ANATOMICAL AND IMMUNOHISTOCHEMICAL STUDIES OF THE SPINAL CORD OF THE GRASSCUTTER (*Thryonomysswinderianus*)

BY

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DEPARTMENT OF HUMAN ANATOMY,
FACULTY OF BASIC MEDICAL SCIENCES,
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ZARIA, NIGERIA

DECEMBER, 2019

DECLARATION

I declare that the work in this thesis entitledentitled Anatomical and Immunohistochemical Studies of the Spinal Cord of the Grasscutter *(Thryonomysswinderianus*) was performed by me in the Department of Human Anatomy. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree or diploma in another University.

Felix UdawnojoEnemali _____ _____

Sign Date.

CERTIFICATION

This thesis entitled ANATOMICAL AND IMMUNOHISTOCHEMICAL STUDIESOF THE SPINAL CORD OF THE GRASSCUTTER (*Thryonomysswinderianus*) by Felix Udawnojo ENEMALI meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my loving parents, Mr and Mrs I.A. Enemali and my dear siblings; Very Rev. Fr. Wilfred Enemali, Dr. Godwin I. Enemali, Dr. Joseph Enemali, Apostle Paul Enemali, Apostle Peter Enemali, Emmanuel Enemali and my beloved sister, Mrs Grace John.

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ABSTRACT

Grasscutters are currently being explored as laboratory models for scientific, medical and behavioural researches. In the present study, the anatomical, histological, histochemical and immunohistochemical studies of the spinal cord of the grasscutter was evaluated. Twelve adult Grasscutters comprising six males and six females were utilized for the study. The studies on the spinal cord of Grasscutter were done by gross observations and light microscopical methods. The animals were weighed and sacrificed by dissected. The spinal cords were removed by splitting open the vertebrae to expose the spinal cords which were then cleaned by removing the meninges surrounding them. The gross morphology of the spinal cord was studied while the segments were fixed, processed, sectioned and were taken for histological, histochemical and immunohistochemical studies using H and E, Cresyl fast violet, Golgi-Cox and Alpha-1 antityrosine hydroxylase methods respectively for detailed studies of the spinal cord segments of the Grasscutter. Histometrical evaluation wasdone for detailed studies on the areas and perimeters of the various neurons in the spinal cord segments of Grasscutter. The results showed that the mean weight of the males was significantly higher than the females ($P<0.05$) while the mean weight of the spinal cord did not show sexual dimorphism. The mean weight of the Grasscutter was 4700.27±0.05 g and 3633.40±0.80 g for males and females respectively. The spinal cord of the Grasscutter was observed to be made up of 28 segments which are regionally divided into 7 cervical, 13 thoracic, 6 lumbar and 2 sacral spinal segments. Two enlarged regions were observed in the spinal cord of Grasscutters namely, the cervical enlargement which was observed between C_2 and C_7 and lumbosacral enlargement observed between L_1 and S_2 spinal cord segments. The result showed that the histological features of the spinal cord of the Grasscutter with H & E method, has inner gray matter core surrounded by an outer white matter. No histological difference was observed between male and female Grasscutter. The basic structural pattern

was the same throughout the length of the spinal cord of the Grasscutter. The present study using Cresyl fast violet method showed that the gray matter was stained dark purple due to the abundant presence of Nissl bodies in the neuronal cell bodies while the outer zone of white matter stained relatively lighter due to the presence of nerve fibers in the region. The neurons in the anterior gray horns of cervical, thoracic, lumbar and sacral segments of the spinal cord are multipolar motor neurons with prominent vesicular nucleus while cells in the posterior gray horns of cervical, thoracic, lumbar and sacral segments are sensory neurons. The spinal cord of the Grasscutter when stained with Golgi-Cox method revealed neurons which are of various shapes in the gray matter of the spinal cord of the Grasscutter. The gray matters of the spinal cord which had posterior and anterior horns were seen to contain neurons which were composed of several dendrites and one axon. Neurons found mainly in the posterior horns of the spinal cord of Grasscutter especially in the cervical and lumbar spinal segments were unipolar neurons while neurons of the anterior horns of the spinal cord of cervical, thoracic, lumbar and sacral spinal segments of Grasscutter were multipolar neurons. The result of immunohistochemical studies of the spinal cord of the Grasscutter treated with alpha-1 antityrosine hydroxylase, showed that dopaminergic neurons are present in the spinal cord of Grasscutters. The dopaminergic neurons were deeply stained in the lumbosacral segments than in other spinal cord segments of Grasscutters. The result of the histometrical studies of cell areas and cell sizes of the spinal cord of Grasscutters showed that the greatest value for mean for cell perimeter and cell area was observed in the lumbar spinal cord segment to be 9.30± 0.67 and 3.28± 0.59 respectively while the least values cell perimeters and cell areas were calculated to be 3.86± 0.24 and 0.83± 0.09 respectively were observed in the cervical spinal cord segment. Using *t*- test analysis the cell area in cervical ventral horn wassignificantly higher than cell area in the sacral ventral horn ($P<0.05$) and cell area in the dorsal horn of cervical spinal cord was significantly higher than the cell

area in the dorsal horn of the thoracic spinal cord segment of Grasscutter. The cell perimeter of the cells of the ventral horn of lumbar spinal cord segment was significant larger than cell perimeter of the cells of the ventral horn of the cervical spinal cord segment ($P<0.05$) and the cell sizes of the dorsal horns of lumbar spinal cord segment was significantly higher of the dorsal horn cell of the cervical spinal cord segment ($P<0.05$). The anatomical, histological, Histochemical, immunohistochemical and histometrical studies on the spinal cord of Grasscutter (*thryonomysswinderianus*) showed unique features which include cervical and lumbosacal enlargements, presence of Nissl bodies in the cell bodies of neurons and abundance of dopamergic neurons in the cervical and lumbosacral regions. These features could be helpful in understanding the biology and behaviour of this rodent.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of study

The African Grasscutter also called the grasscutter or the greater cane-rat (AGC) is a rodent, domiciled in Africa. It is a wild herbivorous rodent regarded by some people as a large version of the rat (Nyameasem *et al.,* 2018). The genus, *Thryonomys,* also known as 'cane rat' in Southern Africa or Grasscutter in West Africa is a genus of rodent and the only member of the family *Thryonomidae* and the suborder *Hystricomorpha* (Adebayo*et al.,*2014). Although many varieties have been described, there are probably only two species namely; the greater cane rat (*Thryonomys swinderianus*), weighing about 9kg or more and has a head-and-body length of up to 60 cm and the lesser cane rat (*Thryonomys gregorianus*) weighing about 3.5-5 kg and may occasionally reach up to 8 kg and a body length of about 50 cm (Aluko, 2014). Grasscutter is the second largest African rodent after the Porcupine (Abioye*et al.,* 2008). Grasscutters are being hunted aggressively in the wild because of their meat value, leading to setting of bushfires by hunters (Alamuoye,2017).

