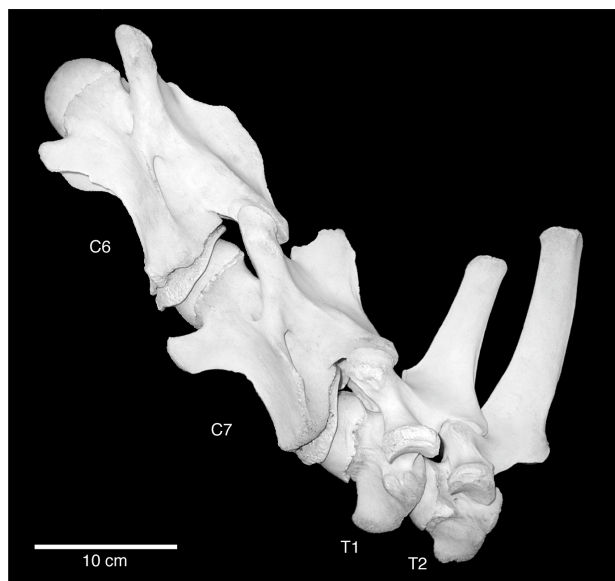


Figure 2: Photographs of the lateral aspect of non-articulated giraffe vertebrae C6, C7, T1 and T2, demonstrating the osteological differences between cervical and thoracic vertebrae. Note the size of the transverse foramen in C7 through which the vertebral artery passes and the lack of a transverse foramina in T1 and T2. Also note the longer of the spinous process of T1 compared to C6 and C7.

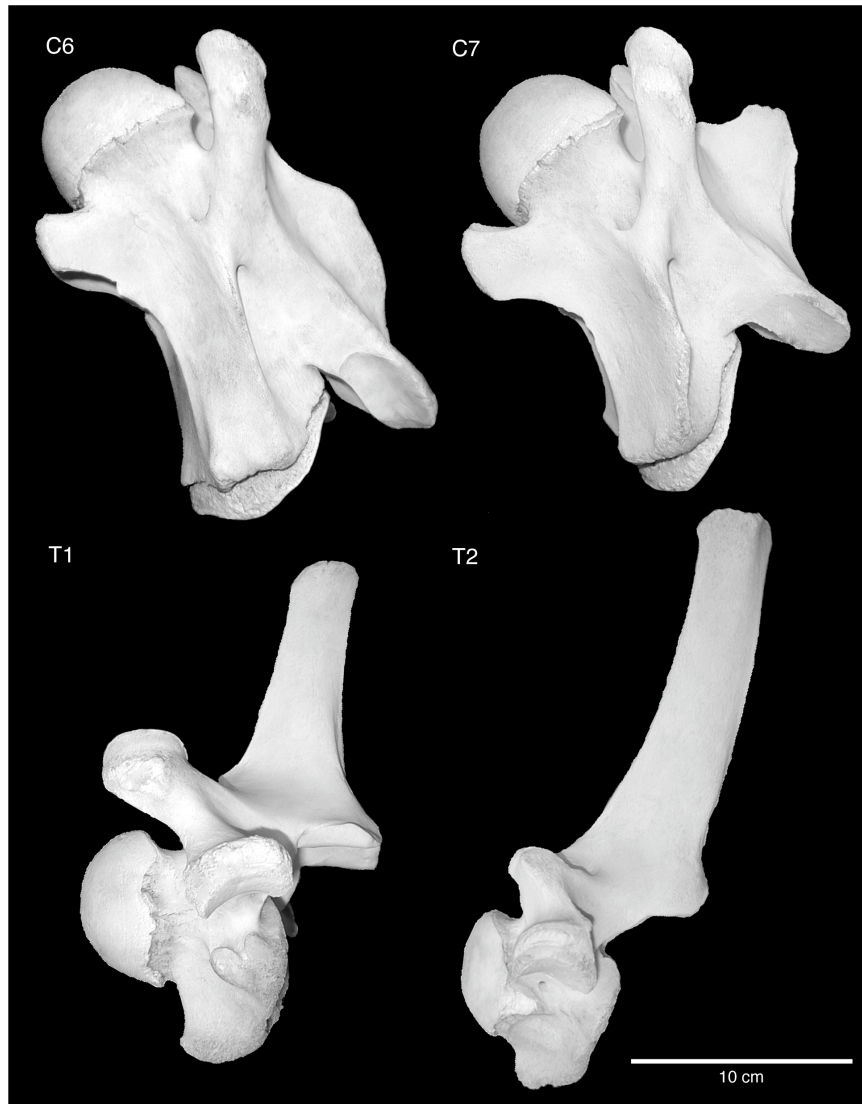


Figure 3: Upper Graph: the percentage contribution of the vertebral regions to the entire length of the vertebral column of giraffe aged from newborn to adult (ages are estimates). In the newborn, the cervical vertebrae occupy around 45% of the total vertebral length. As the animal matures, the proportion increases to between 52 and 54%.

Lower Graph the percentage contribution of the vertebral regions to the entire length of the vertebral column of the extant ungulates studied compared to the adult giraffe. Note that only in the giraffe the cervical vertebrae occupy half of the entire vertebral column. **c** – cervical; **t** – thoracic; **l** – lumbar; **s** – sacral.

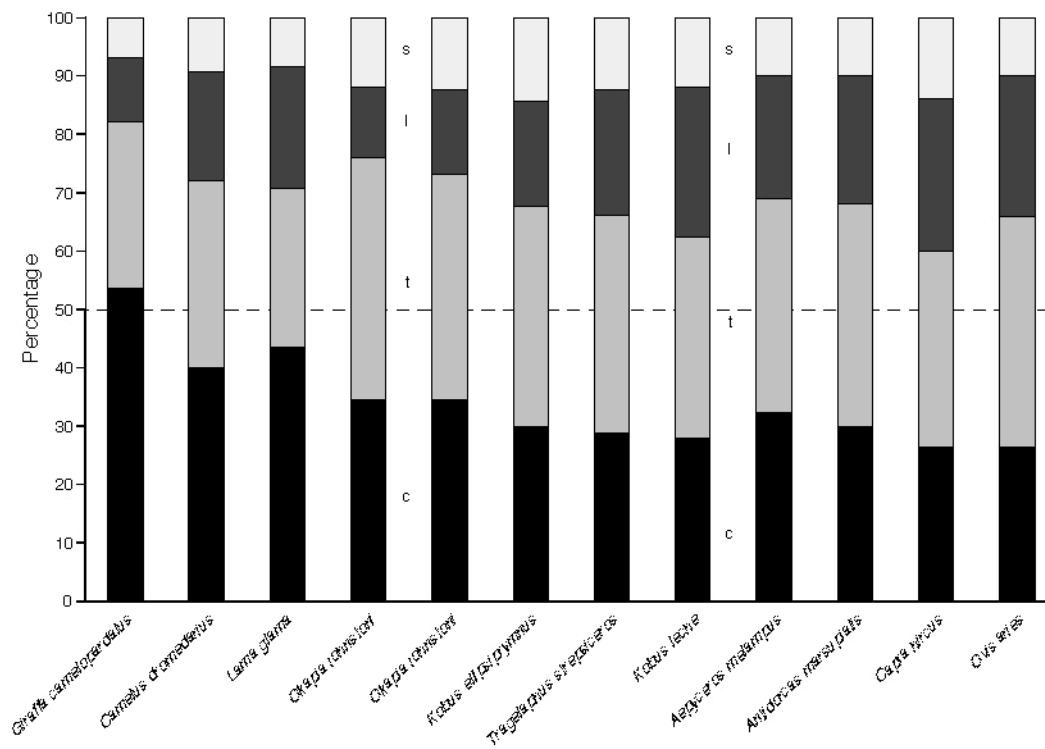
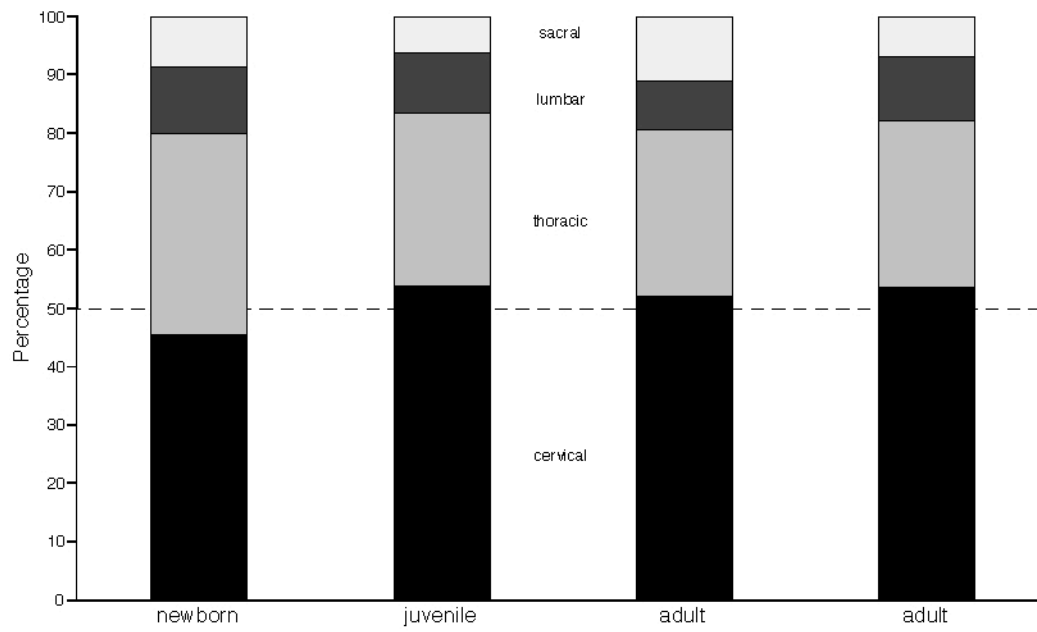


Figure 4: Top Graph: the percentage contribution of the remaining vertebral regions to the entire vertebral column length excluding the cervical vertebrae of giraffe aged from newborn to adult.

Bottom Graph: the percentage contributions of the remaining vertebral regions to the entire vertebral column length excluding the cervical vertebrae of the extant ungulates studied compared to the adult giraffe. The percentage occupied by the giraffe in comparison to the other ungulates studied falls in the same ranges when the cervical vertebrae are not included in the calculation of the total vertebral column length. **t** – thoracic; **l** – lumbar; **s** – sacral.

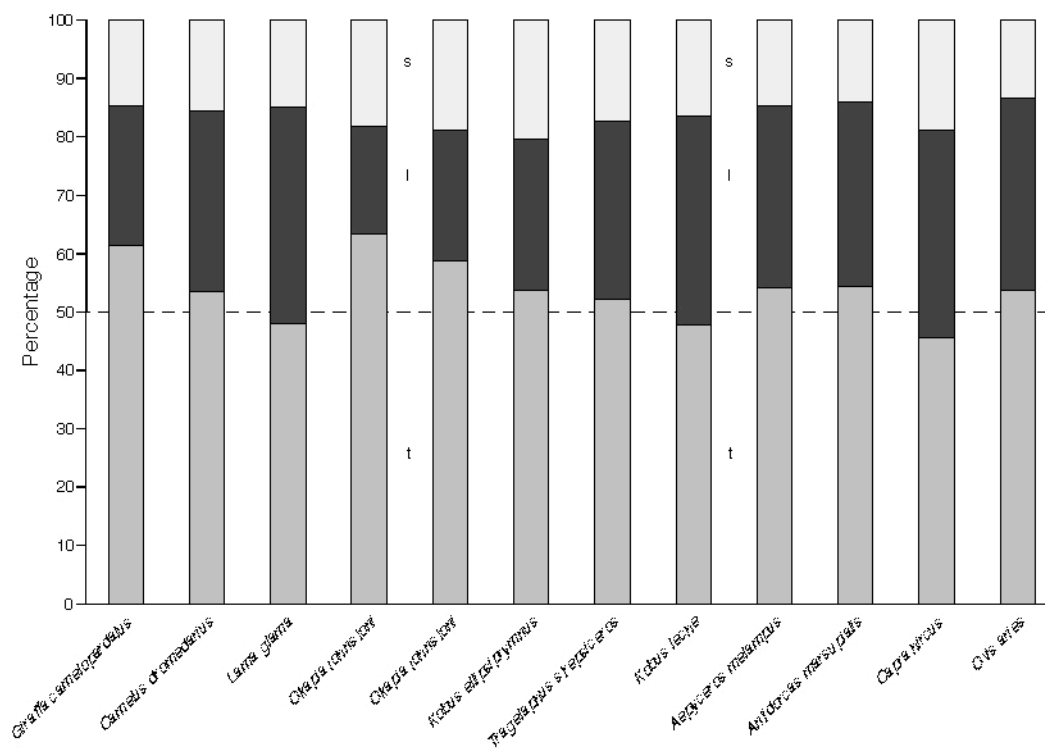
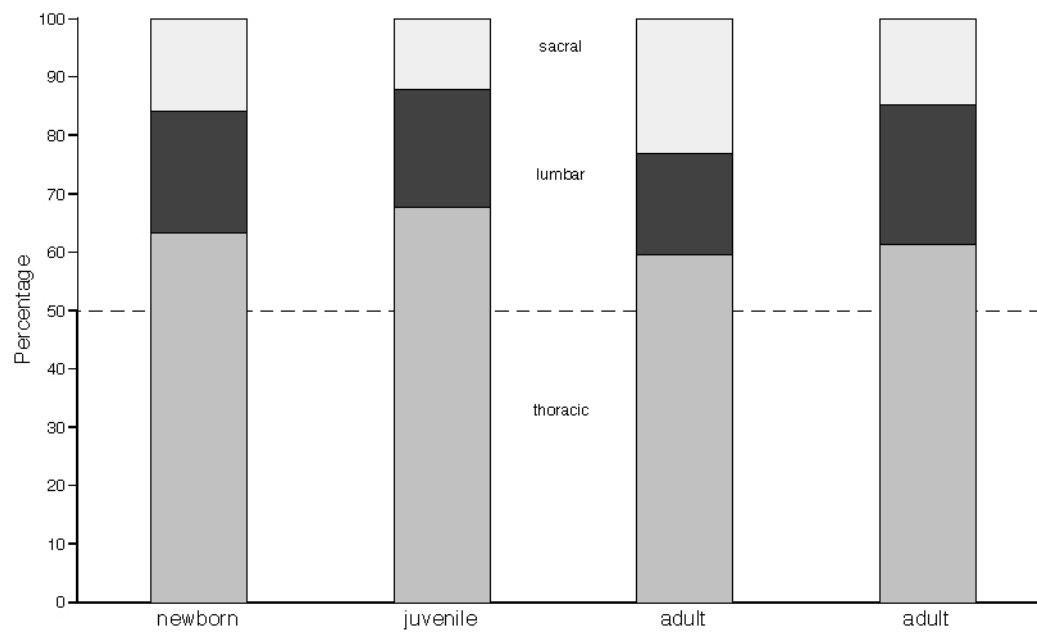


Figure 5: Graph of total cervical vertebral length (TCL) versus individual vertebral length of all the extant specimens studied. Note how the giraffe cervical vertebrae scale in accordance with that seen in the other extant ungulates studied.



Figure 6: Graphs of total vertebral column length plotted against the body lengths of C2 to C7 of all the extant specimens studied. Other ungulates represent all species except the giraffe, camel and llama. Note how for all specimens of the giraffe the vertebral lengths are longer than one would predict on the basis of a generalized ungulate regression and scale more steeply than the ungulates. The dotted line on the ungulate plot is an extension of the ungulate regression that allows us to establish a comparison to the camel.

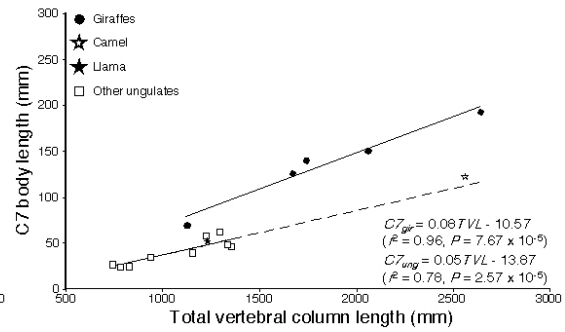
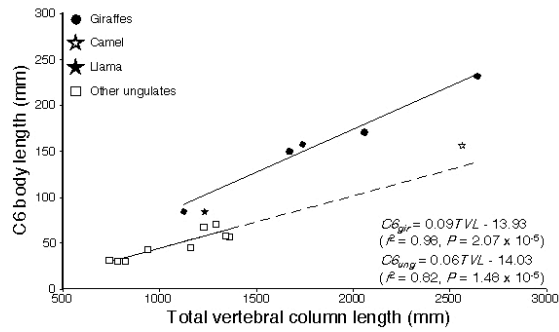
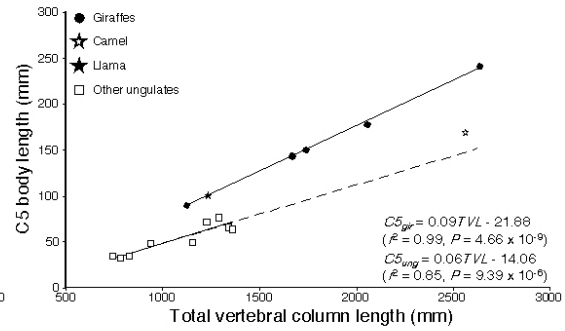
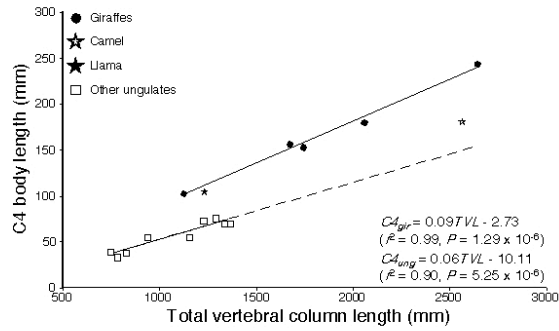
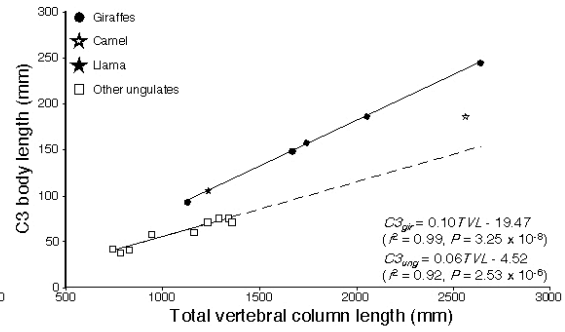
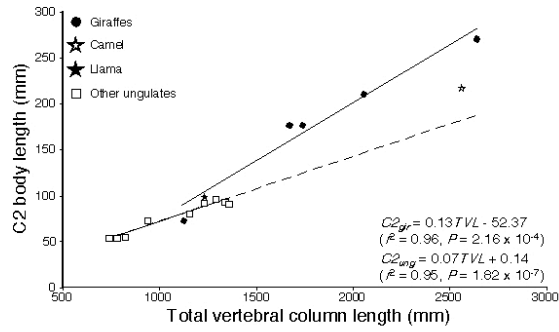


Figure 7: Graphs of normalized vertebral column length (total vertebral column length minus cervical vertebral length) plotted against the body lengths of C2 – T9 of all the extant specimens studied. Other ungulates represent all species except the giraffe, camel and llama. Note how for all cervical specimens of the giraffe the vertebral lengths are longer than one would predict on the basis of a generalized ungulate regression and scale more steeply than the ungulates. However, at the transition from cervical to thoracic, the slope for giraffe becomes near parallel to that of other ungulates. The dotted line on the ungulate plot is an extension of the ungulate regression that allows us to establish a comparison to the camel.