Although much effort has been made to domesticate this rodent, the efforts has not been successful in the past (Eben, 2004,Opara *et al.,* 2006,Alamuoye,2017). The rodent isthe most preferredmeat in West Africa, including Nigeria andcontributes to both local and export earnings of most West African Countries(Martin, 1983, Baptist and Mensah,1986; Asibey and Addo, 1999, Addo *et al*.,2001).

The African Grasscutters have peculiar behaviour in the wild and captivity which may be different from the other rodents. Although Grasscutters commonly forage in groups, they are generally solitary. In addition, they are nocturnal and travel at night through trails in reeds and grasses (Adu *et al*.,2017).Most of the animals seen in the markets are males according to

National Research Council (1991), possiblybecause males lead the groups and are thus most prone to being captured (Aluko, 2014). Grasscutters have a long gestation period, about five months and a relatively small litter size, maximum of four in the wild but up to twelve have been recorded in captivity (Agwunobi *et al.*, 2009, Adebayo*et al*., 2014).

The young are born with their eyes open and the fur coat fully developed and is capable offollowing the mother immediately after birth. Females can be re-fertilised after parturition if paired with the male, thus two litters are possible in a year (Asibey and Addo, 1999).Grasscutters are herbivorous and their favourite foods are the savannah grasses (Asibey, 1974). In captivity, they can take sugar cane, corn stalks and cassava peelings (Akinloye, 2005).The animal doesnot burrow but may shelter in hollows and burrows made by other animals (Asibey and Addo, 1999).

The spinal cord represents the most caudal part of the central nervous system. It is located within the canal of the vertebral column surrounded by cerebrospinal fluid (Eric, 2006, Amene and Nima,2017). During its embryonic development, there is surprisingly little change in the internal anatomical organization of the spinal cord. The peripheral nerve fibers are grouped into segmental spinal nerves that emerge from the vertebral canal between the individual vertebrae. The human spinal cord segments are subdivided into 8 cervical (C_1-C_8), 12 thoracic (T_I-T_{12}), 5 lumbar (L_1-L_5), 5 sacral (S_I-S_5) and 1 coccygeal segments. In the center of the spinal cord lies the central canal, which is a remnant of the space within the primitive neural tube (Acer *et al*., 2008, Silva *et al*.,2014).

The spinal cord is bilaterally symmetrical and consists of a centrally located gray matter, which is characterized by a large number of neuronal cell bodies. This is surrounded by white matter, which is characterized by a large number of nerve fibers. Sensory information from the skin, jointsand muscles and to some extent, the viscera of the trunk and limbs, enters the spinal cord by way of the dorsal roots (Anderson, 2016). The motor neurons in the spinal

cord constitute the final common pathway for issuing commands for muscle contraction by way of the ventral roots (Anderson, 2016). In the spinal cord there is an orderly arrangement of the sensory and motor nuclei controlling the limbs and trunk. In addition, the spinal cord contains various ascending pathways that convey sensory information towards the brain, and descending pathways which originate in higher parts of the brain and terminate within the spinal cord (Cadotte and Fehlings, 2011,Anna *et al.*, 2017).

The enlargements at the cervical and lumbosacral segments provide innervations to the fore and hind limbs respectively; contributing to the brachial and lumbosacral plexuses in rodents (Bjugn *et al.,* 1989;Bjugn and and Gundersen,1993; Agbeluski. 1997, Rahmanifar *et al.,* 2008). The extent of the enlargements has been used to describe characteristic limb efficiency in rodents. Olude *et al*., (2015) used the spinal cord enlargements to explain limb efficiency in the African giant rats.

The animal can take a bunch of grass in their fore limbs, sit upright on their haunches and begin to feed the grass into their mouth slowly cutting it up into small bits and when fighting Grasscutters have shown the wonderful use of the hind limbs by standing upright on both limbs(Anon, 2013). These abilities suggest limbs efficiency which may be as a result of the innervations of the fore and hind limbs from the cervical and lumbosacral enlargements of the spinal cord of the rodent. These wild rodents have great potentials as laboratory animals and this has led to an increasing amount of interest in its biology.

1.2 Statement of Research Problem

The domestication of the African Grasscutters has been encouraged because of their highly rated delicacies and research potentials.Although literatures on the anatomy of the African Grasscutters are gr*adu*ally building up (Asibey and Addo 1999, Eben, 2004, Opara *et al*.,2006; Opara *et al*.,2008, Opara and Fagbemi 2008; Byanet *et al*., 2009, Opara *et al*.,2010,

Bello *et al.*, 2012;Adenyo *et al.*,2012,Adenyo *et al.*,2013, Adu *et al*., 2017) there is paucity of information on the anatomy of its spinal cord which could lead to the understanding of some of its behaviours and the biology of the animal in general.

1.3 Significance of the Study

The present study seeks to describe and document the gross architecture and cellular morphology of the spinal cord of the African grasscutter. The findings from the present study could be useful in understanding the biology of the rodent.

1.4 Aim and Objectives of Study

1.4.1 Aim of study

The aim of the study was to investigate the Anatomical andimmunohistochemicalstudies of the spinal cord of the African Grasscutter.

1.4.2 Objectives of the study

The objectives of the study wereto

i. study the gross anatomical features (morphometrics) of the spinal cord of AGC;
ii. histological features of the gray and white matter at segmental vertebral levels of the spinal cord of the AGC using H and E;
iii. srudy histochemistry of neuronal cell bodies using Cresyl fast violet stain and distribution of neuronal cell types using Golgi techniques in the spinal cord.
iv. Study the histometrical features of neurons in the horns of the spinal cord;
v. Localize of dopaminergic neurons (DPN) in the AGC spinal cord using immunohistochemical (IHC) techniques: *Alpha-1* anti-tyrosine hydroxylase.

1.5 Hypothesis of study

The anatomical features of the spinal cord of African grasscutter is not similar to that of other rodents.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Grasscutter

Grasscutter is a wild herbivorous rodent found in the sub-Saharan region of Africa including Nigeria, Togo, Benin and Ghana. It is the biggest rodent after porcupine in the rodent family. It is referred to as African Grasscutter, Cane rat, cutting grass or Grasscutter (Agwunobi*et al*., 2009). Scientifically, it is referred to as *Thryonomys swinderianus*. Its sub-order is that of Hystricomorpha (porcupine relatives) and the super family is Petromuroidea (Rock rat- like), with genus *Thryonomys*. The species are of two types Thryonomys *swinderianus* Temminck which is the greater cane ratand *Thryonomys gregorianus* which is the lesser cane rat (Simpson, 1974).

Kingdom : Animalia

Phylum : Chordata

Subphylum : Vertebrata

Class : Mammalia

Order : Rodentia

Sub-order : Hystricomorpha

Family : Thryonomyidae

Genus : *Thryonomys*

Species : *Thryonomys swinderianus*

2.1.1 Physical description of Grasscutter

The body length of Grasscutter (*Thryonomys swinderianus)*ranges from 25-70 cm, with an average of 48 cm and their tail reaches 0.65-0.26cm in length (Mpoame, 1994,Asibey and Addo,1999). The total body weight of adult Grasscutter ranges between 4-12 kg. The heaviest body has an average weight of 4.5 kg in male grasscutter and 3.5 kg in female Grasscutter (Merwe, 2000), which looks like a giant guinea pig with a short tail. The body is heavily built, small round with bristle fur and coat and a circular ear (Howell,1981,Akinloye, 2005). The fur reflects the general colour of the animal, with brownish colour from the base to the middle of the fur, while the upper fur appears light yellow to reddish. The combination of these colours gives the animal brownish yellow or red colour. Besides, these colours in Grasscutter range from grayish black to brown exist genetic improvement on breeding has produced other colours such as black, yellowish red and white coloured Grasscutters (Onwuama *et al.,* 2015; Al-Dawood, 2017).

Furthermore the skin of Grasscutter is always white after processing and they have rounded nose, short ears and incisors that grow continuously (Mills, 1997). The pelage is coarse, with flattened bristle- like hairs that grow in groups of five or six. The fore limbs are smaller than the hind limbs and have three well developed middle digits with the first and fifth digits greatly reduced. The hind limbs have no first digit and all digits have heavy claws (Asibey and Addo,1999). The arrangement of the Grasscutters digits and pads on the fore limbs allows a stem to be gripped in one paw only, while being fed into the mouth unlike other rodents that use both paws to hold a single stem (Asibey and Addo,1999).

The dental formula for *Thryonomys swinderianus* is 1/1, 0/0. 1/1, 3/3 (Merwe, 2000). Grasscutters are endothermic and bilaterally symmetrical. Grasscutters run quickly when disturbed. Their nipples are arranged in lateral position and this makes the young ones to

suckle their mother from the side (Onwuama *et al.*, 2015). The head is broad with short flattened muzzle and with small eyes and ears. Its tail is covered with small hairs (Asibey, 1974; NRC, 1991). The photographs of the African grasscutters are shown in Fig. 2.1 and Fig 2.2.



Fig 2.1: African Grasscutter.

Image Source: www.dailytrust.com.ng



Fig 2.2: A family of three female Grasscutters living together.

Image Source: www.dailytrust.com.ng

2.1.2 Behaviour of Grasscutter

Thryonomys swinderianus is a semi nocturnal animal and they live in colonies comprising one male and several females in each colony and the young ones from more than one generation. Even though it possesses digits equipped with power and sharp claws, thefemalesdo not crawl on vertically erect wall or object unlike African giant rat. It does notburrow into the ground, but can temporarily shelter in hollow made by other animals. It is sensitive to noise and almost very frightened when it senses danger (Asibey and Addo,1999). The animal always shelters itself with dry grass to provide warmth since the animals is allergic to cold weather (Ogunsanmi*et al.*, 2002, Abioye *et al*., 2008). Grasscutter cannot jump any obstacle above 3 feet. Many of the animal's weaknesses make it prone to easy capture when spotted by hunters, capture team, or predators. Nevertheless, Grasscutter has the ability of freezing up while hiding under grasses without being detected when it is being hunted (Akpan *et al*., 2009, Adu *et al.*, 2017).

The animal tends to always protect its home range (feeding, nesting and mating territory) against another animal of their kind. That is why Grasscutter's house and enclosure are divided into many partitions suitable for easy grouping of the animals to avoid cannibalism and fighting among animals, more especially during mating (Abioye *et al*., 2008).