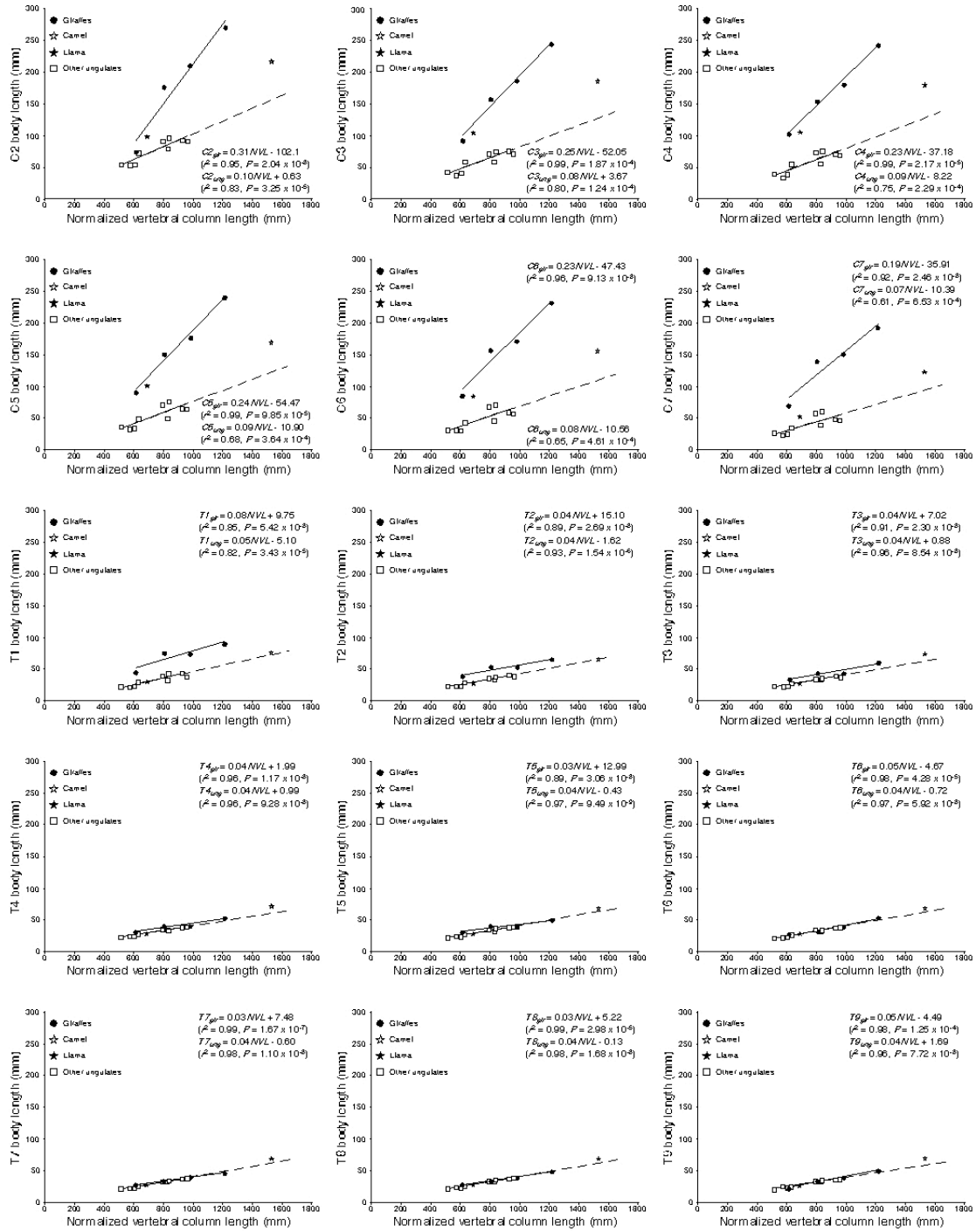
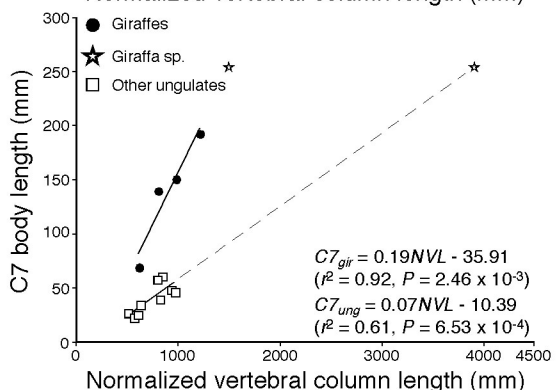
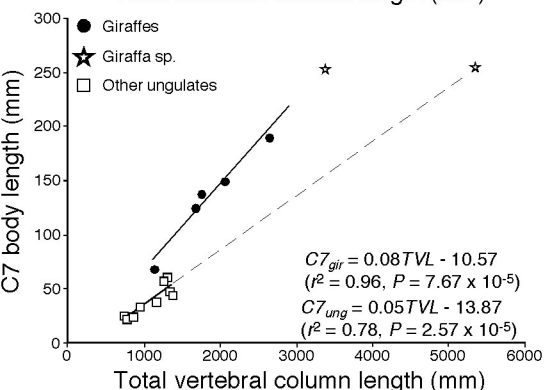
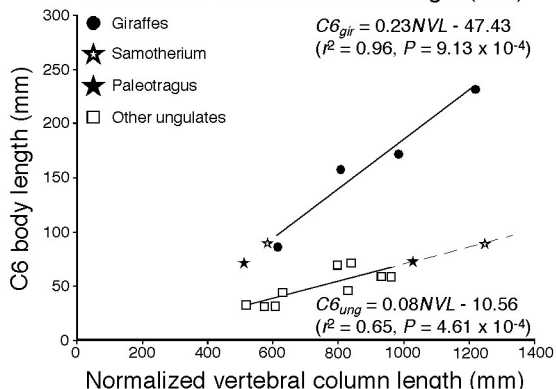
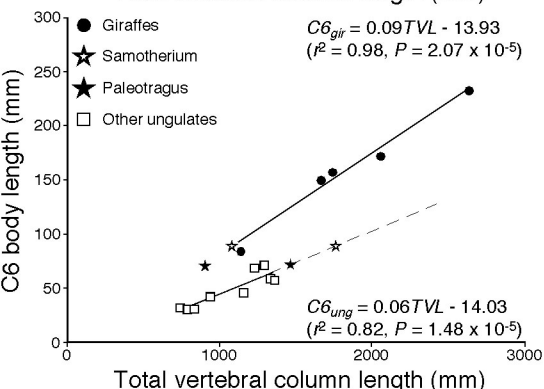
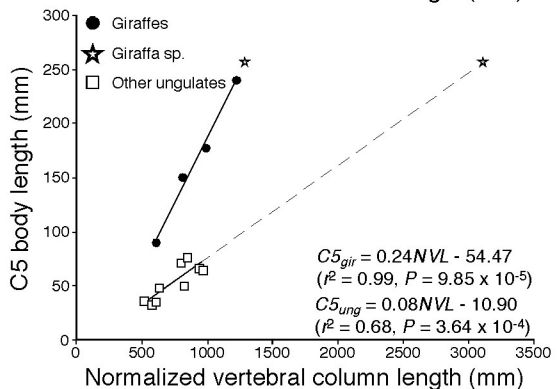
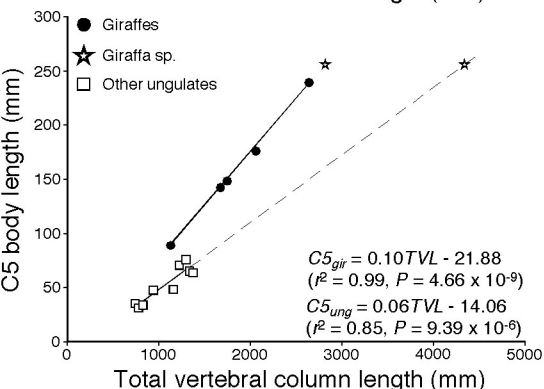
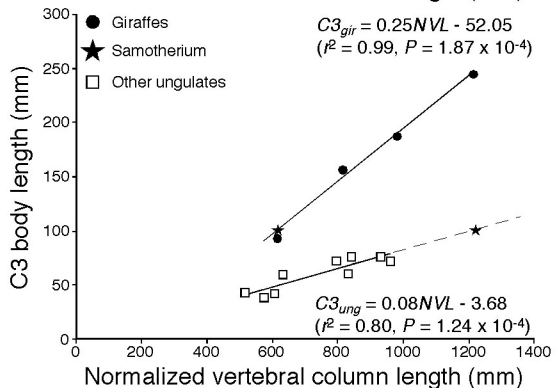
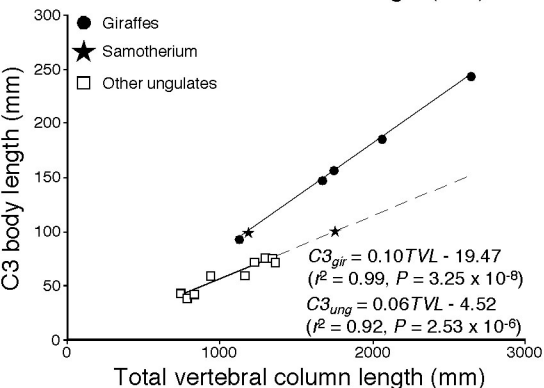
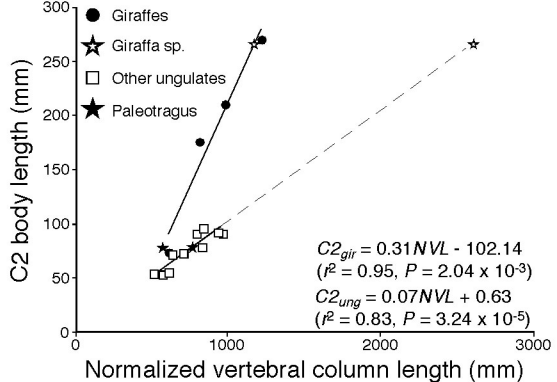
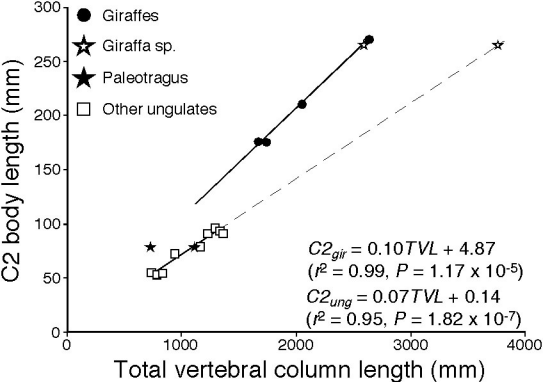


Figure 8: This figure shows a comparison of the measured cervical vertebrae lengths of fossil Giraffids compared with extant giraffes and the “other ungulate” group used in this study. The measurement of total and normalized vertebral column lengths for the fossil giraffids were generated from the regressions derived for extant giraffes or “other ungulates”, while that of the individual cervical vertebrae was taken from the literature (see Table 4).

Left column: Graphs of total vertebral column length plotted against the body lengths of the cervical vertebrae (excluding C1 and C4) of modern giraffes and other extant ungulates compared to cervical vertebral lengths measured in fossil *Giraffa* sp, *Paleotragus*, and *Samotherium*.

Right column: Graphs of normalized vertebral column length (total vertebral column length minus cervical vertebral length) plotted against the body lengths of the cervical vertebrae (excluding C1 and C4) of modern giraffes and other extant ungulates compared to cervical vertebral lengths measured in fossil *Giraffa* sp, *Paleotragus*, and *Samotherium*. Note how in each case the fossil *Giraffa* sp. appears to represent either a long-necked form or an extremely large “normal-necked” ungulate and that for *Paleotragus* and *Samotherium*, the specimens indicate either a medium sized “normal-necked” ungulate or a very small long-necked giraffe.



2.4 Discussion

The osteological observations carried out in the present study clearly indicate that giraffe have seven cervical vertebrae. When we compare the giraffe to 11 other ungulate species, including the okapi, the results show that the lengths of the individual cervical vertebrae in the giraffe are indeed uniquely long, while the remainder of the vertebrae are, for the most part, what would be expected in an ungulate. This indicates that the overall length of the giraffe neck, or neck considered as a whole, may be a unique morphology found only in the giraffe to the exclusion of other ungulates. The camel and llama also show an interesting lengthening of the neck, but this is qualitatively and quantitatively different to that observed in the giraffe. Comparisons with three fossil giraffe species indicate that these ancient animals may be considered either short or long necked forms. The overall length of the giraffe neck may be considered as either adaptive or as resulting from changes affecting developmental processes; however, a variety of features indicate that during the course of evolution of the giraffe neck both phylogenetic constraints and structural laws of form played a major role in the construction of the final form.

2.4.1 Seven cervical vertebrae as a phylogenetic constraint

Detailed examination of the osteological features of the cervical and thoracic vertebrae in the giraffe reveal that it is most parsimonious to conclude that this animal has seven cervical vertebrae. This is in agreement with observations made by previous authors (e.g. Mitchell and Skinner, 2003). On the whole, the morphology of the cervical vertebrae of the giraffe resembles that of the extant ungulate species studied.

All the cervical vertebrae had foramina in their transverse processes and the spinous processes were shorter than that of the thoracic vertebrae. The two major differences between the cervical vertebrae of the giraffe and the extant ungulate species studied were: (1) in the giraffe, the cervical vertebrae were longer and more robust; (2) C7 had a large transverse foramen through which the vertebral arteries pass (Solounias, 1999) and this was not seen in the other extant ungulate species studied.

Solounias (1999) states that the giraffe has eight cervical vertebrae and that an additional vertebra has been added somewhere between C2 and C6 thus making C7 the eighth vertebra (which he calls V8). He states that C7 replaces T1, making it homologous with the eighth vertebra in other mammals (Solounias, 1999) and argues that the giraffe C7 vertebra resembles, “a typical C6 [vertebra in other mammals] in that it has a normal foramen transversarium containing the vertebral artery...” (Solounias, 1999). The eight cervical vertebrae proposal in the giraffe remains unclear in that Solounias (1999) fails to mention exactly where that extra vertebra is added.

Our comparisons of the individual cervical vertebrae of the giraffe to those of the extant ungulates studied revealed that although much longer, each giraffe vertebra scaled appropriately for that particular vertebra (Fig. 5). This scaling is in line with the other extant ungulate species studied and is as predicted for an ungulate with the neck length exhibited by the giraffe. Our findings therefore, dispute Solounias’ (1999) proposal of an additional vertebra being added between C2-C6. The claim that C7 of the giraffe replaces T1 is also negated by our results since it showed the same scaling as the C7 vertebrae of the extant ungulate species studied and was not homologous in its structure to their T1 (Figs. 5, 7).

It is well known that all mammals, except for three genera, have seven cervical vertebrae (Galis, 1999). A variation in the number of cervical vertebrae is associated with neurological and metabolic anomalies in mammals. This is due to a change in the *Hox* gene patterning in development that plays a vital role in the stability of the nervous system and axial skeleton (Galis, 1999).

Within the cervical vertebrae there is also a scaling law of form indicating that alterations in the length of the neck are not achieved by a single vertebrae, but rather the entire suite of cervical vertebrae change length (Fig. 5). This scaling law of form for the cervical vertebrae might also be considered a constraint on the length changes of the neck in ungulates. From these observations, we conclude that there are at least two constraints that the evolution of a long neck in the giraffe has to work within. The first is the class level phylogenetic constraint of seven cervical vertebrae, the second is the appropriate scaling law of form within ungulates for the length of each individual cervical vertebra for a given ungulate neck length.

2.4.2 Cervical vertebrae in the giraffe are uniquely long

This study compared the individual cervical vertebrae of the giraffe to those of several extant ungulate species, including the okapi, to determine how these vertebrae scaled in the giraffe compared to the ungulates studied in terms of the overall length of the vertebral column. When compared to the entire vertebral column (total vertebral length, TVL) and the non-cervical vertebrae (normalized vertebral length, NVL), we found that each of the giraffe cervical vertebrae were uniquely long in comparison to those of other ungulates studied. The camel and llama are two exceptions, and these will be discussed below. Even though the giraffe cervical

vertebrae are uniquely long in comparison to other ungulates, they did show a strong relationship to both TVL and NVL. This indicates a scaling law of form, and potential phylogenetic constraint that is qualitatively different to that seen in the other ungulates, as the slopes of the regression are clearly steeper in the giraffe when compared to the other ungulates studied. These observations show that the development of the giraffe neck occurs in a manner that is comparable in some respects to that of other ungulates, but not in other respects. In all species, including the giraffe, the length of the cervical vertebrae scale with TVL and NVL, this appearing to be a constraint in the evolution of neck length, but the manner in which this scaling constraint is evidenced is different for the giraffe in comparison to the other ungulates studied. The remaining vertebrae of the giraffe appear to scale in a manner that is in accordance, both qualitatively and quantitatively, with the other ungulates studied. Thus, the thoracic vertebrae of the giraffe follow a scaling law of form that is consistent across ungulates, and this may represent an order level phylogenetic constraint.

Thus, we find that the giraffe cervical vertebrae do demonstrate uniqueness in terms of their absolute length and the manner in which they scale with size. But this absolute length does show constraints in relation to the length of the vertebral column. It then follows that the evolution of the neck length most likely occurred by a unique mechanism in the giraffe.

2.4.3 Evolution of “long-neckedness” in giraffe – current observations

In terms of understanding the evolution of the long neck in the giraffe, we could only find published data of fossil giraffe cervical vertebrae from *Giraffa sp.*,

Paleotragus primaveus and *Samotherium africanum*. The *Giraffa* sp. was recorded as being from 3.4 mya (Feibel, Brown and McDougall, 1989), while the *Paleotragus primaveus* and *Samotherium africanum* specimens were from about 14 mya (Churcher, 1970), leaving a 10.6 million year gap between these Giraffid forms. Mitchell and Skinner (2003), in reviewing Giraffid evolution, indicate that *Paleotragus primaveus* had a “slightly elongated” neck; however, they make no comment on the neck length of *Samotherium africanum*. Churcher (1970) indicates that *Samotherium* possessed both elongated limbs and necks. Our analyses, based on calculating TVL and NVL from modern species for these fossil forms, shows that both *Paleotragus* and *Samotherium* could readily be giraffids with the typical “short neck” of ungulates. Their body size interpolated from the “short-necked” regression, would indicate that *Paleotragus* was around the size of the modern Greater kudu (*Tragelaphus strepsiceros* – 200-250 kg), and that *Samotherium* was about twice as large, ranging from around 400 to 600 kg. These estimates of size are consistent with descriptions given in the literature of the size of the extinct forms (Mitchell and Skinner, 2003). It is unlikely that *Paleotragus* and *Samotherium* would be Giraffids with long necks due to the small body size attained, at least in terms of the regressions calculated for the extant giraffe (Fig. 8), which indicates a body size close to or smaller than a newborn giraffe, at around 100 kg. However, these comparisons do not rule out the possibility that both *Paleotragus* and *Samotherium* may have been forms with slightly longer than normal necks, compared to other ungulates, and are precursors to long-necked giraffes. From the phylogeny of the Giraffids provided by Mitchell and Skinner (2003), it appears that the modern Okapi is derived from the Paleotraginae. Given the observations made in the present study, that the length of the extant Okapi neck is indistinguishable from that of other modern ungulates (to the

exception of the giraffe, llama and camel), it would be parsimonious to conclude that the Paleotraginae were indeed “short-necked” ungulates, rather than a form with a slightly elongated neck. The same rationale cannot be directly applied to the Samotheriinae, as they are also descendents of the Paleotraginae, but appear to be on the direct lineage to modern giraffes (Mitchell and Skinner, 2003). It is therefore possible that the Samotheriinae may have possessed some elongation to the neck. There is consensus that *Giraffa sp.* was a long necked form (Churcher, 1970; Mitchell and Skinner, 2003). Our analysis shows that the *Giraffa sp.* was most likely a long necked form, and is slightly larger than modern giraffes, this being consistent with current descriptions (Churcher, 1970; Mitchell and Skinner, 2003). If the *Giraffa sp.* specimen analysed in the present study was a “short-necked” ungulate, it would have been extremely large, and this is not consistent with the descriptions of the size of the appendicular skeleton.

2.4.4 Evolution of “long-neckedness” in giraffe – microevolutionary scenario

Paleotragus most likely gave rise to *Samotherium*, which later gave rise to *Bohlinia* (8mya), a “giraffe-like” ancestor of modern giraffes, which eventually gave rise to the extant giraffe (Mitchell and Skinner, 2003). Mitchell and Skinner (2003) note that *Bohlinia* had a long neck, but we could not find measurements of fossils to compare to our regressions calculated on the extant species. There is a gap of 10.4 million years between the species for which measurements are available, and this makes the evolution of the long neck unclear as to whether it is a microevolutionary or a punctuated event. Thus in the literature, there is a tendency to argue towards the microevolutionary occurrence, which is a slow accumulation in the length of the giraffe neck. This tendency is evidenced by the indication of “slightly-elongated”

necks for both the Paleotraginae and Samotheriinae (Churcher, 1970; Mitchell and Skinner, 2003).

Our comparison of the fossil giraffids, *Paleotragus primaveus* and *Samotherium africanum*, indicates that they are not clearly long-necked forms as in the extant giraffe and *Giraffa sp.* from 3.4mya, and that the Paleotraginae were probably short-necked forms. But, it is possible that the Samotheriinae, as indicated in the literature (Churcher, 1970), may have had slightly elongated necks. If microevolutionary processes were at play in the evolution of neck length in the giraffe, it appears that these changes may have begun prior to the period when the Samotheriinae evolved, sometime during the Paleotraginae – Samotheriinae transition, and accrued over time until the appearance of *Bohlinia*. This would leave a time period of approximately 2 to 3 million years for the gradual accumulation of increased cervical vertebral length, without any specific changes in the remainder of the vertebral column. It is possible that the neck length of *Bohlinia* is slightly shorter than the modern giraffe, which if is the case adds several more million years to the time span free for accumulation of micro-changes in neck length to be passed on over generations.

Mitchell and Skinner (2003) summarize the evidence for consistent changes in the environment of the evolving Giraffid lineage, which appears to have begun approximately 10 million years ago, coincident with the appearance of the Samotheriinae. Approximately 8 million years ago, there were also dramatic changes in the available flora for browsing, and this is coincident with the appearance of the “true *Giraffa*” (Mitchell and Skinner, 2003). This consistent ecological variability over the period when the long neck may have evolved through microevolutionary accumulation of small changes appears to represent a potential driving force for the

evolution of long-neckedness in the giraffe. Thus, it is possible that the overall length of the giraffe neck was acquired through microevolutionary processes, but as highlighted in the present study, this potential adaptive elongation of the neck occurred within the restrictive constructs of several phylogenetic constraints and structural laws of form. If this microevolutionary scenario holds true, then fossil evidence of individual cervical vertebral lengths and entire vertebral column lengths should track the gradual lengthening of the neck of the Giraffid lineage, and demonstrate that lengthening of the neck was indeed a series of adaptive morphological changes associated with climatic and vegetative variation.

2.4.5 Evolution of “long-neckedness” in giraffe – punctuated scenario

A second scenario that has yet to be discussed in the literature concerning the giraffe neck is that of a punctuated evolutionary event leading to the genesis of a long neck in the modern giraffe. In the present study we demonstrated that the cervical vertebrae of the giraffe occupy approximately 50% of the entire vertebral column length, and that this situation is qualitatively different to that seen in all the other ungulates studied, as the scaling of the cervical vertebrae is clearly different to that of other ungulates with longer necks such as the llama and camel (see below). While more information may be required to solidify this conclusion, it does appear that the giraffe neck length morphology is unique, both quantitatively and qualitatively. Moreover, there is currently no convincing evidence for gradual accumulation of neck length in the fossil Giraffids, with the Samotheriinae not showing any substantive signs of a lengthening of the neck (see above). Given this, and the 10.4 million year gap between the short-necked Paleotraginae and the long-necked *Giraffa*, we can

entertain the possibility that a punctuated evolutionary event occurred. In fact, given that all descriptions of *Bohlinia* indicate that it is long-necked, we can propose that such a punctuated event may have occurred at the Samotheriinae-Giraffinae transition approximately 8 million years ago (Mitchell and Skinner, 2003).

While at first glance, such a punctuated event may seem highly improbable, recent experimental observations on the development of the skeletal system may lend support to this line of reasoning. Early in embryonic development, before segmentation of the vertebral column, *Hox* genes appear to pattern the presomitic mesoderm into cervical, thoracic, lumbar, sacral and caudal regions (Kieny et al., 1972; Nowicki and Burke, 2000). The boundaries between the various vertebral regions are marked by the anterior expressional limits of specific *Hox* genes (Krumlauf, 1994). It is possible to imagine a situation during the presomitic stage in giraffe development, where the cervical-thoracic border delineated by expression of specific *Hox* genes, becomes located in a position slightly more caudal in the overall presomitic mesoderm to that seen in other ungulates, such as the Okapi. This may result from under-expression of the specific cervical-thoracic border *Hox* gene markers, or may result from alteration in the upstream regulators of *Hox* genes (Krumlauf, 1994) causing this presomitic border to move to a location that is more caudal in comparison to the antero-posterior location of the cervical-thoracic border in the presomitic mesoderm found in other ungulates.

A caudal shift in the cervical-thoracic border would then lead to a greater proportion of the axial skeleton being devoted to cervical vertebrae, such as is the situation seen in the extant giraffe where 50% of the axial skeleton is cervical. Subsequent somitogenesis would more than likely occur as normal, as evidenced by the existence of 7 cervical vertebrae in the giraffe, the standard ungulate appearance

of the remaining giraffe vertebrae, and the location of the fore and hind limbs in relation to the vertebral regions (Krumlauf, 1994). The slightly larger amount of presomitic mesoderm devoted to the cervical vertebrae would result in the overall longer cervical vertebrae seen in the giraffe. Given this proposed type of change occurred during embryonic development, at the presomitic stage, the actual dimensions of the potential caudad shift in the cervical-thoracic border may only be in the millimetre range.

If such a qualitative change in the pattern of *Hox* gene expression were to occur and lead to the genesis of a longer neck, it is then likely that this type of change would have passed rapidly through the pre-Giraffinae population, at the Samotheriinae-Giraffinae transition approximately 8 million years ago (Mitchell and Skinner, 2003). This transition is strongly marked by environmental changes, and these environmental changes may have led to dietary changes in the late Samotheriinae – early Giraffinae. Such changes may introduce mild toxins into the diet, potentially even seasonally, and these mild toxins may lead to the proposed alteration in *Hox* gene expression patterns. This potential scenario would account for the rapid change in qualitative lengths of the various vertebral regions. If this change occurred at the proposed transition, and became stable in the Giraffinae, subsequent enlargements in body size would magnify the effect, resulting in an obviously lengthened neck.

This scenario, while highly speculative, is no less so than the microevolutionary scenario. At present we do not have sufficient data on either the fossil record of the giraffe vertebral column, or the molecular biology of the giraffe and other ungulate embryos to truly decide which is more the more plausible scenario. The punctuated scenario does not require an underlying adaptive reason for the

evolution of the neck length of the giraffe, which in some senses may be attractive. Both scenarios are testable, but it would appear that gaining more fossil evidence of neck evolution would be the simplest way forward, as experimental embryology on giraffe may prove difficult.