2.1.3 Biology of Grasscutter

Thryonomys swinderianus live in groups of males and females during the breeding season and during the dry season; the males separate from the groups and live by themselves while the females live together afterwards (Jori *et al*., 2001). The wet season of the year is usually the breeding season (Oduor-Okelo and Gombe, 1982). The females are spontaneous ovulators and usually two litters are possible in a year, but the females must be flushed before refertilization for high prolificacy(Addo *et al*., 2001, Olumu *et al.*,2003). Sexual maturity is

attained by 6 months for males and 5 months for females but there may be slight variations due to their feeding habit and patterns(Asibey and Addo, 1999). The gestation period is 5 months (152± 2 days). The average litter size is four in the wild but up to twelve litters size has been recorded in captivity (Addo, 2002; Abioye *et al*., 2008). Growth continues at old age but very slowly and the oestrus cycle lasts for a period of 6.62 days while the offspring weighing about 1.29 g and relatively well developed. The offsprings are precocious, fully furred and capable of running and have their eyes open at birth. Sexual determination in the Grasscutter is done at birth on the basis of the pro-genital distance, which is larger in males than in females as well as a dark brownish stain around the genital part of the mature buck (Addo *et al*., 2001, Van *et al*., 2005). Flushing of female Grasscutter is necessary to allow for proper breast feeding of the kids and this can last for about three to six weeks. The female Grasscutter possesses three pairs of mammary glands by the side of the belly. Apart from the anogenital colour and distance, the animal size can be a criterion for sex differentiation, where the males are larger in size than the females (Addo *et al*., 2001). Shape of the animal's nose is also a criterion for sex differences while the males have blunted and muscular nose shape the female have a pointed nose shape. However, the shape of the animal's nose becomes more conspicuous when the animal is in motion or when the animal adopts a standing position(Agbeluski, 1997;Agwunobi *etal.*, 2009). Another criterion for sex determination is the peculiar sound produced by the males and females, especially during courtship (Asibey, 1974).

2.1.4 Life span/longevity of Grasscutter

The average lifespan of a captive-reared Grasscutter ranges from 7-9 years while they can live up to 12 years depending on health care given to the animals during their service lives(Opara *et al.,* 2008, Aluko, 2014). The total life span is measured from the period of parturition to death while the service life is the period when the animal reaches maturity to

the time when it can no longer reproduce. That is the period of efficient reproductive performanceof the animals(Aluko, 2014).

2.1.5Characteristics and habitat of Grasscutter

In Nigeria the Grasscuuters live in most habitats from rain forests of the South to the semi-arid regions of the extreme north (Asibey,1974). Most of the Grasscutters are ground living and some of the Grasscutters are arboreal (Aluko, 2014). Most distribution of Grasscutters are in dense grasslands and secondary growth in rainforest, derived and guinea savannah zones like Aba, Baissa, Bali, Calabar, Choko Choko, Ede, Elele, Gambari, Ibadan district, Igangan, Ilaro, Ilesha, Ilo, Jalingo, Kanji, Lake Kangoro, Kudu, Lagos, Mamu river (Ogun-Osun river), Muri, Nikrowa, Nsukka, Oba hills, Okogere, Ondo, Owerri, Pandam, Sapoba, Serki, Shasha, Upper Ogun and Yankari (Asibey,1974,Dwarika *et al.*,2008). Asibey*et al.*,(1999), observed that these animals are found mostly in areas consisting of South Eastern part of Nigeria and South Western Cameroon.

The animals are observed to be present in primary and secondary forest adjacent to major plantations especially in new oil palm plantation. In suitable habitat, they are quite common and their presence is usually revealed by the cut on grass stem, by their characteristic faecal pellets scattered on the ground besides the cut grasses and their runaways through the vegetations. Cane rats are heavily-built rodents, with bristly brown fur speckled with yellow or grey (Anon, 2013) (Fig 2). Grasscutter furs comprise a mixture of brown reddish and gray hairs that vary depending on its habitat (Jori and Chardonnet, 2001). They live in marshy areas and along river and lake banks and are herbivores, feeding on aquatic grasses in the wild (Anon, 2013). In agricultural areas they will also as the name suggests, feed on the crops in cane plantations, making them a significant Pest (Awah-Ndukum *et al.*,2001).

Cane rats are widely distributed and farmers expend substantial energyfencing the rodent out of their fields, but they are also valued as a source of "bushmeat" in West and Central Africa (Anon, 2013). In the savanna area of West Africa, people have traditionally captured wild Grasscutters and fattened them in captivity (Anon, 2013). More recently, intensive production of Grasscutters has been undertaken in Countries such as Benin and Togo and agricultural extension services in Cameroon, Gabon, Ghana, Nigeria and Democratic Republic of Congo have also encouraged farmers to rear these rodents in rural and peri-urban areas (Anon, 2013).

Grasscutters live in small groups led by a single male (Anon, 2013). They are nocturnal and makes nests from grasses. Individual may live in excess of four years (Anon, 2013). If frightened, they grunt and run towards water. The Grasscutter expanded from their native reeds into plantations, particularly the sugar cane plantation from which they derived their name (Anon, 2013). Their tendency to adopt plantations as habitat, where they feed on agricultural crops such as maize, wheat, sugarcane, and cassava often earns them the label of Agricultural pest (Anon, 2013).

In Nigeria and other regions of West Africa, they are called Grasscutters (Anon, 2013). Cane rat earned their African nick name of "Grasscutter" because of their method of eating after using their powerful incisors to cut grasses at their base, the animal take the bunch of grass in their fore feet, sit upright on their haunches and feed the grass into their mouth slowly cutting it up into small bits (Anon, 2013). When eating and when relaxed they make soft grunting noises. When threatened, cane rats thump their powerful rear feet on the ground to alert others while emitting a piercing whistling sound. Although its teeth are formidable, a

frightened cane rat will virtually always run with great speed into dense vegetation and towards the nearest open water rather than turning to fight (Byanet *et al*., 2009).

If captured, the Grasscutters thrash frantically and are frequently injured. When enclosed in a box or crate, the rat often uses their padded nose as battering rams to try to escape. Primarily nocturnal, cane rats create and use narrow trails through the grass and reeds to move around their territories (Anon, 2013). Biologists think they live in groups of not more than twelve individuals. Males, who live with their young and a few mature females, do not tolerate the presence of other mature males, aggressively defend their family groups. Males fight by pressing their padded noses together until one ease up on the pressure, at which point his opponent may swiftly swing his rump around to knock the weaker rat off balance (Anon, 2013). Despite their well-developed claws, cane rats use burrowing only as a last resort for shelter and even then, would rather use abandoned porcupine or aardvark burrows or holes in stream banks caused by erosion if dense vegetation for hiding is absent (Anon, 2013).