2.4.6 Evolutionary occurrences of long neckedness in ungulates compared to other vertebrates.

The present study clearly demonstrates that one occurrence of the evolution of long-neckedness in the ungulates is the giraffe. The neck occupies 50% of the vertebral column in the giraffe, making it, of the ungulates studied to date, the relatively and absolutely longest-necked ungulate species. Moreover, the manner in which the cervical vertebrae scale in comparison to other ungulate species indicate a qualitative change in neck development and evolution (see above). The results further indicate the probability of a second evolution of “long-neckedness” arising in the *Camelidae* family (to which the camel and llama belong) in a different and unique way to that seen in the giraffe (with around 40% of the vertebral column being cervical). The C2-C5 vertebral lengths of the camel and llama are longer than the “typical” baseline ungulates, but below the regression derived for the giraffe development series (Figs. 6, 7). If one interpolates a line through the data obtained for C2-C5 for these two members of the *Camelidae*, this line falls almost parallel to, but above that, of the other ungulates. This, in contrast to the scaling seen in the giraffe where the regression slope is steeper than that of the other ungulates, indicating a change in quantity, and not quality in these two members of the *Camelidae*. Thus, while the neck is longer, this increase in length may be associated

with a quantitative change in the Camelidae, as opposed to both a quantitative and qualitative change in the Giraffidae. Further studies on other species of *Camelidae* (for example guanaco and vicuna) including a developmental series may give clues as to this second possible mechanism of evolving a longer neck in the ungulates. While not specifically examined in the present study, another long necked ungulate, the gerenuk (*Litocranius walleri*) may show yet another independent evolution of lengthening of the neck. Thus, within the ungulates, there are possibly three independent evolutionary occurrences of “long-neckedness” and it would be of great interest to investigate these further.

The evolutionary occurrences of long-neckedness in the ungulates is clearly different to that seen in birds (such as the Ratites), reptiles (such as the extinct aquatic forms and dinosaurs), as it appears that these species can add cervical vertebrae to increase neck length, whereas this possibility is not available for mammals. Narita and Kuratani (2005) also note that the long neck in some dinosaurs such as the *Tanystropheus* was due to the elongation of individual cervical vertebrae and this is similar to what is seen in the giraffe. Hence, neck elongation in reptiles can occur in at least these two patterns.

2.4.7 Future Directions

The current study raises a number of issues, not only about the giraffe, but also about other ungulates and the evolution of long necks in general. For the giraffe, two potential scenarios for the evolution of the long neck are outlined, a microevolutionary scenario and a punctuated scenario. Our current state of knowledge does not allow either theory to be supported over the other, but does

suggest many ways forward to resolving the manner in which the length of the giraffe neck was attained. Greater knowledge of both the paleobiology and developmental biology of the giraffe and other ungulate species will ultimately resolve the questions left surrounding the evolution of long necks in the giraffe. Determining the manner in which the giraffe neck evolved is an important theoretical and practical issue for evolutionary biologists, as it has been demonstrated in this study that many phylogenetic and developmental constraints, as well as scaling laws of form, play important roles in both evolutionary scenarios. Taking an approach that considers these constraints and scaling laws, will resolve to what extent the length of the giraffe neck is an adaptation, or whether it arose as a by-product of an altered developmental pattern that was initially non-adaptive, or neutral and that later was useful in opening previously unavailable ecological niches (Gould, 2002) that arose as a result of ecological change (Mitchell and Skinner, 2003).

It is also interesting to examine further the evolution of neck length in the Camelidae and other potentially long-necked ungulates such as the Gerenuk, as it appears that the mechanisms through which lengthening of the neck was attained in these species may differ, both quantitatively and qualitatively, in comparison to the giraffe, i.e. there may be more than one way to evolve a long neck. These independent evolutions, and potentially differing mechanisms, of evolving a long neck in ungulates, may be directly contrasted with those seen in extant birds, such as the Ratites, as the birds do not have the same phylogenetic constraint of only having seven cervical vertebrae to work with as is the case for mammals (Galis, 1999). Such a comparative approach makes the giraffe neck, and indeed the other long necked species, heuristically useful models in understanding evolutionary mechanisms and the balance between phylogenetic and developmental constraints, structural laws of

form and adaptive pressures that drive the evolution of all biological structures
(Gould, 2002).

3 Chapter 3: The Frontal Sinus of the Giraffe (*Giraffa camelopardalis*) Skull

3.1 Introduction

The skull of an adult giraffe (*Giraffa camelopardalis*) presents an interesting morphology, which has a very extensive and seemingly comparatively large frontal sinus. Historically, several authors have examined the anatomy of the giraffe frontal sinuses from as early as 1938 where Colbert comments that they are “extremely large... [are] above the brain and [extend] into the occipit.” (Colbert, 1938). Churcher (1976) also notes that the giraffe has a “highly developed sinus”. Despite these statements, no quantification of this expansive sinus in the adult or developing giraffe has been undertaken. Moreover, the extent to which this sinus in the adult giraffe is “extremely large” has not been quantified in comparison to related species.

There still remains no clear functional rationale as to why the giraffe evolved this very large frontal sinus, but a number of theories have been forwarded postulating the possible function. One suggestion is that the frontal sinus is a cooling mechanism for the brain (Ganey, *et al*, 1990), as the head is under constant heat exposure from the sun due to its height (as they do not always benefit from adequate tree cover). Mitchell and Skinner (2003) state that the sinuses increase “head volume without increasing [the] weight [of the skull]” and also that “a large head volume improves temperature regulation, olfaction and chewing” (Mitchell and Skinner, 2003). Finally, large sinuses in the skull are reported to be “an important prerequisite for neck elongation” (Mitchell and Skinner, 2003). There is an uncertainty regarding the cooling effect of the sinuses since air is a heat insulator rather than an efficient conductor, and it seems unlikely that there is a constant and rapid enough airflow through the sinus that would continuously replenish the air in the sinus that has been “solar-heated” with cooler air. It is also difficult to imagine that the brain would be

adequately cooled in this fashion. As the brain receives ample blood supply from the *rete mirabile* (Mitchell and Skinner, 1993), it is more likely that this would be a more efficient cooling mechanism for the brain.

In the current study CT scans and image reconstruction programs were employed to investigate the development of the frontal sinus in the giraffe ranging from a newborn through to an adult and accurately quantify its size. Moreover, the skulls of several species of ungulates were also scanned and the frontal sinus quantified in order to provide a baseline for comparison of the size of the giraffe frontal sinus.

3.2 Materials and Methods

Specimens

Skulls of 27 ungulates representing 13 species were used (Table 5). Specimens were obtained from the Comparative Osteological Collection of the School of Anatomical Sciences, University of the Witwatersrand and the Transvaal Museum in Pretoria, South Africa. The specimens included six giraffe ranging from a newborn to a large adult, one adult Okapi, 10 white rhinoceros (also ranging in age from a newborn to a very large adult) and 10 other species representing a range of sizes across ungulates.

CT Scans

A Phillips Brilliance 6 180P₃ CT Scanner located at the University of the Witwatersrand Donald Gordon Medical Centre in Johannesburg, South Africa was used

to take CT scans of all the skulls. The slice thickness of the scans varied from 1-3mm depending on the size of the specimen.

Each specimen was placed in the anatomical position with the frontal bone facing superiorly, resting on the mandibular teeth. The mandibles were excluded from the scan. The skulls were scanned from the nasal region to the occipital condyle.

The scans were reconstructed automatically by the scanner and the images saved onto a compact disc for later analysis and digital reconstructions. Each reconstruction was loaded with a Philips MxLite View program that enabled us to measure the area of the frontal air sinuses at each section. To digitally reconstruct the skulls and fill in the frontal air sinus, we used a Slicer 2.4 program (available online at www.slicer.org). This program allowed us to rotate the skulls in the desired planes and mark out the frontal sinus by filling in that region using a contrasting medium (Figs. 11, 12, 13).

Calculation of frontal sinus volume

The frontal sinus volume of each skull was calculated by measuring the area of the frontal sinus taken from the reconstructed images and then multiplying that value by the slice thickness at which the specimen was scanned. All calculations were recorded on a Microsoft Excel spread sheet. A trial calculation of the frontal sinus volume was made on one giraffe skull, where the area of each slice through the frontal sinus was measured and then multiplied by the slice thickness at which the skull was scanned to obtain the volume of the frontal sinus. This volume was compared with the sinus volume calculated from taking the measurements at 10 slice intervals and (then multiplying that with the slice thickness at which the skull was scanned) and the results showed no considerable difference in the volumes. Hence, for expediency, the areas of the frontal sinuses of the skulls studied were measured at 10 slice intervals and the

volumes then calculated. Each sinus volume (mm^3) was calculated as follows: sinus area (mm^2) x slice thickness (mm). For example: $933.4\text{mm}^2 \times 33\text{mm} = 30802.2\text{mm}^3 = 30.8\text{cm}^3$.

Mass of the skull

The dry mass of each specimen was taken by weighing the skull without its mandible and the values were recorded on a Microsoft Excel spread sheet for later analysis.

Nuchal attachment area

The nuchal attachment area is the region on the occipital bone where the nuchal ligament and posterior neck muscles attach to the skull. This area increases as the skull (and hence frontal sinus volume) increases in size. This area was calculated by taking digital photographs of the occipital region of each skull and using the *Image J* software program to measure the nuchal attachment area. The values were recorded on a Microsoft Excel spread sheet. The area was corrected for by taking its square root and then cubing that value to use in the relevant plots. This was done to convert the area to units appropriate for direct comparison with weight and volume.

3.3 Results

Preliminary observations of the adult giraffe skull revealed a large frontal sinus that extends to the most caudal end of the skull, into the occipital bone, completely overlying the brain (Fig. 9). CT scanning and reconstruction of various aged giraffes confirmed this caudal sinus extension, which becomes more prominent with age, hence increasing a sinus volume with age (Figs. 10, 11, 12; Table 5). The

frontal sinus of adult giraffe is far larger than the adult Okapi (Fig. 12) and is far larger than several other ungulates studied (Figs. 12 and 13). Our results showed strong correlations between the skull mass and nuchal attachment area (NA) across all the ungulates studied, including the giraffe. There was also a strong relationship between the skull mass and frontal sinus volume for all the ungulates studied, but the giraffe and buffalo exhibit a different relationship to that seen in those ungulates (Fig. 14). The sinus volume versus NA correlation was again strong among all the ungulates studied, but the giraffe and buffalo again showed a different relationship to that seen in the other ungulates studied.

3.3.1 Anatomy of the frontal sinus in the adult giraffe

Several previous authors, from as early as Colbert (1938), and in more recent studies (Churcher, 1976; Ganey, *et al.*, 1990; Mitchell and Skinner, 2003), have all recorded that the frontal sinus of the giraffe is large and extensive. Our observations show that the adult giraffe frontal sinus is very large compared to the other ungulate species studied, including the Okapi, the giraffe's closest living relative. In this study, the frontal sinus was taken as one continuous anatomical region and was not divided into its various parts as done in other descriptions (Constantinescu and Constantinescu, 2004). The frontal sinus of the giraffe begins in the frontal bone, just anterior to the dorsal chonchal sinus. It then extends caudally into the parietal and interparietal bones. It also extends laterally into the temporal bones (Constantinescu and Constantinescu, 2004). From the parietal bone, it extends over the brain case and into the occipital bone. In the region of the parietal bone, the skull expands dorsally, resulting in a dome shaped region above which the ossicones are attached. The

frontal sinus extends superiorly into that dome and appears to, but does not (as confirmed by CT scans) enter the ossicones (see Fig. 10). The sinus has numerous trabeculae (Fig. 9) as a result of the inner and outer bony mantles separating dorso-ventrally as the sinuses invaginate into the spongy bone (see below). In the midline of the skull there is a bony septum that completely divides the sinus into left and right halves (see Figs. 9, 10). This septum begins at the nasal bone and extends caudally to the occiput.

3.3.2 Development of the extent of the giraffe frontal sinus

As mentioned above, the adult giraffe has an extensive frontal sinus that overlies the brain case and extends laterally into the temporal bone (see also Figs. 9, 10). This expansion, however, is not seen throughout the developmental series of the giraffe, which shows that as the animal matures the sinus expands caudally and laterally to reach the form seen in the adult (see Fig. 11).

In the newborn, the frontal sinus extends partly into the parietal bone and does not extend caudally over the braincase. At this stage of development there is also a partial extension of the sinus into the temporal bone (Fig. 11). CT scans of the newborn skulls did not reveal the large separation of the inner and outer bony mantles seen in the adult skull (see Figs. 9, 10) with the region overlying the braincase composed of a thick layer of spongiform bone.

CT scans of the juvenile skull revealed a greater separation of the bony mantles compared to the newborn. These scans also showed a more caudal extension of the sinus over the brain case as well as a more lateral expansion into the temporal bone compared to the newborn (Fig. 11). As the animal matures to a sub-adult, the

frontal sinus becomes more extensive over the brain case and into the temporal bone compared to the juvenile and newborn animals (Fig. 11). It also starts to expand into the occipital bone thus increasing its volume. Finally, in the adult, the sinus completely overlies the brain, having extended into the occipital bone as well as extending into the temporal bone (see Figs. 9, 10, 11). The region into which the frontal sinus expands during ontogeny is composed of spongiform bone, and it appears that the inner and outer mantles of the calvarium split to accommodate the incursion of the frontal sinus.

There are three main enlargements of the frontal sinus and they add considerably to the total volume. The first one is located at the crest just anterior to the ossicones (see sub-adult reconstruction in Fig. 11) and directly beneath the ossicones (see Figs. 10, 11), giving rise to the nasal and parietal “bulges”. The second is the lateralward expansion of the sinus into the temporal bone (Figs. 10, 11), which while not as expansive as that just mentioned is a significant departure from that seen in the other ungulates studied (see below). The third significant expansion is the caudal extension of the frontal sinus over the braincase that occupies the occipital bone. This extension, while seen in other ungulates (see below), is qualitatively different in its form from these other species. Of great significance is the dorso-ventral separation of the inner and outer bony mantles that appear to enable the caudal expansion of the frontal sinus into the occipital region and the enlargement of the naso-parietal portion of the sinus in the adult giraffe.

3.3.3 The frontal sinus in the okapi

CT scans and reconstructions of the Okapi skull reveal a large frontal sinus compared to most of the other ungulates studied (see Table 5). These results confirm Colbert's (1938) observation where he described the frontal sinus of the Okapi as being "large [and] anterior to the brain". The sinus however, is not as extensive as that of the adult giraffe as it does not reach the occipital region (see Fig. 9). Like the giraffe skull, the frontal sinus also extends superiorly into the dome shaped part of the parietal bone directly inferior to the ossicones (Fig. 12). The sinus does not extend significantly into the temporal bone and there are no noticeable swellings as seen in the giraffe skull.

3.3.4 The frontal sinus in other ungulates

It is clear from the literature and our observations that the frontal sinus is present in all ungulates and what differs is its extent in these species. As with the giraffe, the sinus of ungulates begins in the frontal bone, just anterior to the dorsal chonchal sinus in all the ungulates studied. It then generally extends to the parietal bone and overlies the brain case (or part of it). It does not extend laterally into the temporal bone as seen in the giraffe skull (see Fig. 13).

While this is the case for most of the ungulates examined, there are some exceptions. In bovids such as the buffalo (see Fig. 13) and domestic cow, the sinus extends into the horns and this increases the volume of the sinus compared to that of other ungulates studied (see Table 5). In the buffalo we observed a significant dorso-ventral enlargement of the sinus in the naso-parietal region overlying the braincase, and this is most dramatically visualised when comparing this directly with the extent of the frontal sinus in the wildebeest (Fig. 13). In the warthog and rhinoceros, the

frontal sinus extends into the nuchal crest formed from the occipital bone (Fig. 13). Interestingly, in the developmental series of the rhino, from newborn to adult, we saw that as the nuchal crest increased in size, the extent of the frontal sinus in this crest also increased, resulting in a greater sinus volume (Table 5).

3.3.5 The relationship of skull mass to nuchal attachment area (NA)

In the ungulates that were studied, there was a strong relationship between skull mass and nuchal attachment area (NA), whereby NA increased linearly with increasing skull mass (see Graph 1 of Fig. 14 and Table 5). It is important to note that the masses of the skulls used are just the masses of the dry bones without mandibles, ossicones (in the case of giraffe and okapi), and horns (in the rhinoceros). The lack of soft tissue structures such as muscles, tongue and blood vessels and the lack of horns, ossicones and mandible, (which all contribute to the overall mass of the skull) may lead to errors in the exact relationships described; however, the overall concept of head mass (for which we are using skull mass as a proxy) relating to nuchal attachment area should still hold. Despite these potential inaccuracies, the dry mass provides a baseline for comparison across the various species as the dry mass of the various skulls is readily obtainable from many museum collections. The giraffe as a subgroup also conformed to this relationship of increasing NA with increasing skull mass (Fig. 14).

3.3.6 The relationship of skull mass to frontal sinus volume

For most of the ungulates studied a strong relationship between an increasing skull mass and increased volume of the frontal sinus was seen (see Graph 2 of Fig. 14 and Table 5). Thus, generally speaking, if one knows the mass of the skull of a particular ungulate, the volume of the frontal sinus can be reasonably accurately predicted. There are, however, two exceptions to this generalized ungulate trend – the giraffe and Cape buffalo.

In the giraffe, a very small increase in the skull mass was strongly correlated with very large increases in the frontal sinus volume in comparison to the other ungulates studied (see Graph 2 of Fig. 14 and Table 5). Although the giraffes examined are a developmental series from newborn to adult, it shows that the giraffe as a subgroup deviate dramatically from the ungulate norm. These plots show that the giraffe, especially the adult, has a far larger than expected volume of the frontal sinus in comparison with a baseline derived from a range of other ungulates. The manner in which this sinus develops, from newborn giraffe falling exactly on the regression line derived from other ungulates, and then proceeding on a linear and predictable trajectory that results in the oversized frontal sinus of the adult giraffe, indicates that the size of the frontal sinus in giraffe is a species specific phenomenon.

The one Cape buffalo for which we have data also plots well above the regression line derived for most ungulates. It lies well above the generalized ungulate regression line but still below that of the giraffe developmental series regression (Graph 2 of Fig. 14 and Table 5). Again, this indicates a species specific case for the volume of the buffalo frontal sinus.

3.3.7 The relationship between frontal sinus volume and nuchal attachment area (NA)

As there is an increase in frontal sinus volume as the mass of the skull increased, and an increase in NA with increased skull mass, in all the species studied (see Graph 1 of Fig. 14 and Table 5), we hypothesized that an increase in frontal sinus volume may be related to an increase in NA. This hypothesis is supported when examining as a group the ungulates we studied minus the giraffe and buffalo. This trend was especially strong in the rhinoceros, where a small increase in the sinus volume was related to a large increase in NA (Graph 3 of Fig. 14 and Table 5). As seen earlier (see above results), the increased frontal sinus volume in the rhinoceros (and other species) can be directly related to an increased size of the nuchal crest.

The giraffe and buffalo, however, showed a different relationship to this generalized ungulate pattern (see Graph 3 of Fig. 14 and Table 5). While the relationship between the sinus volume and NA was strong in the giraffe, only very large increases in sinus volume led to an increase in the NA (see Graph 3 of Fig. 14 and Table 5). However, when we compare this to what is seen in the remaining ungulates studied, including the Okapi, it is difficult to conclude that there is a causal relationship, whereby in the giraffe increased NA and sinus volume do not appear to be related in the potentially causal way in which they are related in the other ungulates. The buffalo also appeared to follow a scaling similar to that seen in the giraffe, deviating from the generalized ungulate pattern (Graph 3 of Fig. 14 and Table 5).

Table 5: Raw data of skull mass, frontal air sinus volume and nuchal ligament and musculature attachment area of the ungulates studied.

Species	Skull Mass (g)	Frontal Air Sinus	Nuchal Attachment
		Volume (cm ³)	Area (cm ²)
<i>Giraffa camelopardalis</i>	460	262.1	17.4
<i>Giraffa camelopardalis</i>	880	2298.1	30.8
<i>Giraffa camelopardalis</i>	1280	3229.0	44.6
<i>Giraffa camelopardalis</i>	1400	4715.6	53.7
<i>Giraffa camelopardalis</i>	2380	5824.7	69.9
<i>Giraffa camelopardalis</i>	2780	8222.2	71.0
<i>Okapia johnstoni</i>	2500	1214.1	106.0
<i>Diceros bicornis</i>	7850	1856.1	112.1
<i>Syncerus caffer</i>	4800	4740.4	48.7
<i>Camelus dromedarius</i>	2800	219.9	39.6
<i>Connochaetes taurinus</i>	1960	695.6	10.6
<i>Bos taurus</i>	1420	1173.1	84.7
<i>Phacochoerus aethiopi</i>	1260	403.0	72.6
<i>Tapirus terrestris</i>	1260	364.5	32.8
<i>Equus caballus</i>	1060	490.2	37.3
<i>Potamochoerus porcus</i>	540	280.8	21.7
<i>Sus scrofa</i>	240	53.2	11.6
<i>Ceratotherium simum</i>	14150	2587.7	208.8
<i>Ceratotherium simum</i>	10900	1966.8	183.5
<i>Ceratotherium simum</i>	8000	1170.1	142.3
<i>Ceratotherium simum</i>	7000	431.1	79.3
<i>Ceratotherium simum</i>	6050	748.7	99.1
<i>Ceratotherium simum</i>	5950	638.1	82.3
<i>Ceratotherium simum</i>	5050	614.9	81.6
<i>Ceratotherium simum</i>	4150	534.3	59.3
<i>Ceratotherium simum</i>	3300	301.0	55.4
<i>Ceratotherium simum</i>	1280	262.7	26.9

Figure 9: Photographs of mid-sagittal sections of an adult giraffe (*Giraffa camelopardalis*) skull showing the extent of the frontal sinus that extends over the brain case and into the occipital bone. A bony septum divides the right and left halves of the frontal sinuses and trabeculae can be seen through the sinus. Note the size of the endocranial volume in relation to the size of the skull. Scale bar = 10 cm.

Right half



Left half



Figure 10: CT scans of an adult giraffe skull showing the extent of the frontal sinus that extends from the frontal bone and caudally into the occipital bone (**A** and **H**). **C** and **D** show the lateral extension of the sinus into the temporal bone. **E** and **F** show the dorsal expansion of the sinus resulting in a dome shaped region in the skull above which the ossicones are attached. Note the presence of the bony septum and trabeculae. Scale bar = 10 cm.