Cane rats have been observed gnawing on rocks, pieces of tusk and bones, presumably to sharpen their teeth. The cane rat mates with multiple partners throughout the year, although primarily during the rainy season when more food is available (Anon, 2013). Though nocturnal, after a certain period of acclimatization to their enclosure, Grasscutters in captivity have also started to feed by day.

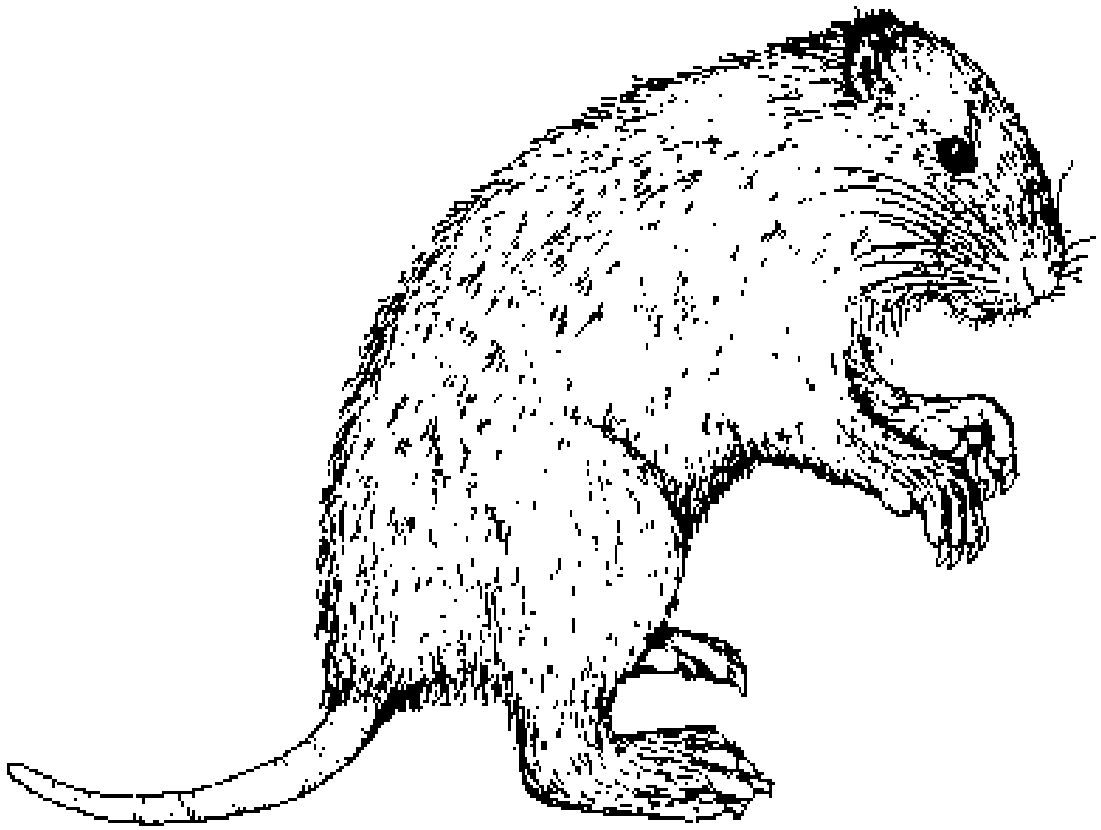


Fig 2.3: A figure showing Grasscutter standing in its hindlimbs.

Image Source: www.ispotnature.org

The greater cane rat (*Thryonomys swinderianus)* and the lesser cane rat (*Thryonomys gregorianus)*; both inhabit non forested sub-Saharan African except for Namibia and most South Africa and Botswana. The two species are found together in certain regions but they occupy different habitats (Owen and Dike, 2012). In Ogun State, Nigeria; Aluko (2014) observed the shape of head, eye placement, ear shape, body pelage, tail shape, breed, tail colour in *Thrynonomys swinderianus swinderianus* (Tss) and *Thryonomys swindrianus gregorianus* (Tsg). In her study, the Tss population had more of the broad heads than the narrow heads. The broad headed animals had broad tip ear, brown and yellow-belly and white body colour. Their tail is broad based and thintapers out to tip although a few had broad base and even out to tip.These broad headed animals had black and hairy tail. She further stated that in the Tsg population, most of the animals had narrow heads than broad heads. These narrow heads animals had ear with broad tip and have black and yellow – belly white body colour. The tail is broad based and thin out to tip. All these narrow heads, black and yellow-belly whitebodies' animals had black and hairy tail. The greater cane rat lives along rivers and lakes and in swamps, reed beds and tall, dense grass with thick cane like stems where as the lesser cane rat prefer grassy ground in moist savannas and tall grass on rocky hill sides (Fayenuwo *et al*., 2013, Igado*et al.,* 2016). Cane rats are swift and agile on land and swim very well. Though primarily nocturnal, they are occasionally active during the day. Depending on the season, greater cane rats are solitary or communal (Anon, 2013). Lesser cane rats live in small family groups, usually denning in thick vegetation, although, termite mounds and the abandoned burrows of aardvarks or porcupine are also used. Their diet consists of grass, other plants, and sometimes barks and fruits (Eben, 2004). Cane rats also eat crops and can become serious pests in regions where corn (maize), sugar cane, pineapples, cassava and egg plant are cultivated. On the other hand, these large rodents provide a significant source of protein for Africans and are intensively

hunted throughout their range (Anon, 2013, Poku *et al.,* 2013). *Thryonomys gregorianus* also called lesser cane rat occurs throughout Eastern African and into Western African countries of Cameroon, the Central African Republic, Chad, the Democratic Republic of Congo, Kenya, Malawi, Sudan, Tanzania, Uganda and Zambia. An isolated population also exists in Zimbabwe and Mozambique. It is likely that population exists in Angola, however no records have been collected of the Zambian border (Skinner and Chimimba, 2005). Lesser cane rats, occasionally referred to as savanna cane rats, inhabit most grasslands dominated by elephant grass (*Pennisetum purpureum*). They are also occasionally found along the fringes of swamps and in wooded areas (Antonanzas *et al.*, 2004).