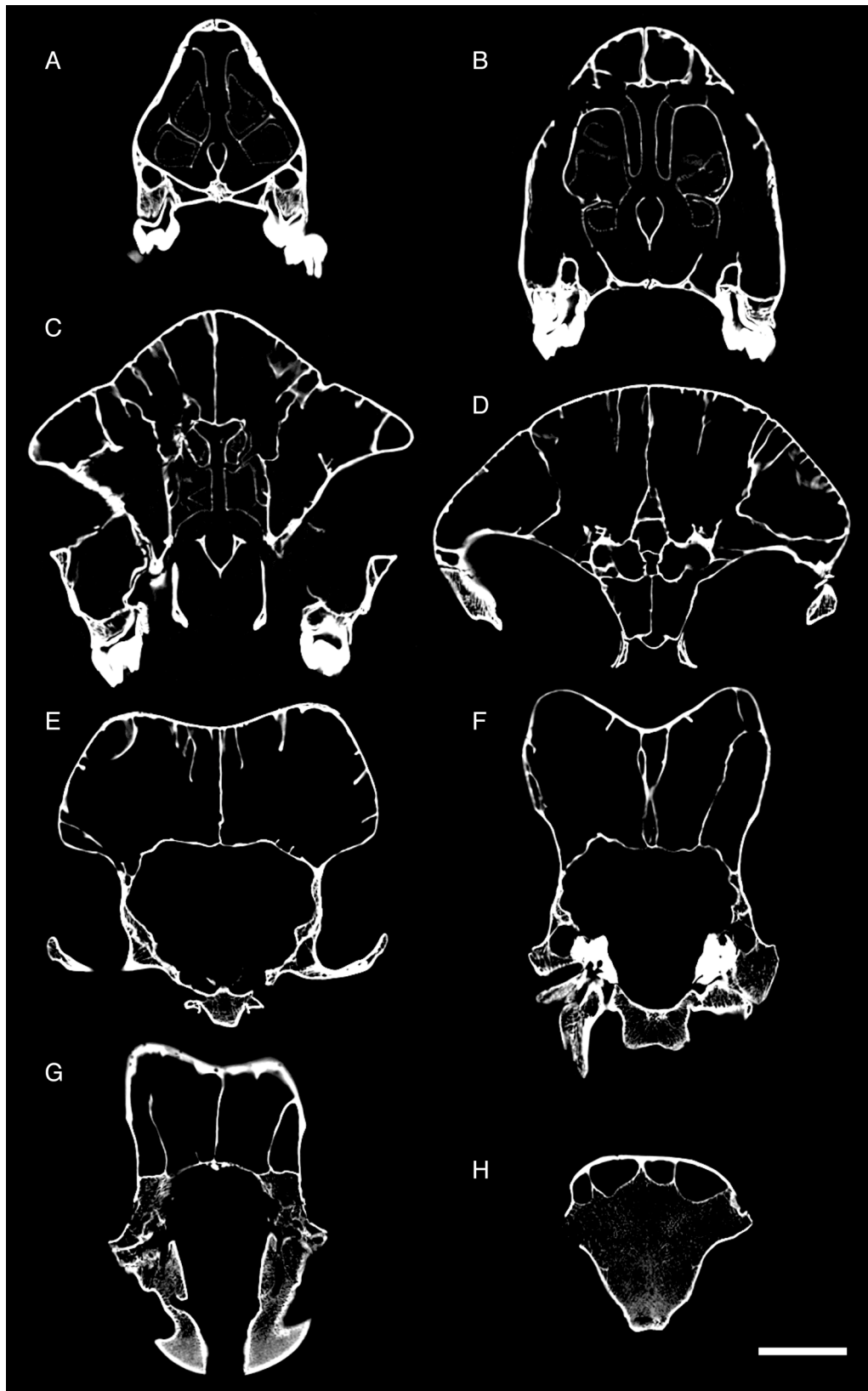
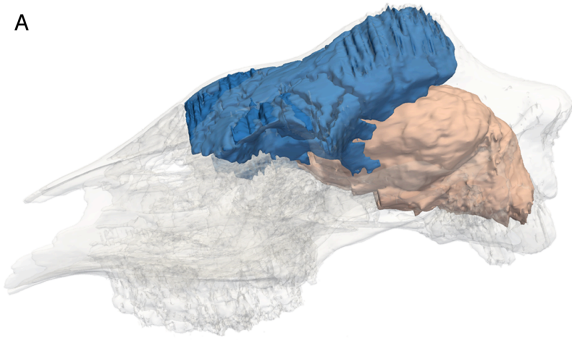
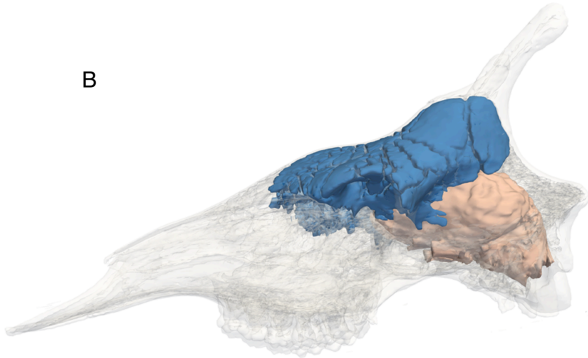


Figure 11: Digital reconstructions of giraffe (*Giraffa camelopardalis*) skulls aged newborn to adult showing the growing caudal expansion of the frontal sinus (shown in blue) as the animal matures (lateral views). In the newborn, the sinus does not extend completely over the brain case. As the animal matures, the sinus reaches the occipital bone and completely overlies the brain case (shown in orange).

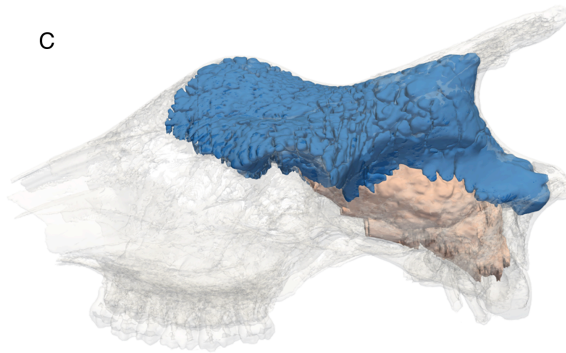
A



B



C



D

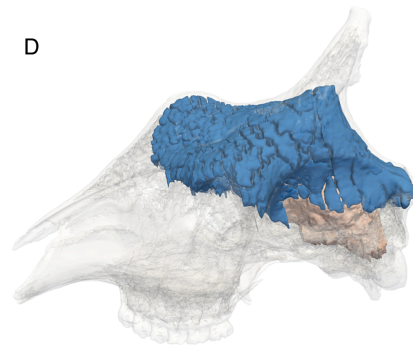


Figure 12: Digital reconstructions of a newborn (top row) and adult giraffe skulls (middle row) and an Okapi (*Okapia johnstoni*) skull (bottom row) showing the extent of the frontal sinus (shown in blue). In each row, the left images are dorsal views, the middle are lateral views and the right images are ventrolateral views. In the adult okapi, the frontal sinus does not overlie the brain case (shown in orange) as is seen in the giraffe.

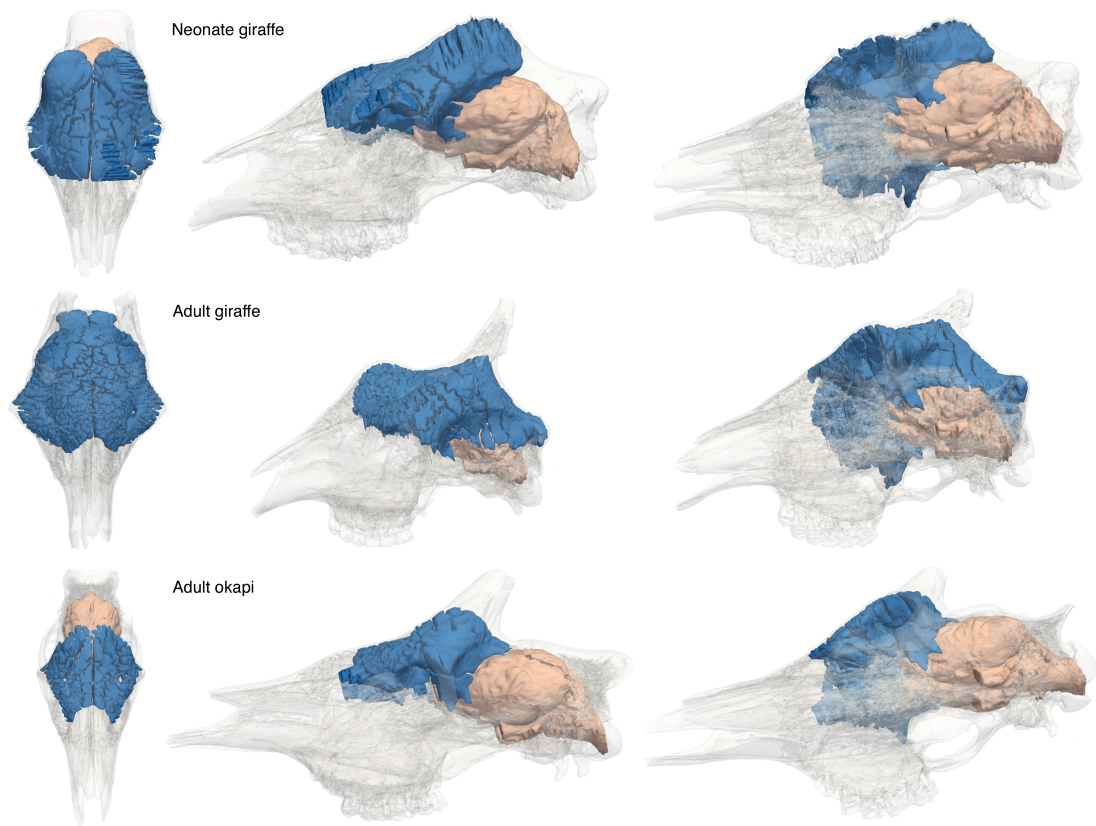


Figure 13: Digital reconstructions some of the ungulates skull studied showing the extent of the frontal sinus. In each row, the left reconstructions are dorsal views, the middle are lateral views, and the right reconstructions are ventrolateral views. The frontal sinus is shown in blue, and the endocranial cavity in orange.

Top row: Cape buffalo (*Syncerus caffer*) skull. Note the expansion of the sinus into the horns as well as over the brain case, but not extending to the caudal aspect of the occipital bone.

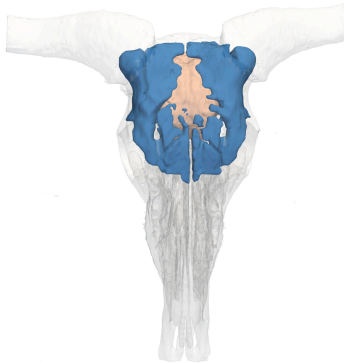
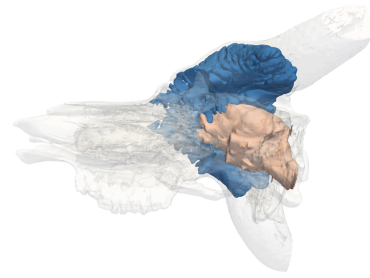
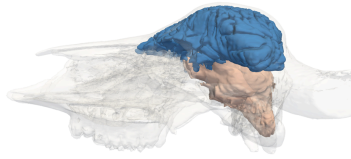
Second row: Blue wildebeest (*Connochaetes taurinus*) skull. The sinus does not extend as far into the horn as seen in the buffalo skull. There is also a region in the middle of the skull that is not occupied by the frontal sinus, which was not seen in any of the other ungulates studied.

Third row: Warthog (*Phacochoerus aethiopus*) skull. The sinus extends into the nuchal crest as well as over the brain case, possibly increasing the size of the nuchal attachment area.

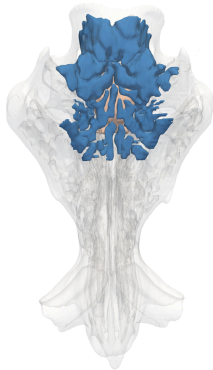
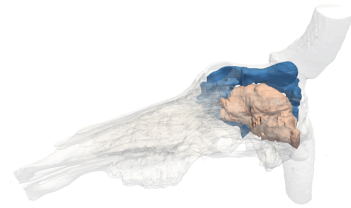
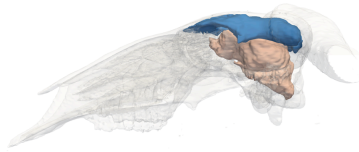
Bottom row White rhinoceros (*Ceratotherium simum*) skull. The sinus extends into the nuchal crest as well as over the brain case, possibly increasing the size of the nuchal attachment area.



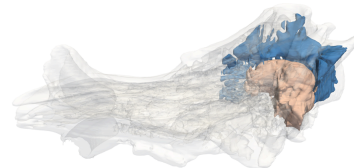
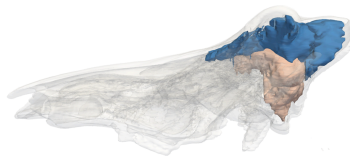
Cape buffalo



Wildebeest



Warthog



White rhinoceros

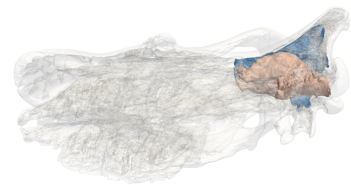
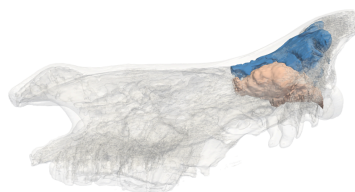
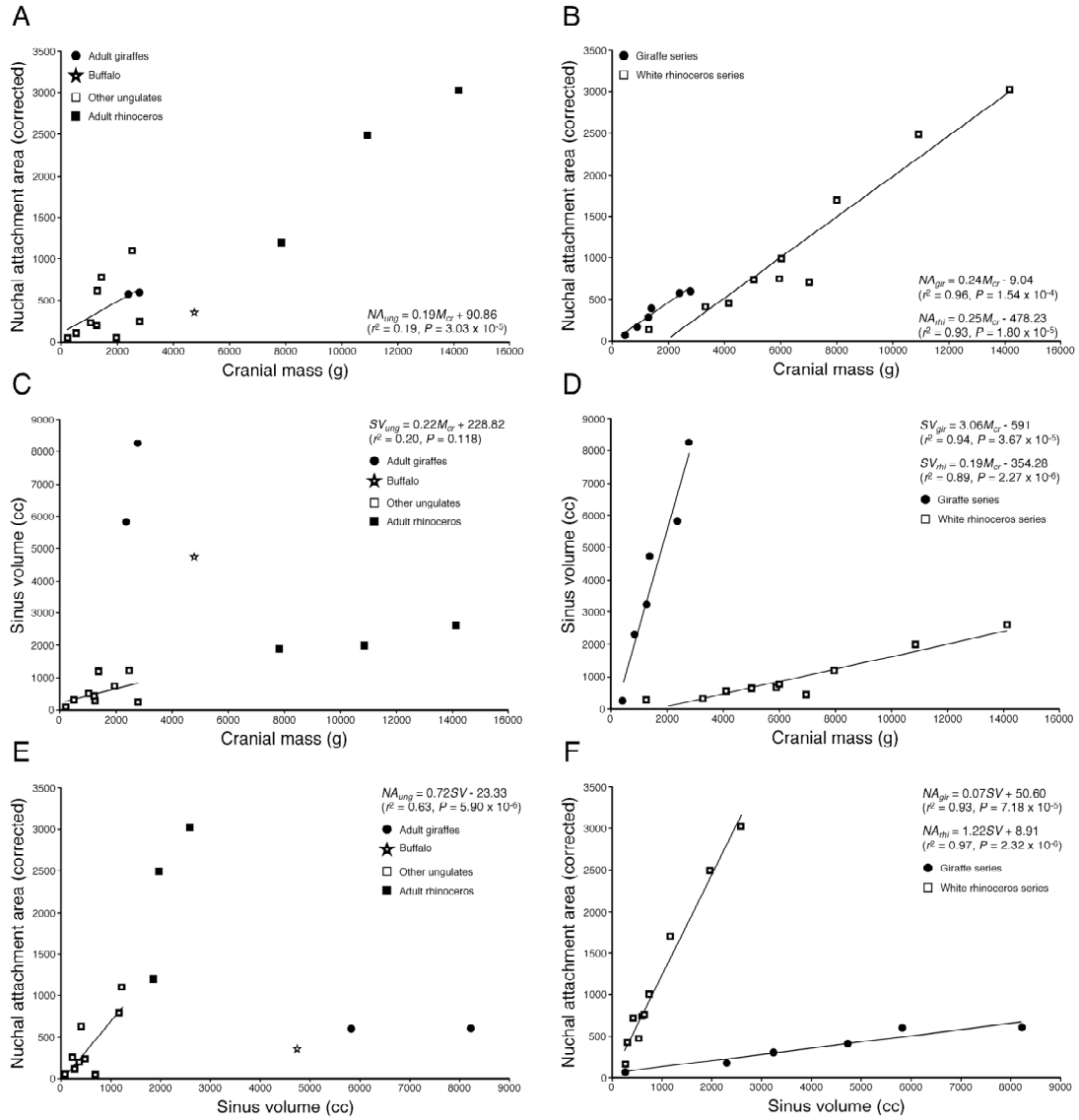


Figure 14: Graphs showing the relationships between skull mass, nuchal attachment area and frontal sinus volume in the species studied.

Top: Graph of skull mass vs. nuchal attachment area. Note that as the skull increases in mass, the extent of the nuchal attachment area increases in proportion.

Middle: Graph of skull mass vs. sinus volume. Note that for most ungulates, that as the mass increases, the volume of the sinus increases in small increments. For the giraffe and buffalo, the size of the frontal sinus is extremely large for the mass of the skull in comparison to the other ungulates studied.

Bottom: Graph sinus volume vs. nuchal attachment area. For most ungulates, the increase in sinus volume is related to large increases in the nuchal attached area, however for the giraffe and buffalo, large increases in sinus volume only lead to small increases in nuchal attachment area.



3.4 Discussion

The current series of analyses and comparisons indicate that frontal sinus of the giraffe is unusually large both in absolute and relative terms in comparison to other ungulates. In all species examined there was a strong relationship between the mass of the skull and the nuchal attachment area. This finding is to be expected, as with increasing skull mass a greater amount of the *Ligamentum nuchae* and posterior neck musculature would be required to maintain the posture and mobility of the head. In the rhinoceros and warthog, the size of the frontal sinus was strongly correlated to the size of the nuchal attachment area in a manner suggestive of a causal relationship whereby increases in the size of the frontal sinus may allow increased nuchal attachment area for greater nuchal musculature as the head increases in overall size. This supposition is supported by the observation of the frontal sinus extending into the nuchal crest in these two species. This relationship between the frontal sinus volume and nuchal attachment area appeared to hold for most of the other ungulates, but in these cases the CT scans revealed that the frontal sinus may not contribute directly to increases in nuchal attachment area. This latter observation is also the case for the giraffe and the buffalo, however in these two species there are dramatic increases in frontal sinus volume with small increases in nuchal attachment area, and this pattern differs from that seen in the other ungulates, including rhinoceros and warthog. The findings and comparisons made in this study indicate that the size of the frontal sinus in the giraffe and buffalo may have evolved to subserve an alternative function to that indicated by the study of other ungulates, especially so the rhinoceros and warthog.

3.4.1 Correlations of frontal sinus volume, nuchal attachment area and skull mass in the rhinoceros and warthog

In the developing series of rhinoceros and the adult warthog studied, the analysis demonstrated a strong correlation between increasing nuchal attachment area (NA) and increasing frontal sinus volume, with both parameters correlated to increasing skull mass. CT scans of the adult rhinoceros and warthog skulls revealed a significant intrusion of the frontal sinus into the nuchal crest. In the developing rhinoceros series the extent of the nuchal expansion of the frontal sinus increased with increasing skull mass. These observations lead to the conclusion, that at least for these two species, the increase in the frontal sinus volume may be causally related to the increase in the nuchal attachment area. This potentially causal relationship would indicate that as the skull, or wet mass of the head, increases, the need for greater support through increased nuchal musculature is accommodated for by increasing the nuchal attachment area *via* the mechanism of increasing the frontal sinus volume and its intrusion into the nuchal crest. The large horns (rhinoceros) and tusks (warthog) will substantially increase the overall mass of the head, and increasing nuchal attachment area through increasing the volume of the frontal sinus would be an advantageous way to increase nuchal attachment area while economizing on overall mass of the head. This mechanism will allow for the maintenance of head posture and mobility as the overall weight increases.

3.4.2 Correlations of frontal sinus volume, nuchal attachment area and skull mass in other ungulates studied

For the other ungulates studied (except giraffe and buffalo), the relationships described for the rhinoceros and warthog are maintained, including the relationship between nuchal attachment area and skull mass (which as mentioned earlier is common to all ungulate species). But in most cases, as revealed by CT scanning, the frontal sinus does not reach the nuchal crest. Thus, the frontal sinus may not have a direct effect on increasing nuchal attachment area as is postulated for the rhinoceros and warthog, but may be secondary in terms of biomechanical moments and transfer of skull mass to the nuchal musculature. However, it is not possible to conclude that increased frontal sinus volume will directly lead to increased nuchal musculature as is seen in the rhinoceros and warthog. In these remaining species it is difficult to assign a clear functional role in terms of biomechanics and weight bearing as is possible in rhinoceros and warthog. In these cases it may simply be that the size of the frontal sinus is related to overall skull size and the relationship seen with nuchal attachment area is not causal. This indeterminate relationship seen in these ungulate species may have been taken advantage of by the rhinoceros and warthog as a starting point allowing an increased overall head mass as required for the lifestyle of these two species.

3.4.3 Correlations of frontal sinus volume, nuchal attachment area and skull mass in giraffe

The frontal sinus in the giraffe is very extensive, but the relationships between the volume of this sinus and the other parameters of the skull measured are different to the other ungulates studied, except the buffalo (see below). Although an increase in skull mass is strongly correlated to an increase in sinus volume in the giraffe, very small increases in skull mass were matched to very large increases in sinus volume. This differs dramatically from the situation seen for the remaining ungulates studied. Moreover, small increases in nuchal attachment were also matched by very large increases in sinus volume, again differing to the relationship seen in other ungulates. These differing patterns from the rest of the ungulates studied, indicate the potential for an adaptive evolution of the increased volume of the frontal sinus in the giraffe that differs from the situation seen in ungulates in general. If this is the case, the adaptive function of this extensive sinus is yet to be determined.

Previous studies suggest that the extent of the frontal sinus in the giraffe is a result of the “frontal, parietal and supraoccipital bones [growing at] a much faster rate than the rest of the cranium” (Ganey *et al*, 1990); but this observation, confirmed in the present study does not suggest any specific adaptive function to this developmental difference to other ungulates. Others have suggested that the frontal sinus aids in thermoregulation of the brain (Ganey *et al*, 1990; Mitchell and Skinner, 2003), and Mitchell and Skinner (2003) also mention that the frontal sinus reduces the mass of the skull, while allowing for a “large head volume”, plus that a great volume “improves olfaction and chewing”, presumably by increasing the overall size of the

nasal conchae for the olfactory epithelium and increasing the attachment area on the skull for the masticatory muscles.

Thermoregulation as possible explanation for an extensive sinus is problematic due to a lack of adequate airflow within the sinus, as the openings between the frontal and nasal sinuses and the nasal meatuses do not appear large enough to sustain a rapid enough airflow to support this conclusion. Moreover, as in most ungulates, there is a rete mirabile vasculature within the base of the skull (personal observations and Mitchell and Skinner, 1993) and this is more likely to be an efficient regulator of brain temperature than air passed through the sinuses, but air passed over the nasal mucosa may serve to cool the blood in the carotid rete. The reduction of skull mass as a possible explanation for the extent of the sinus is also not entirely convincing as even with the increased size of the sinus, there will still be extra bone forming trabeculae within the sinus, as seen in the CT scans, and this will add weight, even though this additional mass would be far less than if the bone was solid. It would be far more efficient to just have a smaller skull. The masticatory function as an explanation for an extensive sinus does not appear appropriate as the muscles of mastication do not appear to extend over the increased surface area created by an increased frontal sinus volume (personal observations, L. Badlangana). In cases where extra masticatory power is required, as in carnivores, or hippopotami, one finds a significant sagittal crest for attachment of the temporalis muscle, and this is not seen in the giraffe. Moreover, being a browser and a ruminant, it is unlikely that extensive strength in the masticatory muscles is necessary.

3.4.4 Biomechanical moments of a high and heavy giraffe head

The frontal sinus may change the biomechanical properties of the giraffe skull by redirecting the planes of force needed to maintain skull posture and mobility on top of a long neck. Our results show that as the giraffe matures, the neck length (See Chapter 2), skull mass and frontal sinus volume increase. The increasing frontal sinus volume may act to distribute the increasing skull mass along the length of the head. This mass distribution may then enable the head to effectively balance on the atlas (C1 vertebra) with minimal strain (hence efficient energy expenditure) on the nuchal ligaments and muscles involved in keeping the head upright. Our results show that an increase in nuchal attachment area and frontal sinus volume are correlated in the giraffe, but a massive increase in frontal sinus volume is needed to increase the nuchal attachment area. This situation is different to that in the other ungulates studied (except buffalo), making the possibility of the frontal sinus in the giraffe enlarging to allow maximal ligament and muscle attachment on the skull unlikely. While this does not rule out the possibility that changes in the biomechanical properties of the skull/nuchal interface are caused by an increased size of the frontal sinus, it is also unlikely as an explanation for increased frontal sinus volume.

3.4.5 Infrasonic vocalisations in the giraffe

The last possibility to explain why the giraffe evolved such an extensive frontal sinus is that the sinus acts as a resonance chamber for sound production at an infrasonic level. Von Muggenthaler, *et al* (2001) presented data indicating that giraffe do produce infrasonic vocalisations. This type of sound production has also been

observed in the okapi (von Muggenthaler, *et al*, 2001). The infrasonic vocalisations in the giraffe were observed during “neck stretch” and “head throw behaviour” (von Muggenthaler, *et al*, 2001). It is possible that the enlarged frontal sinus is related to vocalization resonance, but our findings also indicate that the Okapi does not have an enlarged frontal sinus for which infrasonic vocalization was also reported. Perhaps the frequency of the infrasonic vocalizations differ between the two species, with the giraffe being capable of producing vocalizations of lower frequency than the okapi. This possibility needs to be independently tested in both species, but does represent an interesting possibility as an explanation for increased frontal sinus volume in the giraffe.

Despite these various possibilities, it is currently unclear whether there is indeed a functional purpose to the large frontal sinus in the giraffe. We do know that it is significantly larger than expected and suppose this to have a functional attribute, but further research is required to determine what this function might be.

3.4.6 The frontal sinus of the Cape buffalo

Interestingly, the Cape buffalo, although just one specimen was studied, also showed a large frontal sinus volume compared to the other ungulates studied. Again, no clear functional attributes are known. Like in the giraffe, thermoregulation, reduction of skull mass, masticatory, olfactory and biomechanical functions all seem unlikely for the same reasons given for the giraffe. Again, the large frontal sinus in the buffalo is a unique morphology and may possess functional attributes that are related to low frequency sound production, but this needs to be tested. It may be that the large frontal sinus in the buffalo, given its location anterior to the horns and dorsal

to the brain, may serve to dissipate force during butting of heads. Speculatively, there may be two functional possibilities, the first being that the buffalo is capable of the production of infrasonic vocalizations, the second is that the enlarged frontal sinus aids in the dissipation of force. Both of these possibilities can be explored by examining skulls of different sexes and ages and through recording of the vocalizations of buffalo herds. The potential for infrasonic vocalization in the buffalo and its relationship to consensus decision making by the herd in terms of where to graze, and when to move, presents an interesting possibility (Prins, 1996; Conradt and Roper, 2005).

Chapter 4: The catecholaminergic and serotonergic systems of the medulla of the giraffe (*Giraffa camelopardalis*)

4.1 Introduction

The phenotypically unusual long neck of the giraffe (*Giraffa camelopardalis*) (see Chapter 2), which can be well over 1 m long, presents a range of potential problems for a variety of neural systems. The spinal cord terminates at the sacral level (see Chapter 5) and in itself can be over 1.5 m in length in sub-adult giraffe. Furthermore, there are nerves that project to the viscera such as the vagus and phrenic nerves that presumably have significantly longer pathways than would be the case in other Artiodactyla and mammals. In this study, the catecholaminergic and serotonergic systems of the medulla of the giraffe were examined using immunohistochemistry for anti-tyrosine hydroxylase to reveal catecholaminergic neurons and anti-serotonin to reveal serotonergic neurons.

The specific nuclei forming the catecholaminergic systems normally observed in the medulla of mammals are the rostral ventrolateral tegmental group (C1), the caudal ventrolateral tegmental group (A1), the rostral dorsomedial group (C2), the caudal dorsomedial group (A2), and the area postrema (Smeets and Gonzalez, 2000). Significantly, of these nuclei, the C1 neurons project to the intermediolateral cell column and the intermediate grey matter of the spinal cord (Smeets and Gonzalez, 2000), and the neurons of area postrema are involved in the regulation of heart rhythm and innervate smooth muscle (Koizumi and Brooks, 1974). Given the spinal projection of the C1 neurons, and the involvement of the area postrema in vascular

control the phenotype of the giraffe may pose a challenge that is reflected in the anatomy of these catecholaminergic nuclei in comparison to other mammals.

While the majority of these catecholaminergic nuclei have been observed in the medulla of sheep (Tillet and Thibault, 1989), it has been reported that sheep lack the C1 group (Tillet, 1988). The reported absence of C1 in sheep is based on a lack of phenylethanolamine-*N*-methyltransferase (PNMT) immunopositive cells in the region where one would expect these neurons to be found. Despite this, Tillet and Thibault (1989), using dopamine- β -hydroxylase (DBH) and tyrosine hydroxylase (TH) immunohistochemistry revealed neurons that are found in a location of the medulla that might be considered C1. One difficulty with these studies is that Tillet (1988) has defined C1 in a way that is based solely on the lack of PNMT immuno-reactivity. While in many mammals the neurons of C1 are immunoreactive for PNMT (Smeets and Gonzalez, 2000), this may not be the case for the C1 neurons of the sheep, as a conformational change in the 3-D structure of the protein reacting with the PNMT antibody may have occurred, leading to a potential false negative in this case. But if there is really a lack of a C1 in the sheep, the identity of the neurons that are immunoreactive to both TH and DBH in the region where C1 neurons would be expected to be located in sheep needs to be established (Tillet and Thibault, 1989). However, if the C1 group does not exist in sheep, the potential challenge of these neurons projecting to the spinal cord may not exist for the giraffe, as the results described for the sheep may indicate that the Artiodactyla as an order lack a C1 group (Manger 2005).

All serotonergic neurons of the medulla, the caudal serotonergic cluster, project to the spinal cord (Tork, 1990). There are normally five serotonergic nuclei found in the medulla which are: the raphe magnus nucleus (RMg), raphe pallidus

nucleus (RPa), raphe obscurus nucleus (ROb) and the rostral ventrolateral (RVL) and caudal ventrolateral (CVL) groups (Tork, 1990; Bjarkam, 1997), all of which are found in sheep (Tillet, 1987). The neurons of RMg project to laminae I and II of the dorsal horn of the spinal cord; RPa and ROb neurons project to layers VIII and IX of the ventral horn of the spinal cord; and the axons of the RVL/CVL neurons project to the intermediolateral column of the spinal cord (Tork, 1990). This intermediolateral projection is of interest specifically in the giraffe in terms of its relationship to blood pressure (Howe, et al, 1983). Again, the giraffe phenotype may pose a challenge to the caudal serotonergic neurons in that they must project over a large distance in order to maintain their specific functions in the spinal cord and this may cause changes in the morphology and organization of these neurons.

4.2 Materials and Methods

One sub-adult giraffe (*Giraffa camelopardalis*) was used in the present study. The animal was male, approximately 400 kg and 2 years old, and died during the process of capture for relocation. The animal was treated in accordance to the University of the Witwatersrand Animal Ethics Committee guidelines for the care and use of animals in scientific experiments. Appropriate permissions for use of the animal were also obtained from the local provincial governmental bodies responsible for wildlife management in South Africa. Upon death, the giraffe was immediately perfused with 0.9% normal saline solution (200 l, or approximately 0.5 l/kg) to flush the blood. This process took approximately 20 mins. This was immediately followed with a perfusion of 200 l of 4% paraformaldehyde in 0.1M phosphate buffer (PB) for fixation, a process which again took about 20 mins. The saline rinse and fixative was

manually pumped through tubes inserted into the carotid arteries. The rinse and fixative was directed rostrally within the arteries, and thus caudally these arteries were severed to allow the escape of blood and excess rinse and fixative. Following fixation the brain as well as the spinal cord was removed. The whole brain and the entire length of the spinal cord were clear of blood and rubbery to the touch indicating a successful whole body perfusion. The perfusion was carried out in the field, with excess fixative being collected using a plastic tarpaulin that was placed under the animal and stored in drums for later appropriate waste disposal. After weighing the brain (509 g), it was then immersed overnight in the buffered paraformaldehyde for further fixation. It was then allowed to equilibrate in 30% sucrose solution in 0.1M PB, and subsequently stored in antifreeze solution at -20°C . The medulla was dissected from the remainder of the brain (Fig. 15) and allowed to re-equilibrate in sucrose solution and then frozen in crushed dry ice.

Serial 50 μm sections of the medulla were made in a coronal plane using a freezing sledge microtome. A one in four series of sections was stained for Nissl (cresyl violet), myelin (modified from Gallyas, 1979), serotonin, and tyrosine hydroxylase (TH) (e.g. Manger et al., 2003, 2004; DaSilva et al., 2006). For immunocytochemical staining, the sections were treated first with an endogenous peroxidase inhibitor (49.2% methanol:49.2% 0.1M PB:1.6% 30% H_2O_2) for 30 min, followed by three 10 min rinses in 0.1M PB. This was followed by a 2 h preincubation (on a shaker), at room temperature, in a solution containing 3% normal goat serum (NGS) (Chemicon Int.), 2% bovine serum albumin (Sigma), and 0.25% Triton X100 (Merck) 0.1M PB. After preincubation, the sections were placed in primary antibody solution containing the appropriately diluted antibody in 0.25%

Triton X-100 in 0.1M PB, 3% NGS, and 2% bovine serum albumin, for 48 hr at 4°C on a shaker.

To reveal catecholaminergic neurons we used anti-tyrosine hydroxylase primary antibody (TH) (AB151, Chemicon) at a dilution of 1:6500. To reveal serotonergic neurons we used anti-serotonin primary antibody (AB938, Chemicon) at a dilution of 1:10000. After the 48 h incubation in primary antibody solution, the sections underwent three 10 min rinses in 0.1M PB, and were then placed in a secondary antibody solution for 2 h at room temperature. The secondary antibody solution contained a 1:500 dilution of biotinylated anti-rabbit IgG (BA 1000, Vector Labs), with 3% normal goat serum, and 2% bovine serum albumin, in 0.1M PB. After a further three 10 min rinses in 0.1M PB, the sections were incubated in AB solution (Vector Labs) for 1 h and rinsed in three 10 min rinses of 0.1M PB. The sections were then treated in a 0.05% solution of 3,3'-diamino-benzidine (DAB) in 0.1M PB for 5min. This was followed by adding 3 µl of 30% H₂O₂ per 0.5ml solution. The development process was followed visually and checked under a low power stereomicroscope. The staining was allowed to continue until the background staining was at a level that would enable us to match architectonic features of the sections with the adjacent Nissl and myelin stained sections without obscuring the immunopositive neurons. The development was stopped by placing the sections in 0.1M PB and then rinsing them in twice in 0.1M PB.

The sections were mounted on 0.5% gel coated glass slides and left to dry overnight. They were then dehydrated in a graded series of alcohols, cleared in xylene and coverslipped with Depex mounting medium. Two controls were employed during the immunostaining. The first control consisted of omitting the primary antibody, the second omitted the secondary antibody.

The sections were examined under a low power stereomicroscope and the architectonic borders were traced according to the nissl and myelin sections using a camera lucida. After completing the traces, the immunoreacted sections were matched to the drawings and the immunopositive neurons marked. Immunopositive neurons were those that clearly showed stained soma and dendrites. The drawings were scanned and redrawn using the Canvas 8 drawing program.

High power photomicrographs were taken using a digital camera mounted on a Zeiss Axioskop. No pixilation adjustments or manipulations of the captured images were made, except for brightness and contrast adjustments using Adobe Photoshop 7. In this study we used the nomenclature suggested by Dahlstrom and Fuxe, (1964) and Hokfelt, *et al.*, (1984) in conjunction with that provided by Smeets and Gonzalez (2000) and Tillet and Thibault (1989) for the catecholaminergic system, and that suggested by Tork (1990) for the serotonergic system (as opposed to that provided by Dahlstrom and Fuxe, 1964; Tillet, 1987; and Bjarkam et al., 1997) (see Table 6 for comparative nomenclatures).

Abbreviations	
AP	area postrema
Cu	nucleus
DC	dorsal cochlear nucleus
DMS	dorsomedial spinal trigeminal nucleus
ECu	external cuneate nucleus
Flm	medial longitudinal fasciculus
Ge5	gelatinous layer of the caudal spinal
Gr	gracile nucleus
icp	inferior cerebellar peduncle
io	inferior olive
LRt	lateral reticular nucleus
MdD	medullary reticular nucleus dorsal part
MdV	medullary reticular nucleus ventral part
n.Amb	nucleus ambiguus
oc	olivocerebellar tract
py	pyramidal tract
pyx	pyramidal decussation
Sp5	spinal trigeminal tract
Sp5c/ SpVc	spinal trigeminal nucleus caudal part
SpVe	spinal vestibular nucleus
vc	ventral cochlear nucleus

Table 6: Comparative nomenclature of the catecholaminergic and serotonergic systems in previous studies.

Name of nucleus in present study	Smeets and Gonzalez, 2000	Tillet and Thibault, 1989	Hokfelt et al., 1974	Dahlstrom and Fuxe, 1964	Tork, 1990	Tillet, 1987	Bjarkam et al, 1997
Catecholaminergic Nuclei							
C1	Rostral ventrolateral tegmental group	Group C1	C1				
C2	Rostral dorsomedial group	Group C2	C2				
A1	Caudal ventrolateral tegmental group	Group A1	A1	A1			
A2	Caudal dorsomedial group	Group A2	A2	A2			
Area postrema	Area postrema	Area postrema	Area postrema	Area postrema			
Serotonergic Nuclei							
Raphe magnus nucleus, RMg				Nuc. Raphe Magnus, RM, part of B3	Raphe magnus nucleus, RMg	Group B1/B3	Raphe magnus nucleus, NRM
Raphe pallidus nucleus, RPa				Nuc. Raphe pallidus, RP, part of B1	Raphe pallidus nucleus, RPa	Group B1/B3	Raphe pallidus nucleus, NRP
Raphe obscurus nucleus, ROb				Nuc. Raphe obscurus, RO, B2	Raphe obscurus nucleus, ROb	Group B2	Raphe obscurus nucleus, NRO

Rostral ventrolateral medulla, RVL				Rostral ventrolateral medulla;part of B3/B1	Rostral ventrolateral medulla, RVL	Group S2	Rostral ventrolateral medulla, NMVR
Caudal ventrolateral medulla, CVL				Caudal ventrolateral medulla;part of B3/B1	Caudal ventrolateral medulla, CVL	Group S1	Caudal ventrolateral medulla, NMVC

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Please notice that C1-C2 were discovered by TH,DBH and PNMT IR .They could not be observed in our original mapping with CA fluorescence histochemistry. (Dahlström and Fuxe 1964).We assume they contain adrenaline but in too low amounts to be demonstrated with the CA fluorescence histochemistry of Falck and Hillarp.This is still a hypothesis.

4.3 RESULTS

The giraffe brain is a typically mammalian brain with the medulla found in the location expected for any mammal (Fig. 15). The present study found immunocytochemically positive catecholaminergic (TH+) and serotonergic (5-HT+) neurons throughout the medulla of the giraffe. The TH+ neurons were for the most part similar to those seen in many other mammals previously studied (Tillet and Thibault, 1989; Smeets and Gonzalez, 2000). There was however, one major difference, this being an apparent lack of the C3 subdivision typically found in rodents (Dahlstrom and Fuxe, 1964; Smeets and Gonzalez, 2000). The serotonergic positive neurons were very similar to those seen in other mammals (Dahlstrom and Fuxe, 1964; Tork, 1990; Tillet, 1987; Bjarkam et al., 1997). The TH+ and 5-HT+ neurons that were observed are described from the rostral to the caudal aspect of the medulla.

4.3.1 Catecholaminergic neurons

The catecholaminergic neurons revealed with TH immunocytochemistry were readily divided into five nuclei based on their anatomical location and neuronal morphology. We found evidence indicating the presence of the C1, A1, C2, A2 and area postrema nuclei.

C1, Rostral Ventrolateral Tegmental Group

The C1 neurons are found in the rostral ventro-lateral portion of the medullary tegmentum extending from the floor of the medulla to approximately the mid dorso-ventral level of the medullary tegmentum. At their most rostral extent they are found surrounding the caudal pole of CNVII (Fig. 16, B-D). Caudal to this they are seen ventral to and surrounding nucleus ambiguus, and the caudal pole of the nucleus ambiguus is the most posterior level at which neurons that form part of this nucleus could be identified (Fig. 16, E-G). The most medially located neurons of this nucleus are found in a position just lateral to the inferior olive and they extend to just lateral of the lateral edges of CNVII and nucleus ambiguus (Fig. 16, B-H). The neurons are for the most part ovoid in shape and range from bipolar to multipolar forms (Fig. 17A). No specific orientation to the dendrites could be observed. The TH⁺ neurons exhibit a low to moderate density throughout the extent of this nuclear grouping.

A1, Caudal Ventrolateral Tegmental Group

The TH⁺ neurons assigned to the A1 nucleus are found as a caudal continuation of those that form the C1 group (Fig. 16H). They are found from the level of the posterior pole of nucleus ambiguus to the anterior level of the cervical spinal cord within the medullary tegmentum (Fig. 16, H-L). They occur in a position that can be described as significantly more lateral within the medullary tegmentum as compared with the neurons the C1 nucleus and extend from the ventrolateral edge of the medulla in a dorsomedial direction approximately halfway across the medullary tegmentum (Fig. 16, K-H). They are found in the region of the lateral reticular

nucleus (LRt) of the medullary tegmentum (Fig. 16, I-L). These TH⁺ neurons are ovoid in shape, range between bipolar and multipolar forms (Fig. 17B), and exhibit no specific dendritic orientation. They have a very low density throughout the range in which they are found.

C2, Rostral Dorsomedial Group

The rostral dorsomedial group is represented by a relatively substantial number of TH⁺ neurons located in the dorsal aspect of the medulla in a position just lateral to the area postrema (see below) and adjacent to the floor of the fourth ventricle. This nuclear cluster is found dorsal and lateral to the anterior pole of CNNX (Fig. 16G). The antero-posterior, medio-lateral, and dorso-ventral extents of this nucleus are less than 1 mm. Within this region the TH⁺ neurons show a moderate to high density. All neurons observed were ovoid in shape and bipolar (Fig. 17C). The dendrites were orientated parallel to the floor of the 4th ventricle.

A2, Caudal Dorsomedial Group

The number of TH⁺ neurons that could be reliably identified as belonging to the A2 nucleus were very few and only found over a very restricted antero-posterior extent. Those neurons assigned to this nucleus were found lying between CNNX and CNNXII close to the central canal (Fig. 16K) and close to the level of the spinal cord at the level of the pyramidal decussation, and slightly lateral to this location within the dorsal medullary tegmentum. These neurons were ovoid and bipolar and exhibited a very low density and had no specific dendritic orientation.

Area postrema

The area postrema was found at the same antero-posterior level as the C2 nucleus, but was located at the midline dorsal to the anterior most part of CNNX and forming part of the floor of the fourth ventricle (Fig. 16, G&H). The TH+ neurons that demarcate the area postrema form two distinct clusters on either side of the midline, but there are several neurons located between these two main clusters, across the midline, that merge at the left and right halves of this distinct structure. There is a moderate to high density of small ovoid to circular shaped TH+ soma that show a mixture of bipolar and multipolar forms (Fig. 17D). The majority of the neurons are bipolar and the dendrites are orientated in a medio-lateral aspect parallel to the floor of the fourth ventricle.

4.3.2 Serotonergic neurons

The serotonergic immunopositive (5-HT+) neurons were readily divided into four nuclei based on their anatomical location and neuronal morphology. These were the: raphe magnus, raphe pallidus, rostral and caudal ventrolateral, and raphe obscurus nuclei.

RMG, Raphe Magnus Nucleus

The neurons of the raphe magnus were found close to the midline forming two columns either side of the midline (Fig. 16A&B). The 5-HT+ neurons forming this

nucleus were first observed at the level of the trigeminal motor nucleus, and continued caudally to the anterior most level of the nucleus ambiguus (Fig. 16, A-E). The neurons comprising this nucleus were all piriform in shape and bipolar (Fig. 18A). For those neurons located immediately adjacent to the midline, the dendrites were orientated dorso-ventrally. The dendrites of those neurons located within 500 μm of the midline were orientated medio-laterally. The neurons had a low density throughout the extent of the nucleus.

RPa, Raphe Pallidus Nucleus

The 5-HT⁺ neurons that form the raphe pallidus nucleus were all found in and around the pyramidal tracts (Fig. 16, A-M). This nucleus exhibited an antero-posterior extent that ranged from the level of the caudal pole of CNVII to the cervical spinal cord (Fig. 16, A-M). The neurons of this nucleus were never seen beyond the inferior olive, either laterally or dorsally. These neurons were medium to large in size and multipolar (Fig. 19A). No distinct orientation of the dendrites could be readily observed.

RVL and CVL, Rostral and Caudal Ventrolateral Nuclei

These two nuclear groups are described together as they appear to be continuous in their distribution and the distinction between them is more or less arbitrary based on anatomical location more so than connections or function (Fig. 16, A-L). The rostrocaudal boundary given for these two nuclei is the trapezoid body in

the rabbit (Bjarkam et al., 1997), but other descriptions do not provide a clearly delimited boundary (e.g. Tork, 1990). The 5-HT+ neurons assigned to these nuclei are found from the level of CNNVII through to the cervical spinal cord (Fig. 16, A-L). The neurons are for the most part found in the ventral lateral portions of the medullary tegmentum, extending approximately 4 mm lateral to the inferior olive. Here they form a continuous antero-posterior column along their extent in this locality.

Anterior to the inferior olive, the neurons of the putative RVL sweep across the midline above the pyramidal tracts, fusing the anterior portions of the RVL. The RVL is then split into left and right halves by the paired inferior olives and the appearance of the raphe pallidus nucleus (Fig. 16, D-A). The RVL continues caudally in the ventrolateral medullary tegmentum to the level of the caudal pole of the nucleus ambiguus, where, somewhat arbitrarily, we can demarcate the beginning of the putative CVL (Fig. 16, D-H). The CVL neurons continue this ventrolateral medullary column of 5-HT+ neurons to the level of the cervical spinal cord (Fig. 16, H-L).

The neurons forming these two nuclei are most numerous anteriorly and steadily decline in number caudally. The neurons of these nuclei are medium to large in size and are multipolar (Fig 18, B-D; Fig 19B), giving the soma a stellate appearance. The dendrites exhibit a rough medio-lateral orientation. At the most anterior extent of the RVL there is a small cluster of 5-HT+ neurons in a position just lateral to CNNVII, and while the neuronal morphology is similar to that of the other neurons of these nuclei, the dendrites are not oriented in any specific direction.