Individuals usually use tall grass for shelter, but have also been found digging shallow burrows or using rock crevices, termite mounds and abandoned aardvark or porcupine holes. This species is considered non-aquatic, differing in habit preference from the semi-aquatic *Thryonomys swinderianus.* Individuals have been recorded up to altitudes of 2,600m(skinner and chimimba, 2005). *Thryonomys gregorianus* family groups have territory sizes of 3,000 to 4,000 square meters. *Thryonomys gregorianus* individual have poor eyesight, but good senses of smell and hearing. Vocalizations include whistling and low hooting grunts. They will also thump their hind feet when alarmed. The greater cane rat (*Thryonomys swinderianus*) lives by reed-beds and river banks in sub-saharan Africa (Anon, 2013). It has rounded ears, a short nose and coarse bristly hair. Its forefeet are smaller than its hindfeet, each with three toes (Anon, 2013).



Fig 2.4: Young Grasscutter eating stem

Image Source: www.ispotnature.org

2.2 Gross organization of the mammalian spinal cord

The spinal cord lies in the vertebral canal and is continuous rostrally with the medulla oblongata just below the level of the foramen magnum. Caudally it terminates as conus medullaris which is continuous with filum terminale and is anchored to the dorsum of the coccyx (Dellmann and McClure,1975, Dyce *et al.,* 2002, Butler and Hodos, 2005, Dyce *et al.,* 2010). The spinalcord length varies in various animals and it has been described in several animals including the rabbits (Gabr,1982, Farag *et al.,* 2012, Elkarmoty,2012), goats (Kahvecioglu *et al.,* 1995; S*adu*llah *et al.,* 2013); sheep (Ghazi and Gholami, 1993, Ghazi and Gholami, 1993), donkeys (Ocal and Haziroglu, 2015), horse Claridge *et al*., 2010). In most animals the spinal cord is ensheathed with spinal meninges which are continuous with the cranial meninges through the foramen magnum(Bjugn *et al.,* 1989). The spinal cord is approximately circular in cross section and its diameter varies according to the level. It bears two enlargements which are: cervical and lumbar enlargements from which the innervations of the fore and hind limbs arise.

The anatomical location of the cervical and lumbosacral enlargements - between cervical (C4) and thoracic (T1) and between lumbar (L2) and sacral (S3) respectively were typical with laboratory rats (Bjugn *et al.,* 1989). These anatomical positions seem characteristic of rodents as several authors have reported slightly different positions in most domestic animals. The cervical enlargement is found in pigs (Dellmann and McClure, 1975), in the rabbits (Farag *et al.,* 2012), in the dogs (Miller *et al.,* 1964), in buffalo and camels (Abu-zaid, 1982; Mansour, 1980) and in Indian sheep, donkeys (Mansour, 1980; Rao, 1990), and man Kameyama,*et al*.,1994).

While the lumbosacral enlargement was observed in African giant rat (AGR), the donkeys (Mansour, 1980), the camels (Mansour, 1980), the rabbits (Farag *et al.,* 2012), the buffalo (Abu-zaid, 1982), the sheep (Rao, 1990), the pigs and the dogs (Dellmann and McClure, 1975). The enlargements at the cervical and lumbosacral segments provide innervations to the fore and hind limbs respectively; contributing to the brachial and lumbosacral plexuses (Bjugn *et al.,* 1989; Rahmanifar *et al.,* 2008).

The cord ends at differentlevel in different animals like horses, cattle (Deem *et al.*, 2004) and sheep (Rao, 1990), in dogs (Getty *et al.*, 1975), in buffalos (Rao, 1976) andimpalas (Rao *et al.*, 1995), and in humans (Choi *et al.*,1996). The spinal cord is divided into cervical, thoracic, lumbar, sacral and coccygeal parts. The segments correspondto the areas of the spinal cord to which the cervical, thoracic, lumbar, sacral and caudal spinal nerves are connected. In most mammals the spinal cord is seen to bedivided into segments. A spinal cord segment is that part of the cord where a pair of spinal nerve rootlets enters and leaves the spinal cord (Dellmann and McClure, 1975). The segment is named according to the pair of spinal nerves connected to it hence the third lumbar segment is connected tolumbar(L_3) spinal nerve.In man the spinal cord gives rise to 31 pairs of segmentally arranged spinal nerves which are attached to the spinal cord by linear series of dorsal amd ventral rootlets (Moore and Dalley, 2006). The dosal rootlets contain afferent nerve fibers and the ventral rootlet contains efferent nerve fibers group of adjacent rootlets coalesceto for the dorsal or ventral nerve roots (Ramer *et al.,* 2014;Ellingson, *et al.*,2015).

In human embryos, the spinal cord occupies the full length of the vertebral canal thus spinal cord segments lie approximately at the vertebral level of the same number, the spinal nerves passing laterally to exit the corresponding foramen. By the end of the embryonic period about

the 8th week, the tail-like caudal eminence has disappeared, and the number of coccygeal vertebrae is reduced from six to four segments. The spinal cord in the vertebral canal of the coccyx atrophies (Ramer *et al*., 2014).

During the fetal period, the vertebral column grows faster than the spinal cord. At birth, the tip of the medullary cone is at the lumbar spinal cord (L4) level (Rea,2009). Thus, in postnatal life, the spinal cord is shorter than the vertebral column; consequently, there is a progressive obliquity of the spinal nerve root. Because the distance between the origin of a nerve roots from the spinal cord and the nerve's exit from the vertebral canal increases as the inferior end of the vertebral column is approached, the length of the nerve roots also increases progressively. The lumbar and sacral nerve roots are therefore the longest, extending far beyond the termination of the *adu*lt cord at approximately the L2 level to reach the remaining lumbar, sacral, and coccygeal foramina (Kolesar *et al*., 2015, Gregory, *et al.*, 2017).

This loose bundle of spinal nerve roots arising from the lumbosacral enlargement and the medullary cone and coursing within the lumbar cistern of CSF caudal to the termination of the spinal cord resembles a horse's tail, hence its named the cauda equine (Yu *et al*., 1985). Arising from the tip of the medullary cone, the terminal filum descends among the spinal nerve roots in the cauda equine (Eric, 2006).

The terminal filum is the vestigial remnant of the caudal part of the spinal cord that was in the tail-like caudal eminence of the embryo. Its proximal end (the pial part or internal terminal filum) consists of vestiges of neural tissue, connective tissue, and neuroglial tissue covered by pia mater. The terminal filum perforates the inferior end of the dural sac, gaining a layer of dura and continuing through the sacral hiatus as the dural part or external terminal

filum(also known as the coccygeal ligament) to attach to the dorsum of the coccyx. The terminal filum is an anchor for the inferior end of the spinal cord and the spinal meninges (Moore and Dalley, 2006).



Fig 2.5: Gross Anatomy of thehuman spinal cord.

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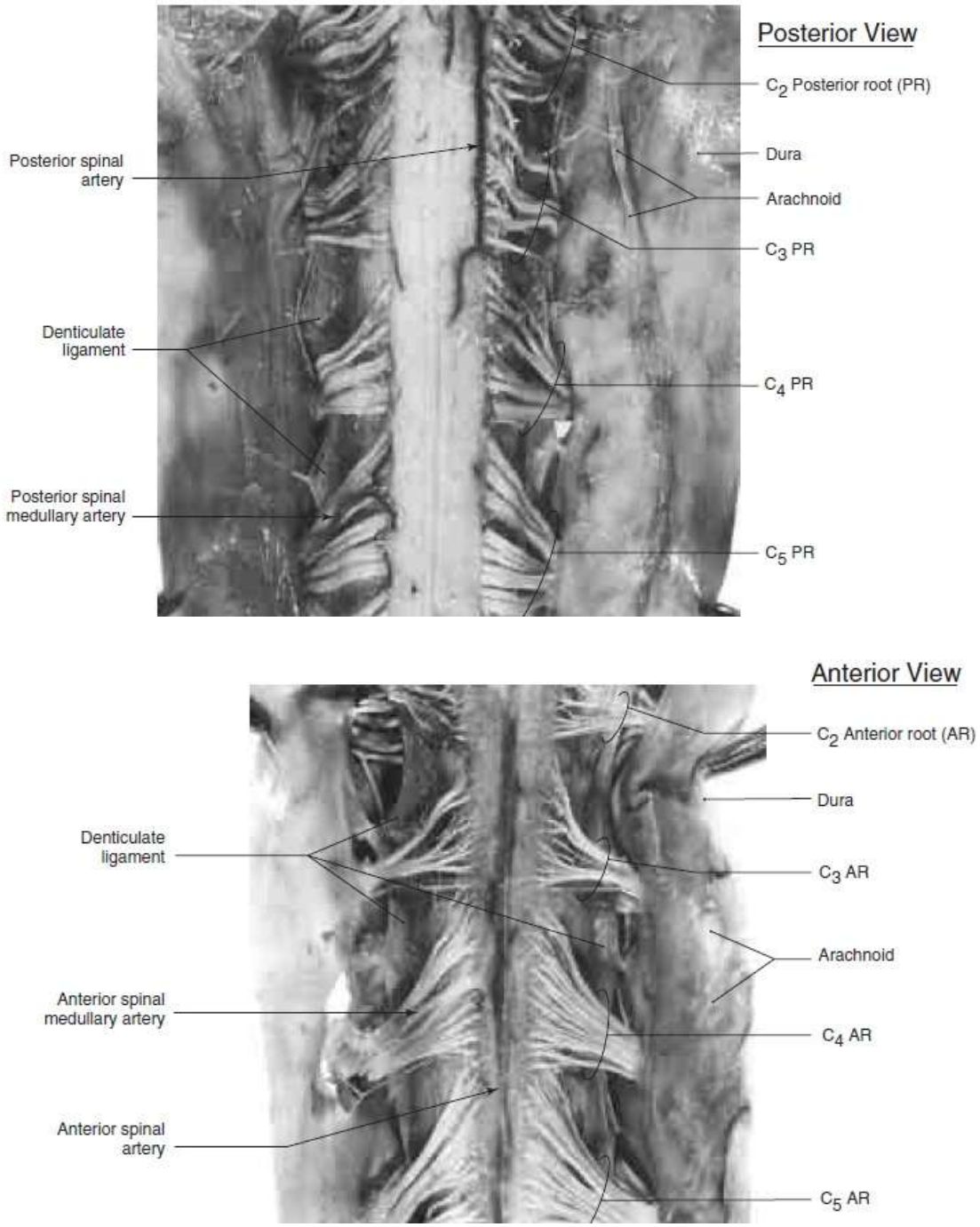


Fig 2.6: Posterior (upper) and anterior (lower) views with the general features of the human spinal cord as seen at levels cervical spinal cord (C2–C5). The dura and arachnoid are reflected, and the pia is intimately adherent to the spinal cord and rootlets.

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2.2.1 Vascular supply to the spinal cord

Spinal blood supply was first studied by Albert Wojciech Adamkiewicza Polish pathologist, in 1881. The great radicular artery is also known as the Adamkiewicz artery(Lip *et al.,* 2017). Knowledge of the blood supply to the spinal cord is important when planning treatment of diseases of the aorta.

Observations in the rabbit confirmed high variability of segmental arteries supplying blood to the spinal cord. On the left side, they occurred in higher number with more uniform distribution. Arteries in the thoracic and lumbar regions ensured segmental supply of the respective sections of the arteria spinalis ventralis and, thus, also the caudal two-thirds of the rabbit spinal cord. Segmental arteries in the thoracic region occurred homosegmental, that is, each spinal cord segment is supplied with one corresponding radicular artery with minimal or none collateral bloodstream.

In the mouse, the artery of Adamkiewicz, regularly observed in man in whom it supplies the spinal cord caudally from the point of narrowing of the arteria spinalis ventralis. In humans the arteries supplying the spinal cord are branches of the vertebral, ascending cervical, deep cervical, intercostal, lumbar, and lateral sacral arteries. Three longitudinal arteries supply the spinal cord: an anterior spinal artery and paired posterior spinal arteries(Hamdy*et al*.,2017).