ROb, Raphe Obscurus Nucleus

The 5-HT+ neurons of ROb are seen to form two columns either side of the midline between CNXII and the inferior olive (Fig. 16, I-K). They are found from the level of the exiting hypoglossal nerve to the anterior most portion of the cervical spinal cord (Fig. 16, I-K). Like RMg, there are neurons located immediately adjacent to the midline and those that are a short distance away from the midline. The neurons are found in a low to moderate density throughout their distribution and all are multipolar. Those neurons located immediately adjacent to the midline are fusiform in shape (Fig. 19C) and the dendrites are orientated dorsoventrally. The neurons located a short distance from the midline are triangular in shape and there is no specific orientation to the dendrites.

Figure 15: Photograph of the left side of the giraffe brain (lateral view) showing the region of the brainstem investigated in the present study.

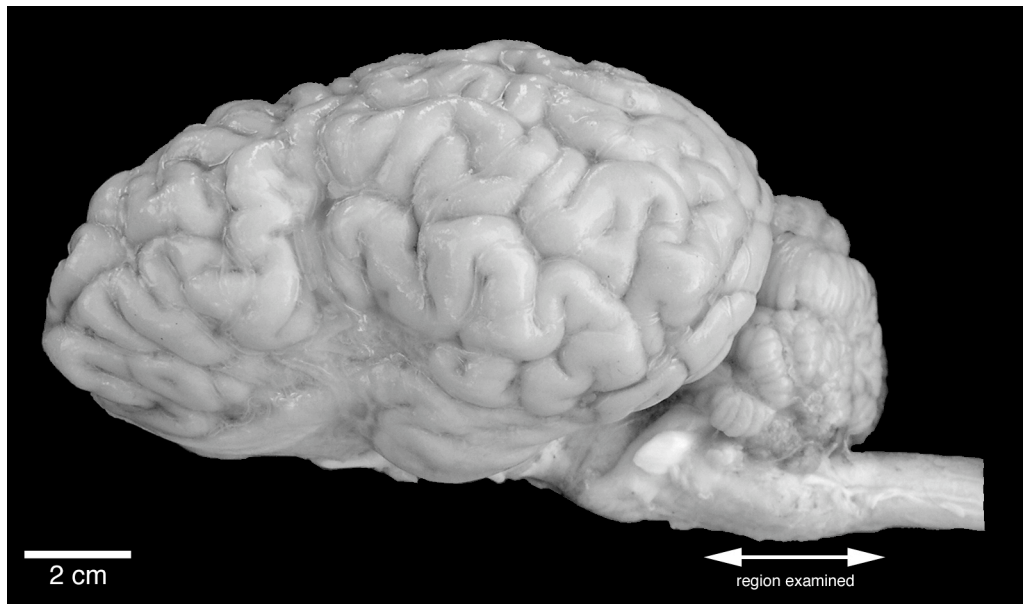
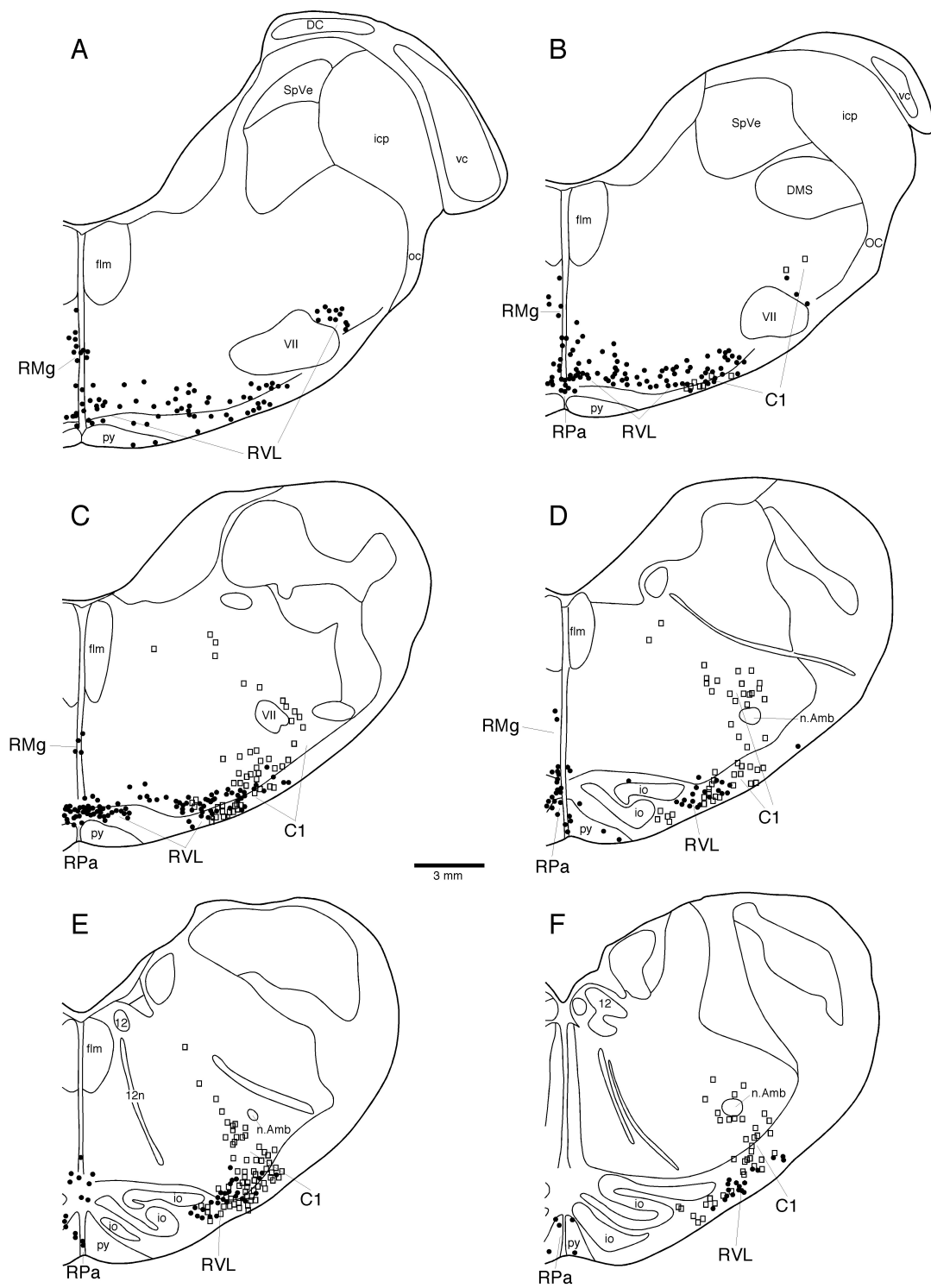


Figure 16: Diagrammatic reconstructions of the left half of the giraffe medulla. The open squares represent neurons immunoreactive to tyrosine hydroxylase and the filled circles represent neurons immunoreactive to serotonin. Section A is the most rostral, M the most caudal, and each section is approximately 1000 μm apart.



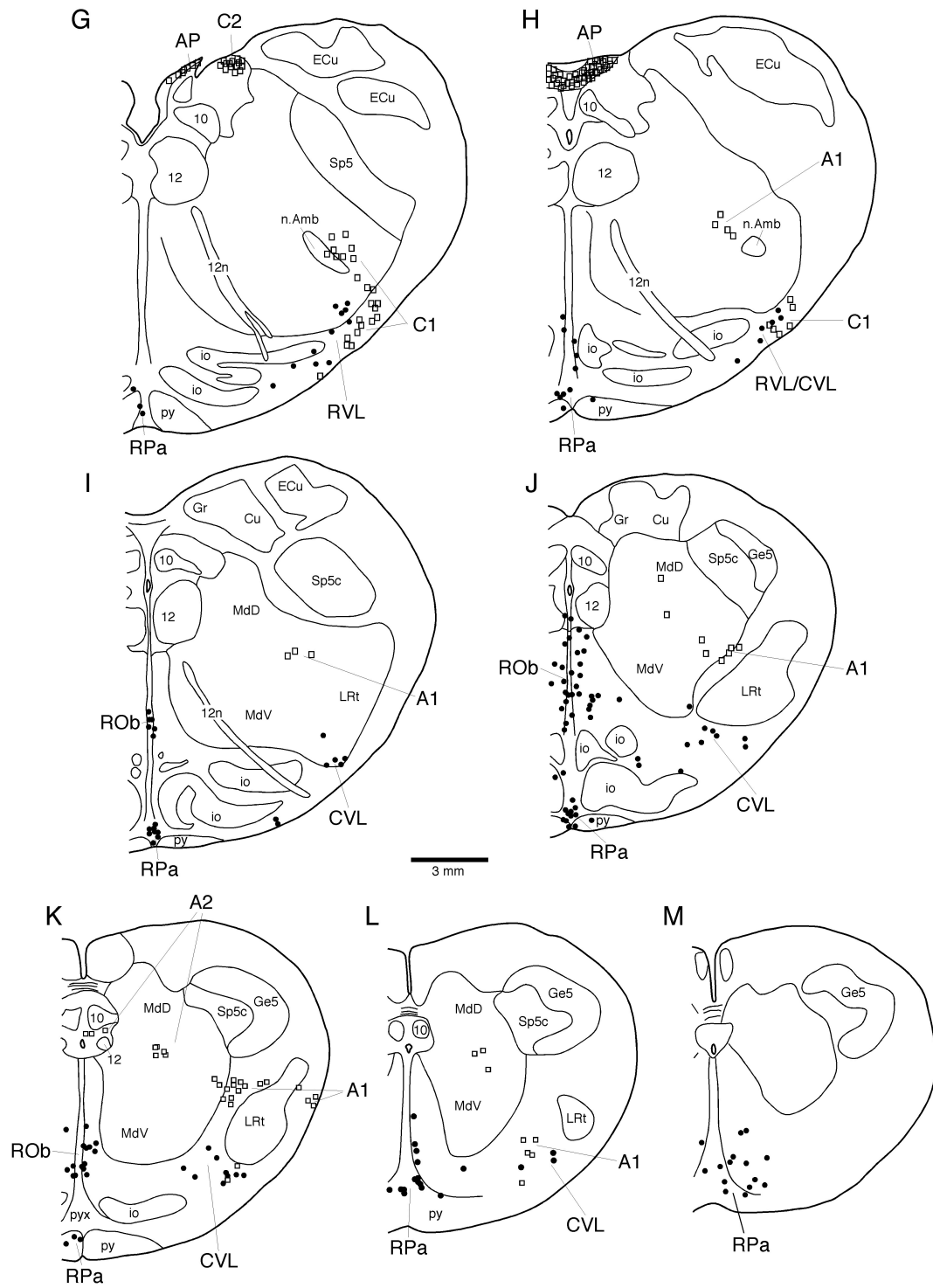


Figure 17: Photomicrographs of the giraffe medulla showing neurons immunoreactive to tyrosine hydroxylase in four locations. **A** – C1, rostral ventrolateral group; **B** – A1, caudal ventrolateral group; **C** – C2, rostral dorsomedial group, and **D** – area postrema. Note the high density of neurons in area postrema. Scale bar = 200 μm .

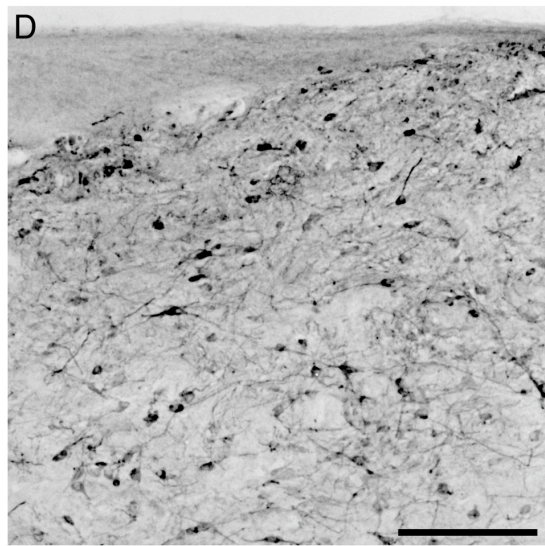
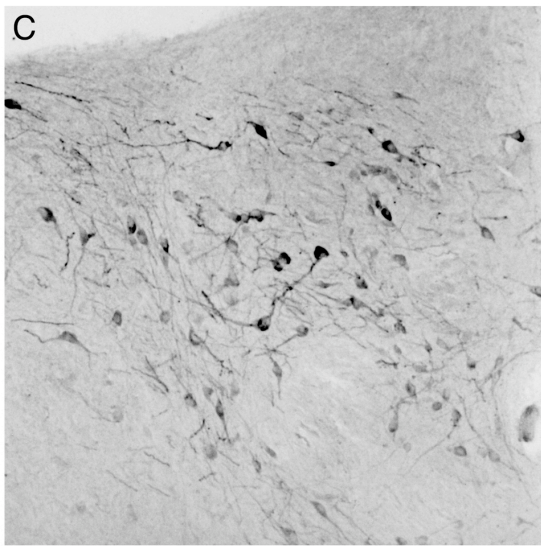
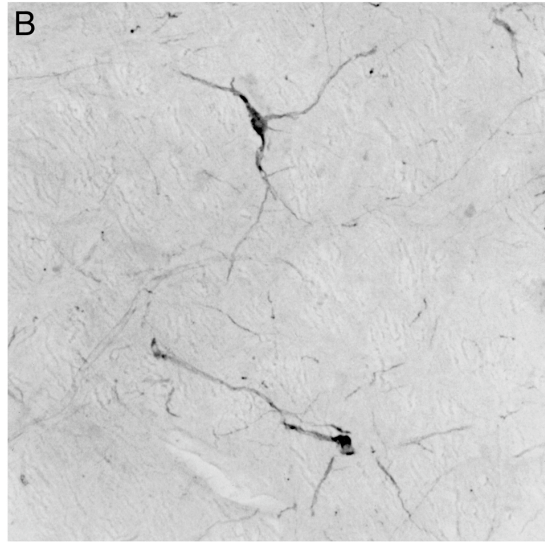
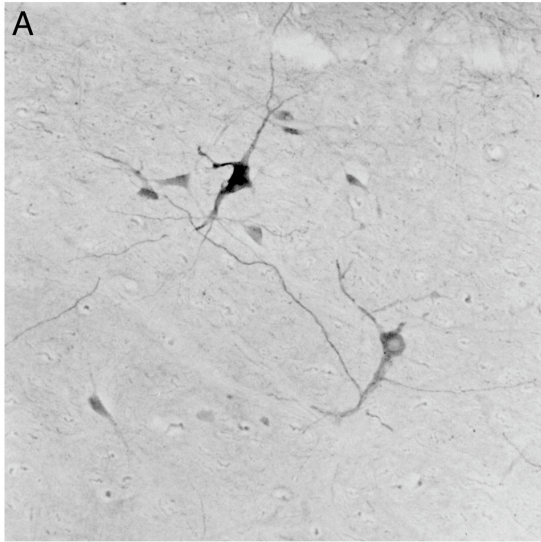


Figure 18: Photomicrographs of the giraffe medulla showing neurons immunoreactive to serotonin in four locations. **A** – RMg, raphe magnus nucleus; **B** – RVL, rostral ventrolateral group, medial portion; **C** – RVL, rostral ventrolateral group, middle portion, and **D** – RVL, rostral ventrolateral group, extreme lateral portion – located lateral to facial nucleus. Note the varying orientation of the dendrites in the different regions. Scale bar = 200 μm .

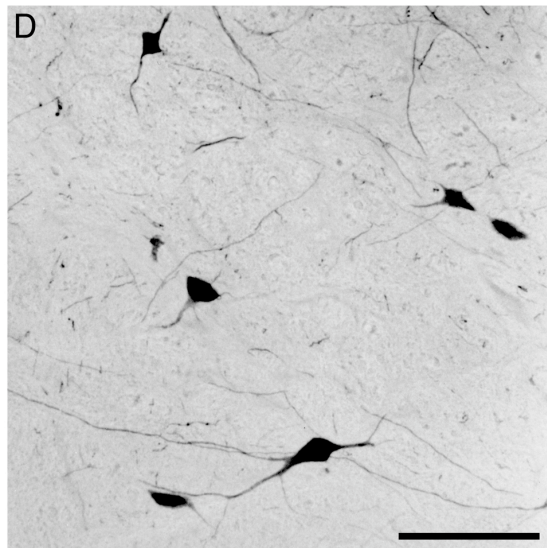
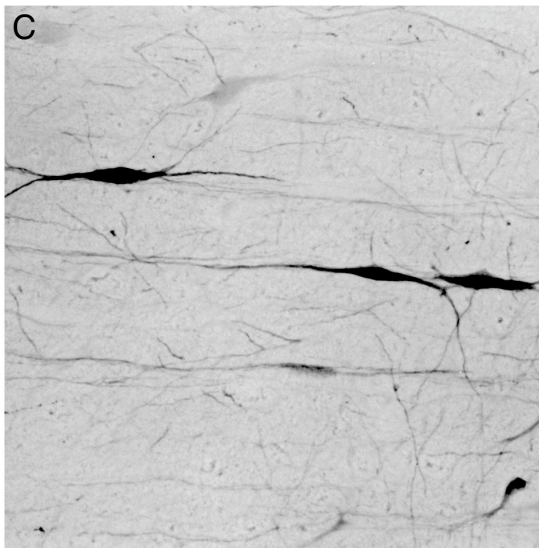
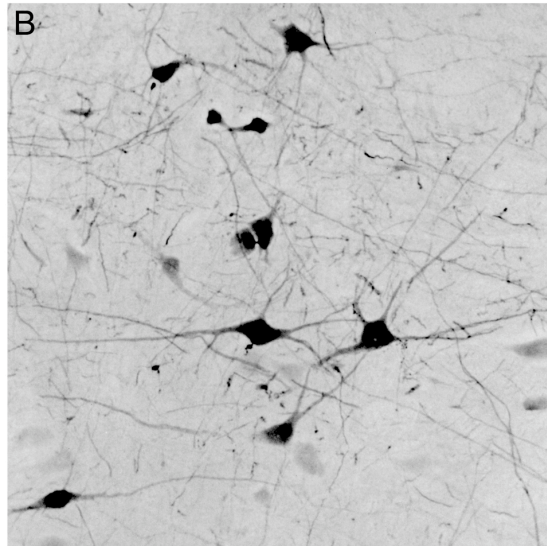
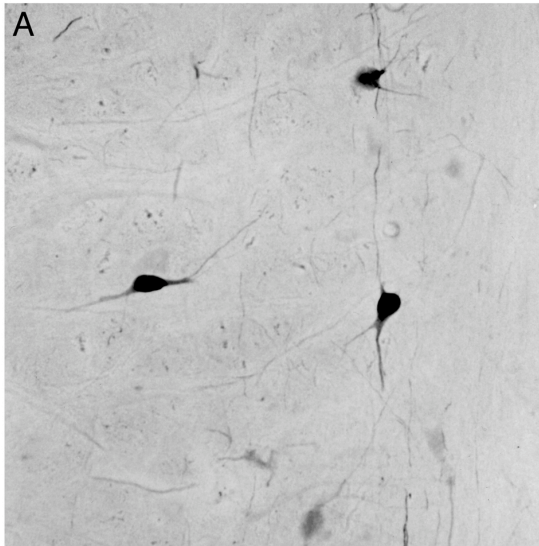
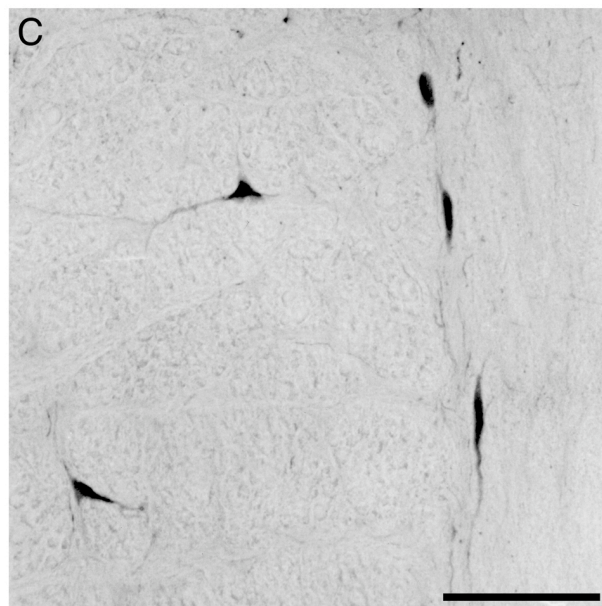
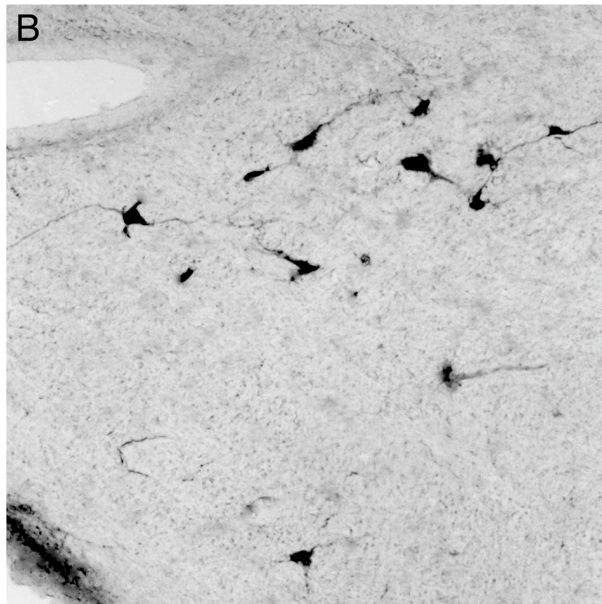
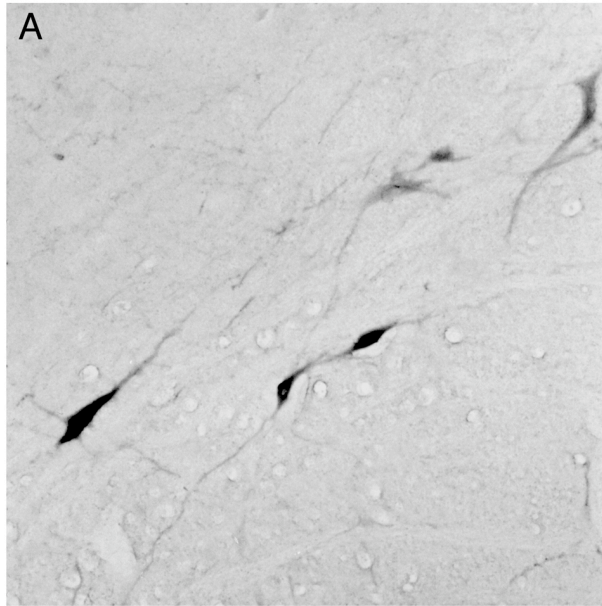


Figure 19: Photomicrographs of the giraffe medulla showing neurons immunoreactive to serotonin in three further locations (see Figure 4). **A** – RPa, raphe pallidus nucleus; **B** – CVL, caudal ventrolateral group; **C** – ROb, raphe obscurus. Note the differing shape of the soma and dendritic orientation between the neurons closest to the midline (fusiform and dorsoventral) as opposed to those away from the midline (triangular and irregular) in the ROb. Scale bar = 200 μ m.



4.4 DISCUSSION

Overall our results show that the catecholaminergic and serotonergic neurons in the medulla of the giraffe are very similar to those seen in the sheep (Tillet, 1987, Tillet, 1988 and Tillet and Thibault, 1989). The catecholaminergic nuclei found in the giraffe medulla include C1, A1, C2, A2 and area postrema regions. We identified the C1 nuclei in the giraffe medulla with tyrosine hydroxylase immunohistochemistry, and this was similarly identified in the sheep (Tillet and Thibault 1989), but in sheep, this nucleus is not as evident with phenylethanolamine *N*-methyltransferase (PNMT) immunohistochemistry (Tillet, 1988). The serotonergic nuclei found in the giraffe medulla include RMg, RPa, RVL/CVL and ROb, all of which were recorded for the sheep (Tillet, 1988) and indeed most other mammals (Tork, 1990).

4.4.1 Catecholaminergic nuclei

The catecholaminergic nuclei found in the giraffe medulla are similar to those seen in most other mammals. The two exceptions are the lack of A3 and C3 nuclei, seen in the lab rat (Dahlstrom and Fuxe, 1964). The A3 nucleus however, has not been found in the lab rat or any other mammal studied with immunohistochemistry (Smeets and Gonzalez, 2000). Thus, the absence of an A3 nucleus in the giraffe medulla is not surprising. The C3 nucleus has been reported to be found in rats, hamsters, and neonatal swine (Smeets and Gonzalez, 2000); however, it has not been reported in monotremes (Manger et al., 2002a), sheep (Tillet and Thibault, 1989), rabbit (Blessing et al., 1981), cat (Poitras and Parent, 1978; Blessing et al., 1980), dog (Barnes et al., 1988; Dormer et al., 1993), tree shrew (Murray et al., 1982), or any

primates that have been studied (Hubbard and Di Carlo, 1974; Garver and Sladek, 1975; Jacobwitz and MacLean, 1978; Schofield and Everitt, 1981; Pearson et al., 1983). The A2 division comprised only a few TH+ neurons, but these were found in an expected location. The neurons forming the A1 nucleus appeared similar to that of other mammals studied. The C2 and area postrema nuclei, while found in the expected locations, appeared to be far more densely populated with neurons compared to other mammals and in particular the sheep (Tillet and Thibault, 1989). Since these two nuclei are involved in cardiovascular functions (Koizumi and Brooks, 1974), this may be expected in the giraffe, because of the greater length of the neck in comparison to other mammals.

A C1 nucleus in the giraffe medulla has been identified in the present study with tyrosine hydroxylase immunohistochemistry. Using TH and DBH immunohistochemistry, Tillet and Thibault (1989) also found immunoreactive neurons in the same location in the sheep; but in the sheep, these same neurons were not immunoreactive for PNMT (Tillet, 1988). This leaves the current designation of C1 neurons open to interpretation. Are they really C1 neurons, could they represent a rostral extension of A1 neurons that are immunoreactive for both DBH and TH, but not PNMT? Further studies in other ungulates should be undertaken using the three immunochemical markers and connectional studies with the spinal cord to determine if C1 is indeed present or absent in all ungulates, and what the exact identity of these TH and DBH immunopositive neurons are in both the sheep and giraffe. Overall, the phenotype of the giraffe does not appear to have had a dramatic effect on the nuclear subdivisions or neuronal morphology of the medullary catecholaminergic neurons in comparison to other mammals, and the strong similarities to that seen in sheep, another member of the Artiodactyla, strengthens this conclusion.

4.4.2 Serotonergic nuclei

All of the 5-HT+ subdivisions (RMg, RPa, RVL/CVL and ROb) observed in the giraffe medulla were found to have direct homologs in the sheep and indeed other eutherian mammals. The CVL appears to be absent in monotremes (Manger et al., 2002b), and the Virginia opossum (Crutcher and Humbertson, 1978), but is present in the wallaby (Ferguson et al., 1999). The one slight difference is that the RVL appeared to be more extensive anteriorly than that seen in other mammals. The projection of the RVL to the intermediate lateral horn of the spinal cord (Tork, 1990), where it regulates blood pressure, may explain the relative expansion of this nucleus compared to the sheep, for example (Tillet, 1987). Despite projecting down a long spinal cord, there was no evidence for abnormally large 5-HT+ neurons in the giraffe medulla.

4.4.3 Order specific patterns in the complement of neuromodulatory systems

Manger (2005) and Da Silva, *et al* (2006) have suggested that animals from the same mammalian order may exhibit the same complement of nuclei in the neuromodulatory systems of the brain regardless of phenotype, life history or brain size. The giraffe has a significantly different phenotype to the sheep in terms of the length of the limbs and neck, has a far larger brain (around 500 g in the giraffe compared to around 150 g in the sheep), and a very different life history (e.g. a browser of trees compared with a grazer of grass). Despite these differences, there is a striking similarity between the morphology and subdivisions of the catecholaminergic and serotonergic systems of the medulla of the giraffe and the

sheep (Tillet, 1987, 1988; Tillet and Thibault, 1989). The only variations seen were in the density of the neurons within various nuclei (e.g. area postrema and the anterior portion of RVL). Thus, regardless of its phenotype and life history, it appears that the giraffe brain is constrained in its ability to construct changes that may be adaptive to its phenotype within a given artiodactyl framework. Galis (1999) has suggested that changing the expression of one gene that may lead to adaptive changes in the morphophysiology of different parts of the body may lead to lethal mutations as there are several other genes that are intricately tied to that one gene and they too would be affected and cause phenotypic change, that may not be compatible with overall organismal success. Given that the neuronal systems examined in the present study appear very early in development, it may be that the consequences of pleiotropy during the evolutionary changes leading to altered morphology may be the factor restricting change in these systems within a mammalian order.

Chapter 5: The Corticospinal Tract of the Giraffe (*Giraffa camelopardalis*): an Immunohistochemical study

5.1 Introduction

The basic function of the corticospinal tract is thought to be the mediation of voluntary movement. In mammals, this tract extends from the primary motor cortex (M1) located in the frontal and parietal lobes to the dorsal, intermediate lateral and ventral horns of the spinal cord (Kiernan, 1998). M1 is the region of the cortex where muscular movements are initiated. Very few corticospinal fibres synapse directly with motor neurons rather they synapse for the most part on the dorsal horn (at the base) as well as in the intermediate grey matter and ventral horn (Kiernan, 1998). The corticospinal tract passes from M1 through the internal capsule and to the cerebral peduncles of the midbrain. The tract then enters the pons as small strands which then unite into a “compact body of white matter” (Kiernan, 1998) to form the pyramidal tracts in the ventral medial portion of the medulla. It then passes from the medulla to form the lateral and ventral corticospinal tract in the grey matter of the spinal cord.

In the primary motor cortex of primates, the corticospinal tract originates at the Betz cells, which are giant pyramidal cells found in layer 5 (Sherwood, *et al*, 2003). It has been shown that the Betz cells that project to a greater distance (e.g. to the lumbar spinal cord) are larger than those that project to say the cervical spinal cord (Murray and Coulter, 1981; Sherwood, *et al*, 2003). Large pyramidal cells in the primary motor cortex of the sheep have been described (Ebinger, 1975); however, they do not appear to have the unique morphology that would allow them to be regarded as homologous to Betz cells. A situation not dissimilar to the sheep is seen

in the primary motor cortex of the cetaceans that have been investigated (Kojima, 1951; Kesarev et al., 1977; , Hof and van der Gucht, 2006).

One might predict that the morphology of the giraffe neck may lead to potential alterations in the form of the corticospinal tract. For example, it may be possible that the giraffe will have enormous giant pyramidal cells, akin to Betz cells, correlating with the long axons required to reach the end of the spinal cord as is seen in primate cortex (Sherwood et al., 2003). The giraffe's uniquely long neck predicts, using adaptive evolutionary rationale, that the cortico-spinal pathway will be exceptionally long, but it is unknown how long the giraffe spinal cord really is. Determining the length of the spinal cord in giraffe forms one part of the present study.

Alternatively, the features of the giraffe cortico-spinal pathway may show features that are constrained in their ability to undergo adaptive change. For example, the primary motor cortex may be very similar in appearance to other members of the order such as sheep (Manger, 2005), or larger phylogenetic groupings such as the cetartiodactyla (Hof et al., 2000). In this second scenario, the evolution of a long neck may not actually lead to significant morphological change in the features of the corticospinal pathway.

Thus, the long neck of the giraffe may serve as a heuristic model in the deduction of evolutionary processes in the evolution of the nervous system. On the one hand, if significant morphological changes are apparent, adaptive evolutionary rationale may best explain these altered features. Alternatively, the nervous system may be subject to numerous phylogenetic constraints and the current study may reveal certain of these constraints and the phylogenetic level at which they are acting (Gould, 2002; Hof et al., 2000; Manger, 2005). The current study examines both the gross and

microanatomy of the corticospinal tract in the giraffe in an attempt to reveal any potential features affected by the evolution of the long neck compared to other mammals, or features that may be constrained during evolutionary change.

5.2 Materials and Methods

Three male giraffe (*Giraffa camelopardalis*) were used in the present study. These animals were all male, ranged in size from approximately 400 kg to 1500 kg and 2 years old to adult, and died during the process of capture for relocation. The animals were treated in accordance to the University of the Witwatersrand Animal Ethics Committee guidelines for the care and use of animals in scientific experiments. Appropriate permissions for use of the animals were also obtained from the local provincial governmental bodies responsible for wildlife management in South Africa. Laminectomies to expose the entire spinal cord were undertaken in the field. We noted the levels of the conus medullaris and cauda equina for comparison with published accounts of other large ungulates. Immediately following death, one of the smaller giraffe was perfused (using a manual pump) with 0.9% normal saline solution (200 l, or approximately 0.5 l/kg) via the paired carotid arteries to flush the blood. This was immediately followed with a perfusion of 200 l of 4% paraformaldehyde in 0.1M phosphate buffer (PB) for fixation. Following fixation the brain as well as the spinal cord was removed. The whole brain and the entire length of the spinal cord were clear of blood and rubbery to the touch indicating a successful whole body perfusion. Both the brain (same specimen as in Chapter 3) and spinal cord of this animal was post fixed in 4% paraformaldehyde in 0.1M phosphate buffer (PB) overnight. The specimens were then allowed to equilibrate in 30% sucrose solution in

PB at 4°C. They were later stored in antifreeze solution (distilled water, glycerol, ethylene glycol and PB) until sectioning.

The entire right cerebral hemisphere was dissected free from the remainder of the brain and placed in 30% sucrose solution in PB at 4°C for 5 days and then frozen in crushed dry ice. A freezing sliding microtome was used to cut 50µm thick sections in the coronal plane. Every 10th section was mounted and stained for Nissl substance (with cresyl violet), and every 41st section was mounted and stained for myelin (a modified silver stain from Gallyas, 1979). In combination with a previous study (see Chapter 3), the medulla was sectioned and every fourth 50µm section was stained for myelin. The other sections were used for the medulla immunocytochemistry study (see Chapter 3). Small blocks of the spinal cord, approximately 10 mm in length from the level of C2, T5, and L3 were dissected, placed in 30% sucrose solution for 5 days and then frozen in dry ice. A freezing microtome was used to serially section 50µm thick slices in the coronal plane. A one in four consecutive series of sections was stained for Nissl bodies, myelin, calcitonin gene-related peptide (CGRP) and vesicular acetylcholine transporter (VChAT).

For immunocytochemical staining, the sections were first treated for 30 min with an endogenous peroxidase inhibitor (49.2% methanol:49.2% 0.1M PB:1.6% 30% H₂O₂) followed by three 10 min rinses in 0.1M PB. This was followed by a 2 h preincubation (on a shaker), at room temperature, in blocking buffer containing 3% normal goat serum (NGS) (Chemicon Int.), 2% bovine serum albumin (Sigma), and 0.25% Triton X100 (Merck) 0.1M PB. After preincubation, the sections were then placed in primary antibody solution containing the appropriately diluted antibody in 0.25% Triton X-100 in 0.1M PB, 3% NGS, and 2% bovine serum albumin, for 48 h at 4°C on an orbital shaker. Anti-vesicular acetylcholine transporter (VChAT) (V5387,

Sigma) at a range of dilutions from 1:500 to 1:16000 was used for all three spinal cord regions. To reveal CGRP immunoreactive axons, anti-calcitonin gene related peptide (CGRP) (AB1971, Chemicon) was used. A range of dilutions from 1:500 to 1:16000 was used for all three spinal cord regions.

This primary antibody incubation was followed by three 10min rinses in 0.1M PB. The sections were then placed in secondary antibody solution for 2 h on a shaker. The secondary antibody contained a 1:500 dilution of biotinylated anti-rabbit IgG (BA 1000, Vector Labs), with 3% normal goat serum, 2% bovine serum albumin, in 0.1M PB. After three further 10 min rinses in 0.1M PB, the sections were incubated in AB solution (Vector Labs) for 1 h and rinsed once more in three 10 min rinses in 0.1M PB. The sections were then treated in a 0.05% solution of 3,3'-diaminobenzidine (DAB) in 0.1M PB for 5 min. This was followed by adding 3 μ l of 30% H₂O₂ per 0.5ml solution. The development process was followed visually and checked under a low power stereomicroscope. The staining was allowed to continue until the background staining was at a level that would enable us to match the sections to the adjacent Nissl and myelin sections without obscuring the immunopositive neurons. The development was stopped by placing the sections in 0.1M PB and then rinsing them twice in 0.1M PB. Two controls were employed during the immunostaining. The first control consisted of omitting the primary antibody, the second omitted the secondary antibody.

The sections were mounted on 0.5% gel coated glass slides and left to dry overnight. They were then dehydrated in a graded series of alcohols, cleared in xylene and coverslipped with DePex mounting medium. The sections with the best reactivity across the dilution runs (see above) were then examined under a low power stereomicroscope and the architectonic borders were traced according to the Nissl and

myelin sections using a camera lucida. After completing the traces, the immunoreacted sections were matched to the drawings and the immunopositive cell bodies and axons marked. Immunopositive neurons were defined as those that clearly showed stained soma and dendrites, while immunoreactive axons often displayed a varicose appearance. These drawings were scanned and redrawn using the Canvas 8 drawing program. High power photomicrographs were taken using a Zeiss Axioskop. No pixilation adjustments or manipulations of the captured images were made, except for brightness and contrast adjustments using Adobe Photoshop 7.

Abbreviations	
bf	basal forebrain
c	caudate nucleus
c	cruciate sulcus
cc	corpus callosum
cl	claustrum
co	coronal sulcus
gp	globus pallidus
ic	internal capsule
imm	intermediate zone of grey matter
IML	intermediate lateral horn
io	inferior olive
lat vent	lateral ventricle
m	marginal sulcus
p	putamen
pir	piriform cortex

5.3 Results

The corticospinal tract in the giraffe, in its overall morphology, resembles that seen in other mammals. The spinal cord is long and extends to the sacrum, a feature typically seen in ungulates (Dellmann and McClure). The primary motor cortex displays the features and location common to the Artiodactyla (Ebinger, 1975) and also seen in the Cetacea (Hof et al., 2005; Hof and van der Gucht, 2006). The descending pathway passes through the internal capsule to form pyramidal tracts in the medulla and then passes onto the spinal cord. The anatomy of the spinal cord is similar to that seen in other mammals (Kappers, *et al*, 1936; Butler and Hodos, 2005).

5.3.1 Length and gross anatomy of the giraffe spinal cord

The spinal cord of the giraffe is continuous with the medulla and found in the vertebral canal as in all other mammals. It is segmented at each vertebral level and the dorsal and ventral roots are clearly visible. From the foramen magnum, it continues as a solid mass, ranging from approximately 1-1.5cm in diameter to the level of approximately S1-S2, where the conus medullaris starts. The conus medullaris tapers to finish at the first caudal vertebrae. The cauda equina begins at S1-S2 and continues into the caudal vertebrae. The overall length of the spinal cord in the animals studied ranged between 1.5 – 3 m. Within the vertebral canal, there appeared to be an extensive vascular supply to the entire length of the spinal cord and large aggregations of white adipose tissue surrounding the spinal cord, especially in the ventral aspect.

5.3.2 Cytoarchitecture and location of giraffe primary motor cortex

The primary motor cortex (M1) of the giraffe showed only five layers of the cerebral cortex. No layer 4 was evident as is typical of all mammals. Layer 1 is very distinct as seen in all cerebral cortices (Fig. 20A). The layer 2/3 boundary is slightly blurred, but readily detectable. Deeper in layer 3, the pyramidal cells increase in size. The layer 3/5 boundary is also somewhat blurred (Fig. 20A). Layer 5 is marked by a low cell density and clusters of relatively large pyramidal neurons that are likely to be the origin of the corticospinal tract (Fig. 20, A-D). Unlike in primates, (Sherwood, *et al.*, 2003) where there are extremely large, but individual and evenly distributed Betz cells in layer 5 of M1 (Nieuwenhuys, *et al.*, 1998; Sherwood, *et al.*, 2003), the giraffe M1 shows clusters of 3-4 large pyramidal neurons approximately 600 μm in area (Sasaki and Iwata, 2001); (Fig. 20, A-D, compare specifically the three large clustered pyramidal neurons with the single smaller one in D). In most cases, the apical dendrites of these giant pyramidal neurons in the giraffe coalesce to form a small fasciculus (Fig. 20D). These clusters are evenly distributed though out layer 5 of M1 in a manner not dissimilar to the distribution of the individual Betz cells in primate M1. The giraffe M1 architecture resembles that closely reported for sheep (Ebinger, 1975) and cetaceans (Hof *et al.*, 2005; Hof and van der Gucht, 2006).

The primary motor cortex, Brodman area 4, was found on the dorsal medial aspect of the anterior half of the cerebral hemisphere (Fig. 21). It is bordered posteriorly by the ansate sulcus, medially by the anterior part of the marginal sulcus, laterally by the coronal sulcus and ends just anterior to the cruciate sulcus (Fig. 21). These sulci are what we believe to be homologous sulci to that seen in other ungulates (Johnson, 1984). M1 is approximately 2cm mediolaterally and 4cm anteroposteriorly

in extent on the dorsal surface of the cerebral hemisphere (Figs. 21 and 22), but its surface area is far greater than this due to the depth and frequency of the sulci in this region of the cerebral cortex (Fig. 22).

5.3.3 Descending pathway of the giraffe corticospinal tract

The descending fibres presumably emanating from the primary and pre-motor cortical regions in the giraffe coalesce to form the internal capsule, which splits the dorsal striatopallidal complex into recognizable caudate and putamen nuclei (Fig. 23). The path continues to form the cerebral peduncles, which then enter the pons. In the medulla, clearly recognizable pyramidal tracts are seen in the ventral midline below the inferior olives (Fig. 24). The pyramidal tracts are somewhat flattened dorsoventrally and elongated mediolaterally. This pathway is similar to that seen in other mammals (Butler and Hodos, 2005). The decussation of the pyramidal tract occurs approximately 2mm rostral of the beginning of the cervical spinal cord. The pyramidal tracts appear to continue into the spinal cord in a manner similar to other mammals (Butler and Hodos, 2005).

5.3.4 Microarchitecture of the giraffe spinal cord

Sections at three levels of the spinal cord were taken, these being C2, T5 and L3. These were stained for Nissl bodies (Fig. 25, A, C, E), myelin (Fig. 25, B, D, E), vesicular acetylcholine transporter (VChAT) (Figs. 26, 27) and calcitonin gene-related peptide (CGRP) (Figs. 26, 28).

A typical mammalian appearance was seen with the Nissl and myelin stains (Fig. 25). The amount of the spinal cord occupied by gray matter increased with increasing distance from the brain, i.e., the lumbar had more gray matter than the

cervical spinal cord both in absolute and relative terms. Neuronal density in each section was not particularly high, but the ventral, intermediate lateral (IML) and dorsal horns could be readily identified (Fig. 25).

VChAT staining revealed large VChAT+ motoneurons in the ventral horn at all levels examined (Figs. 26 and 27). VChAT showed medium sized motoneurons in the IML horn at all levels of the giraffe spinal cord (Figs. 26 and 27); however, in the cervical spinal cord, the IML horn was not as strongly expressed compared to the thoracic and lumbar levels investigated (Figs. 26 and 27). At all the spinal cord levels studied, strong and homogenous VChAT axon terminal staining was seen in the superficial lamina of the dorsal horn. This corresponded to an area that stained weakly for myelin, probably being lamina II (or the substantia gelatinosa). This lamina is similar to that of other ungulates and mammals (Kappers, *et al*, 1936; Butler and Hodos, 2005).

CGRP immunohistochemistry revealed strong axon terminal staining in the substantia gelatinosa (Figs. 26 and 28). However, the staining was not homogenous across this lamina. Rather, there was an appearance of strong staining on the inner and outer borders of this lamina (Figs. 26 and 28). Axonal terminal staining was evident in the remainder of the dorsal horn grey matter, the intermediate grey matter and the IML grey matter, but this staining was weak and sparse (Fig. 26).

Figure 20: A Low power photomicrograph of a coronal section through the giraffe motor cortex (M1) stained with cresyl violet. Note the clusters of giant pyramidal neurons in Layer 5. Scale bar = 1mm.

B & C Slightly higher power photomicrographs of Layer 5 of the giraffe M1 showing the giant pyramidal cell clusters. Scale bar for B & C = 50 μm .

D High power photomicrograph of a cluster of there giant pyramidal cells forming a single cluster. Note how the apical dendrites of each neuron appear to form a small fasciculus. Scale bar = 100 μm .

Figure 21: Diagram showing a dorsal view of a drawing of the giraffe brain. The shaded region on the right cerebral hemisphere depicts the location of Brodman Area 4, or primary motor cortex (**M1**). Primary motor cortex in the giraffe appears to be located more caudally than in the sheep brain (Ebinger, 1975); however, this location in the giraffe is similar to that seen in the horse (Johnson, 1984).

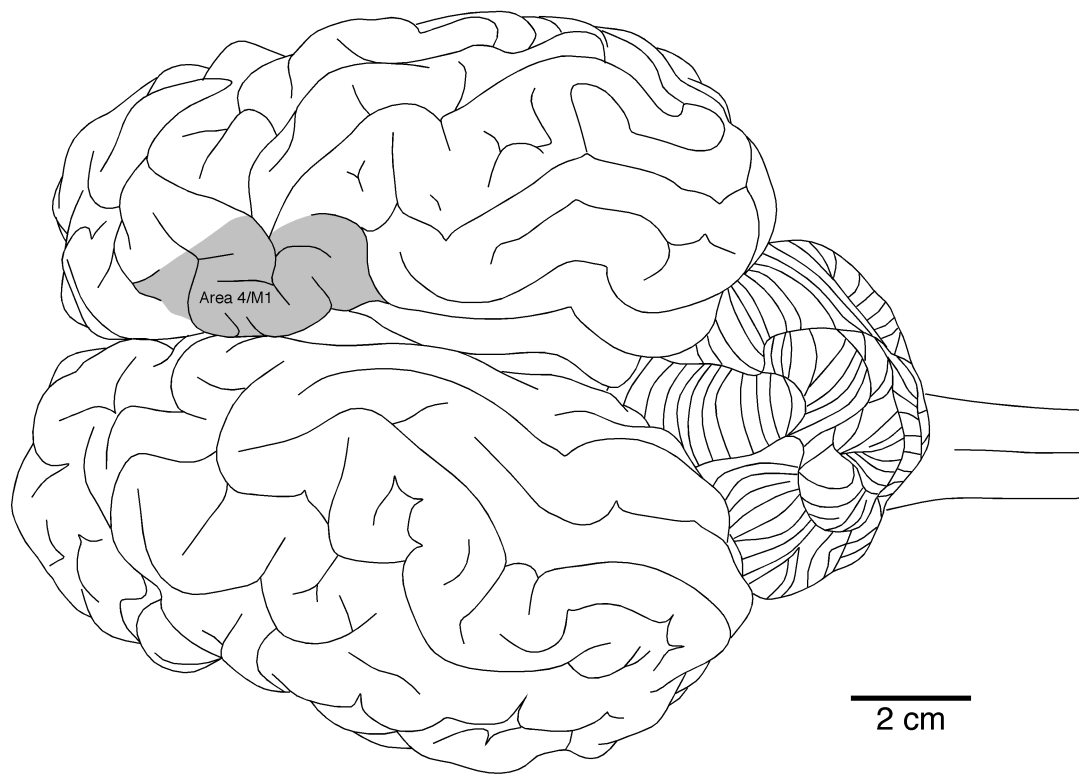


Figure 22: Diagrammatic lateral view of the right hemisphere of the giraffe brain. The arrows labelled A-F correspond to the reconstructed coronal sections shown in the lower half of the figure and indicate the approximate antero-posterior location of primary motor cortex.

A-F Serial drawings of coronal drawings through primary motor cortex, the location of which is indicated by the regions shaded in grey. See table for abbreviations.

Figure 23: Low power photomicrographs of coronal sections through the dorsal striatopallidal complex of the right cerebral hemisphere of the giraffe. The morphology of this region is similar to many other mammals. **A** Nissl stain. **B** Myelin stain. Scale bar = 5mm.

Figure 24: Low power photomicrograph of the ventral half of the midline of a coronal section through the medulla oblongata of the giraffe. This section has been stained for myelin and shows the location of the pyramidal tracts (**py**) which is similar to that seen in other mammals. **i.o.** – inferior olive. Scale bar = 2 mm

Figure 25: Low power photomicrographs of coronal sections of the giraffe spinal cord. **A** and **B** - cervical spinal cord. **C** and **D** - thoracic and **E** and **F** - lumbar. **A**, **C** and **E** are stained for Nissl bodies. **B**, **D** and **F** are stained for myelin. Note the large proportion of grey matter in the lumbar spinal cord compared to the other two regions. Scale bar = 3mm.

Figure 26: Diagrammatic reconstructions of the central grey matter of the cervical, thoracic and lumbar regions of the giraffe spinal cord showing the distribution of neurons and axon terminals immunoreactive for vesicular cholineacetyltransferase transporter (VChAT) (right side) and calcitonin gene-related peptide (CGRP) (left side). The large circles represent motor neurons immunoreactive for VChAT. The smaller circles represent axon terminals immunoreactive for VChat and CGRP.

VChat: In the cervical region there is a high concentration of axons in the superficial lamina of the dorsal horn and throughout the ventral horn. At this level the intermediolateral horn (**IML**), while having structures immunoreactive to VChAT, the number was comparatively low.

In the thoracic region, the axons are again highly concentrated in the superficial lamina of the dorsal horn and throughout the ventral horn. There is an increase in the density of both VChat + motor neurons and axon terminals in the IML horn, and some neurons are seen between the ventral and IML horn.

The distribution of VChAT immunoreactive structures in the lumbar region are similar to that seen in the thoracic region.

CGRP: A high density of CGRP labelled axon terminals was observed in the superficial lamina of the dorsal horn at all levels of the spinal cord examined. Sparse labelling of axon terminals in the IML horn and in the intermediate zone of grey matter was also seen at all levels examined. **OSTt** – origin of the spinothalamic tract.

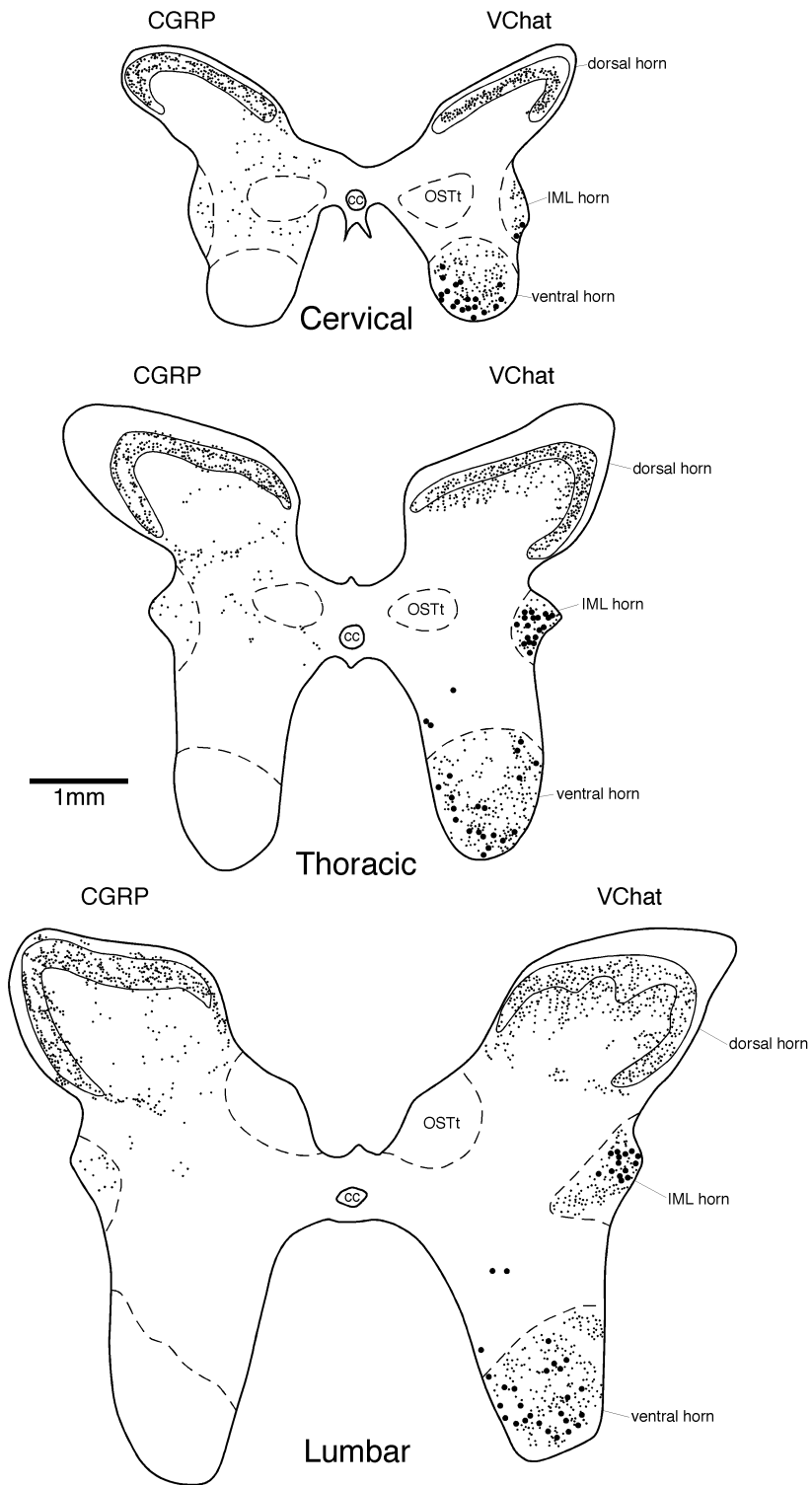


Figure 27: Low power photomicrographs of sections of giraffe spinal cord reacted for vesicular cholineacetyltransferase transporter (VChAT) immunoreactivity. **A-C** – cervical, **D-F** – thoracic and **G-I** – lumbar. **A, D, G** – superficial lamina of the dorsal horn, **B, E, H** – intermediolateral horn, **C, F, I** – ventral horn.

At all three spinal cord levels, there was intense staining of axonal terminals in the superficial lamina, substantia gelatinosa, of the dorsal horn. At all three levels, VChAT immunoreactive neurons and terminals were seen in the IML horn, but these were not strongly expressed in the cervical region (**B**). At all three levels immunoreactive axon terminals and motoneurons were seen in the ventral horn.

Scale bar = 250 μ m.

Figure 28: Photomicrographs of calcitonin gene-related peptide (CGRP) immunoreacted sections of the dorsal horns of the giraffe spinal cord. **A** – cervical, **B** – thoracic, and **C** – lumbar. The axons are distributed in 2 laminae within the superficial layers of the dorsal horn. Scale bar = 250 μm .

5.4 DISCUSSION

The spinal cord of the giraffe extends to the level of the sacral vertebrae, making it very long, but this extension through to the sacrum is similar to that seen in other ungulates. The cyto-, myelo- and chemo-architecture of the spinal cord appears to be similar to that of other mammals. The pathway of the corticospinal tract through the brain is also very similar to that of all mammals, with the internal capsule, cerebral peduncles and pyramids of the medulla all demonstrating a morphology that is not unusual or dramatically different in comparison. The primary motor cortex is distinguished by the appearance of clusters of large, but not extraordinarily large, pyramidal neurons in layer five. This clustering of large pyramidal neurons is quite similar to that seen in the primary motor cortex of the sheep, but it is qualitatively different to that of primates. Thus, while extremely long, the corticospinal tract of the giraffe for the most part resembles that found in other mammals, and more specifically so that of other ungulates.

5.4.1 The spinal cord of the giraffe

Our observations of the spinal cord of the giraffe revealed it is very long in comparison to many other mammals, especially those of a similar body mass but with a much shorter neck. In the largest giraffe, the spinal cord length might be in the range of 2.5 to 3 m and extends to the sacral vertebrae. In all other respects however, the gross anatomy of the spinal cord is similar to that seen in other ungulates and mammals, thus despite the extended neck length there is no specific gross anatomical morphology present to account for or to adapt to this increased length. The

termination of the spinal cord at the level of the sacral vertebrae is also as seen in other ungulates (Dellmann and McClure, 1975a; 1975b). The microanatomy of the giraffe spinal cord was also similar to that seen in other mammals (Kappers, et al, 1936; Butler and Hodos, 2005; Weihe, 1992). The Nissl, myelin, vesicular acetylcholine transporter (VChAT) and calcitonin gene-related peptide (CGRP) stains used revealed no specific differences in the neuroanatomy that would be associated with neck lengthening in the giraffe compared to other mammals.

5.4.2 Pathway of the cortico-spinal tract through the giraffe brain

The pathway of the corticospinal tract appears to begin at the layer 5 pyramidal neurons of the primary motor cortex (discussed below). As seen in most mammals, the descending axons of these pyramidal neurons coalesce to form a clear internal capsule that splits the dorsal striato-pallidal complex into distinct caudate and putamen nuclei. The internal capsule continues to the floor of the brain where it forms the cerebral peduncle that enters the pons as seen in other mammals. Below the pons, the medullary pyramids are clearly recognizable and are seen in the ventral midline below the inferior olives as in other mammals. The pyramids appeared somewhat expansive in a medio-lateral plane, but were foreshortened in a dorso-ventral plane as seen in other ungulates (for comparison see the images located at www.brainmuseum.org). The pyramidal tracts then continued into the spinal cord in a similar pattern to that described for other mammals. There was heavy myelination throughout this pathway and this feature enabled us to trace the tract readily.

5.4.3 Primary motor cortex (M1) of the giraffe

We found clusters of gigantopyramidal cells in layer 5 of the giraffe primary motor cortex (M1). In most clusters, the apical dendrites of these pyramidal neurons, coalesced to form a small externally oriented fasciculus. This formation is different to that seen in primates, where there are giant Betz cells which vary in size according to the length the axon has to travel to reach its target cell (Sherwood, *et al*, 2003). In the giraffe, there was no clear indication of a change in size of the pyramidal cell throughout the M1 that may be associated with the length of the axons projecting to the spinal cord. Neuronal size may be of little importance in the giraffe as evidenced by research conducted on retinal ganglion cells of the cat that found “no positive correlation between soma or axon diameter and intraretinal axon length” (Fitzgibbon, *et al*, 1991). Rather what may be of greater importance is the type of chemical activity within each neuron and within the clusters of neurons that would result in a high speed of conduction of neural impulses to initiate the required movements. The pyramidal neuron clusters of layer 2 were evenly distributed throughout this layer of the motor cortex as seen in primates (Sherwood, *et al*, 2003).

The morphology of the giraffe M1, including the clustering of the gigantopyramidal cells, is of a similar nature to that described for the sheep (Ebinger, 1975). This may indicate an order specific morphology in ungulates (Manger, 2005). A similar description has been given for cetaceans, which also describes the clustering of gigantopyramidal cells (Hof, *et al*, 1999; Hof, *et al*, 2000; Hof and Sherwood, 2005). This suggests that the anatomy of the M1 in ungulates may be a feature of the cetartidactyla superorder, so that the constraint in the appearance of the giraffe M1 could reflect a broader taxonomic grouping (Hof, *et al*, 1999). More research into the

M1 of other ungulates is required in order to determine if the morphology of M1 in ungulates is indeed as conservative as it would currently appear.

5.4.4 Overall Impressions

The present study revealed a great deal of conservatism in the general appearance and specific morphology of the corticospinal pathway and the spinal cord of the giraffe when compared to other mammals. In particular it is noteworthy that these neural features of the giraffe differ very little from that described for the sheep and other ungulates. In this sense, although this pathway is under a strong phenotypic challenge by the lengthening of the neck, the neural systems remain extremely conservative in terms of specific morphological adaptations. Further studies of the neural systems associated with the “extreme” phenotypic appearance of the giraffe may reveal which features are particularly labile in terms of neural evolution and what features are very conservative. Relating these features, both those that are variant and those that are invariant, to specific phylogenetic groupings may lead to interesting insights into the evolution of the neural systems in a broader sense.

6 CONCLUDING CHAPTER

The present study has led to the conclusion that the overall length of the giraffe neck may be considered a unique morphological adaptation; but despite this conclusion, it is clear that the majority of the morphology of the giraffe head and neck adheres to structural laws of form and phylogenetic constraints. Although more studies need to be conducted on the anatomy of other ungulates, overall the results of this study reveal that the anatomy of the giraffe head and neck remains true to a basic ungulate plan, with very little morphologically differentiation, or individualization, representing unique adaptations at organizational anatomical levels below that of the entire head and neck. The observations made in the current study lead to the conclusion that constraints and allometric scaling laws of form play a greater role than previously thought in the evolution of extreme morphologies. These potential avenues of morphological variation must really be taken into account in greater detail when discussing the evolution of the length of the giraffe neck.

6.1 Constraints

In the current study a range of morphological features at different organizational levels were examined. Specific aspects of the anatomy were targeted to examine potentially unique morphological features that may be related to overall lengthening of the neck in the giraffe. What turned out to be more interesting was the fact that the features were similar to that seen in related species of ungulates. These features are thought to be those limited in their variation due to their phylogenetic history. Examples include:

- (a) The giraffe has seven cervical vertebrae as seen in almost all mammals (Galis, 1999; Solounias, 1999; Mitchell and Skinner, 2003).
- (b) Immunohistochemistry of the giraffe medulla revealed that the catecholaminergic and serotonergic neurons were arranged in a similar pattern to that seen in other mammals.
- (c) The corticospinal tract of the giraffe was also found to resemble that seen in other mammals.
- (d) The morphology and distribution of the gigantopyramidal neurons in layer 5 of the primary motor cortex are very similar to that seen in sheep.

6.2 Adaptations

- (a) The current study also managed to identify a range of morphologies that may be considered unique to the giraffe, and in this sense can be termed adaptations. Examples include: The giraffe cervical vertebrae are uniquely longer, scale in a unique manner, and are more robust than those of the other extant ungulate species studied.
- (b) C7 had transverse foramina through which the vertebral arteries passed (Solounias, 1999).
- (c) The frontal sinus of the giraffe was uniquely large and could not be conclusively correlated to increasing nuchal attachment area.
- (d) The immunohistochemistry of the giraffe medulla revealed an unusually high concentration of TH+ neurons in area postrema (Figs. 16H; 17D) possibly as a result of the long neck.

6.3 Chapter 2: Vertebrae study

The contribution of the cervical vertebrae to the total vertebral column of the giraffe in comparison to several extant ungulates and fossil giraffids was examined to better understand the evolutionary processes that result in extreme morphology using the giraffe neck as a model. Three main constraints in the morphology of the giraffe vertebrae were found. The first, and perhaps most significant constraint, is the seven cervical vertebrae which remains similar across most mammals, this being a class level phylogenetic constraint. Secondly, the morphology of all the giraffe vertebrae was similar to that of the extant ungulates studied, an order level phylogenetic constraint. Lastly, the pattern of growth or elongation of each individual giraffe vertebrae was also similar to that of the extant ungulates studied, a second example of order level phylogenetic constraint.

Three adaptations in the morphology of the giraffe vertebrae compared to the extant ungulates studied were also found. Firstly, the seven cervical vertebrae made up over 50% of the total vertebral length in the adult giraffe. In the extant ungulates studied, this contribution was mostly around 30% in other ungulates including the okapi and around 40% in the camel and llama of the total vertebral length. Thus the overall length of the giraffe cervical vertebrae is a unique feature of the giraffe. The giraffe vertebrae were also more robust (larger, thicker and heavier) compared with the extant species studied and this may be related to their extraordinary length and potential weight bearing function. Finally, within the cervical vertebrae there was a scaling law of form indicating that alterations in the length of the neck are not achieved by a single vertebra. Instead, the entire suite of cervical vertebrae changed in length (Fig. 5). This is an example of a unique adaptation in the lengthening of the

giraffe neck, although it should be noted that this follows a structural law of form. These results indicate that the long neck of the giraffe has evolved within a very restricted framework of seven cervical vertebrae and as a result, these vertebrae had to elongate more rapidly than the rest of the vertebrae. There is a mixture of features indicating phylogenetic constraints, structural laws of form and unique adaptations, which make the giraffe neck a very interesting model in terms of the evolution of new and unusual morphologies.

6.3.1 Evolution of long-neckedness in giraffe

Two possible scenarios that may have led to the evolution of the long neck in the giraffe, a microevolutionary scenario and a punctuated scenario, are proposed. The microevolutionary setting argues for a slow accumulation in the length of the giraffe neck which may have come about as a result of climatic and dietary changes (Mitchell and Skinner, 2003) in the giraffe's evolutionary history. The punctuated evolutionary scenario looks at an event such as a change in vegetation that would have triggered certain *Hox* genes to alter their pattern of expression and rapidly lead to the genesis of a long neck in the modern giraffe. Either scenario is potentially plausible given the currently available data and both are worth exploring in greater detail. Further studies are required to investigate the vertebral body lengths of extinct giraffids to establish when in giraffid evolution the long neck may have evolved and possibly determine which of the two evolutionary events resulted in the genesis of the modern giraffe.

6.3.2 Evolution of long-neckedness in mammals

Our results indicate that long-neckedness in ungulates may have evolved separately in three ungulate groups. The giraffe is one case as are the *Camelidae* and the gerenuk (*Litocranius walleri*). Each of these latter cases requires more detailed examination to understand the processes that have lead to lengthening of the neck in ungulates. It is possible that different mechanisms may have resulted in the production of longer than normal necks. By examining these different scenarios in the evolution of longer necks in conjunction with the giraffe, it is possible that insights into evolutionary processes across the remaining ungulates, and more generally mammals, may enable us to better understand how this adaptation arose in various species.

6.4 Chapter 3: Frontal sinus study

In this study, the abnormally large frontal sinus in the giraffe skull was examined with the aim of determining the possible reasons why the giraffe has such an extensive frontal sinus in comparison to other extant ungulates. It was found that although the frontal sinus of the giraffe is large, its size relative to the skull is not unique to the giraffe. The Cape buffalo (*Syncerus caffer*), rhinoceros (*Ceratotherium simum*), and warthog (*Phacochoerus aethiopus*) also exhibit extensive frontal sinuses. However, due to the differences in phylogeny in these species, the evolution of the large size of the giraffe frontal sinus represents a case of an independent evolution of this feature, and thus can be considered an adaptation.

6.4.1 Function of the frontal sinus in the giraffe

We found a strong correlation of the volume of the frontal sinus in the giraffe skull with both skull mass and nuchal attachment area. These correlations were also observed in several ungulate species studied. In other ungulates, such as the rhinoceros and warthog, the size of the frontal sinus was clearly related to skull mass and nuchal attachment area, whereby the larger the skull, the greater the nuchal attachment area, the latter being achieved by increasing the volume of the frontal sinus. This explanation could not however be used for the giraffe, as enormous increases in frontal sinus volume were associated with small changes in both skull mass and nuchal attachment area. It may therefore be concluded that the frontal sinus in the giraffe (and possibly the Cape buffalo) may act as a resonance chamber for infrasonic sound production rather than for primarily biomechanical and weight bearing functions as seen in the rhinoceros and warthog.

6.4.2 Further studies

This study leads to some interesting possible future investigations. The possibility of infrasonic sound production in both giraffe and Cape buffalo needs to be tested. If it could be conclusively shown that these species can produce infrasonic sound, studies on the physics of the frontal sinus in these species, and others producing infrasonic sound, may reveal interesting insights into the evolution of these capabilities.

6.5 Chapter 4: Medulla study

This immunohistochemical study of the catecholaminergic and serotonergic systems in the giraffe medulla was made in an effort to determine whether these neuronal systems resemble those of the mammals. The interest in studying these systems is that parts of both of these systems project to the entire length of the spinal cord, and thus the morphology of the giraffe may present unique challenges to these systems that may result in unusual morphologies. Interestingly it was found that the morphology of these systems appear constrained in that the location and nuclear parcellation of these neurons in the giraffe medulla are similar to that previously described in other mammals, and particularly the sheep (Dahlstrom and Fuxe, 1964; Hokfelt, *et al*, 1984; Tillet and Thibault, 1989; Tork, 1990; Smeets and Gonzalez, 2000). Thus, in spite of facing a strong phenotypic variance, no new or highly unusual morphological features were observed.

Within the existing framework of these systems, it was observed that there was a high concentration of TH⁺ neurons in area postrema compared to the sheep. The catecholaminergic neurons of the area postrema are involved in the regulation of heart rhythm and innervate smooth muscle, thus an increased density and number of these neurons may be related to the phenotype of the giraffe, but this is merely speculation.

6.6 Chapter 5: Corticospinal tract study

This study examined the cortico-spinal tract of the giraffe, as the phenotype of the giraffe may affect the morphology of this neural pathway. In all mammals, this tract contains motor and sensory axons that travel from the cerebral cortex to the

spinal cord. In comparison to other ungulates, no features were noteworthy in terms of morphological adaptation. Most interestingly, the morphology of the layer 5 neurons of the cerebral cortex is identical to that seen in sheep. This indicates that despite the phenotypic challenge of the giraffe, the neural systems (such as neuronal size and density) are strongly constrained in their ability to adapt to these changes.

6.7 Overall conclusion

The current study has allowed a somewhat better understanding of the evolutionary processes that result in extreme morphologies in mammals by using the giraffe head and neck as a model. The observations made have allowed the determination of certain morphological systems within the head and neck that stay true to the basic mammalian or ungulate morphotype (constrained) or show features unique and specifically related to the giraffe (adapted). It was hypothesized at the beginning of the study that much of what will be found in the giraffe head and neck will be elongated typically ungulate (1st level of morpho-constraint) or mammalian structures (2nd level of morpho-constraint). As predicted, there were some anatomical features that were unique to the giraffe as a result of its evolving this unusual neck (evolutionary adaptation), such as greatly elongated and robust cervical vertebrae, an extensive frontal sinus and high concentrations of neurons within the area postrema. These evolutionary adaptations may have played a crucial role in enabling the elongated giraffe neck to remain in its present form; but, of real interest in the current study has been the revelation that a greater number of morphological features are constrained in their evolution, or follow specific structural laws of form occur. These latter avenues of evolutionary change have been downplayed in terms of

the giraffe neck, however, it is hoped that the current series of observations will bring these to the fore. It is only with a broad approach to understanding morphological evolution, such as that attempted in the current study, that a better understanding of the genesis of form will emerge.

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