These arteries run longitudinally from the medulla of the brainstem to the medullary cone of the spinal cord. The anterior spinal artery, formed by the union of branches of the vertebral arteries, runs inferiorly in the anterior median fissure. Sulcal arteries arise from the anterior spinal artery and enter the spinal cord through this fissure. The sulcal arteries supply approximately two thirds of the cross-sectional area of the spinal cord (Hamdy*et al*.,2017). Each posterior spinal artery is a branch of either the vertebral artery or the posteroinferior cerebellar artery. The posterior spinal arteries commonly form anastomosing channels in the

pia mater. By themselves, the anterior and posterior spinal arteries can supply only the short superior part of the spinal cord. The circulation into much of the spinal cord depends on segmental medullary and radicular arteries running along the spinal nerve roots(Moore and Dalley, 2006).

The anterior and posterior segmental medullary arteries are derived from spinal branches of the ascending cervical, deep cervical, vertebral, posterior intercostal, and lumbar arteries. The segmental medullary arteries are located chiefly where there is a need for a good blood supplies to the spinal cord. They enter the vertebral canal through the interveteral dics foramina. The great anterior segmental medullary artery (of Adamkiewicz), which is on the left side in about 65% of people, reinforces the circulation to two thirds of the spinal cord, including the lumbosacral enlargement. The great anterior segmental medullary artery, much larger than the other segmental medullary arteries, usually arises from an inferior intercostal or upper lumbar artery and enters the vertebral canal through the intervertebraldisc foramen at the lower thoracic or upper lumbar level (Moore and Dalley, 2006).

The posterior and anterior roots of the spinal nerves and their coverings are supplied by posterior and anterior radicular arteries, which run along the nerve roots. Most spinal nerve roots and proximal spinal nerves and roots are accompanied by radicular arteries that do not reach the posterior, anterior, or spinal arteries. Segmental medullary arteries occurirregularly in the place of radicular arteries; they are really larger vessels that make it all the way to the spinal arteries. Most radicular arteries are small and supply only the nerve roots; however, some of them assist with the supply of superficial parts of the gray matter in the posterior and anterior horns of the spinal cord. In general, the veins draining the spinal cord have a distribution similar to that of the spinal arteries. There are usually three anterior and three

posterior spinal veins. The spinal veins are arranged longitudinally, communicate freely with each other, and are drained by up to 12 anterior and posterior medullary and radicular veins. The veins draining the spinal cord join the internal vertebral (epidural) venous plexus in the epidural space. The internal vertebral venous plexus passes superiorly through the foramen magnum to communicate with dural sinuses and vertebral veins in the cranium. The internal vertebral plexus also communicates with the external vertebral venous plexus on the external surface of the vertebrae (Lip *et al.*, 2017).

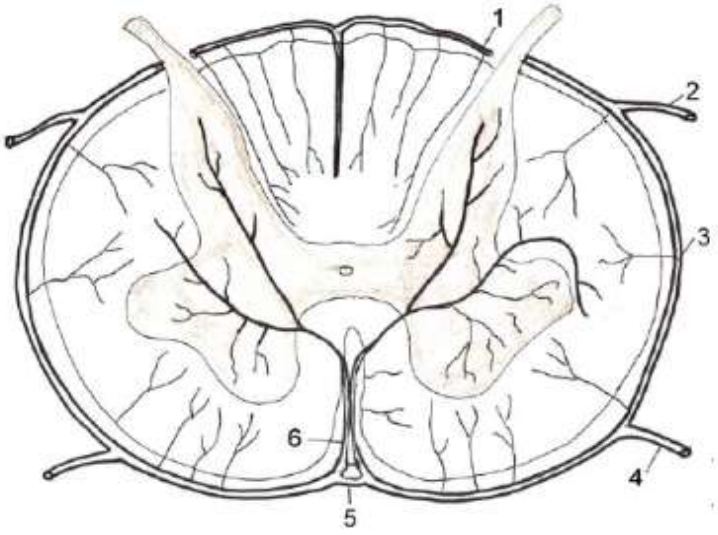


Fig 2.7: Distribution of arteries supplying the spinal cord. of wistar rat**1**: Dorsal Superior Artery, **2**: Dorsal branch; **3**: Spinal arterial ring, **4**: ventral branch; **5**: VSA **6**: Central artery.

2.2.2Spinal meninges

Spinal meninges have been observed in several animals. In fish, there is a single membrane known as the primitive meninx. In other vertebrates – amphibians, reptiles and birds, the meninges include a thick outer dura mater and a thick inner secondary meninx (Lukas *et al*., 2017). In the early 1900s, Giuseppe Sterzi an Italian anatomist carried out comparative studies on the meninges from the lancelet to man. Contrary to previous reports the spinal meninges were seen to be very simple, both in the adult lower vertebrates and in the early developmental stages of the more advanced vertebrates. From the mesenchyme surrounding the neural tube only a single leaflet forms the primitive meninx(Lee *et at.,* 2014).In the following phylogenetic and ontogenetic stages, the latter divides into an internal leaflet: the secondary meninx, and into an external one: the dura mater(Lee *et at.,* 2014).

Finally, in higher vertebrates, even the secondary meninx divides into the arachnoid matter and the pia matter. Mammals as higher vertebrates retain the dura mater, and the secondary meninx divides into the arachnoid and pia mater. Collectively, the dura mater, arachnoid mater, while pia mater surrounding the spinal cord constitute the spinal meninges(Lee *et al.,* 2014). These membranes surround, support, and protect the spinal cord and spinal nerve roots, including those of the cauda equina, and contain the cerebrospinal fluid (CSF) in which these structures are suspended (Bart*et al*.,2001,Hamdy*et al*.,2017).

The spinal dura mater, composed mainly of tough fibrous with some elastic tissue, is the outermost covering membrane of the spinal cord. The spinal dura mater is separated from the periosteum-covered bone and the ligaments that form the walls of the vertebral canal by the epidural space (Lee *et al.,* 2014). This space is occupied by the internal vertebral venous plexus embedded in a fatty matrix (epidural fat). The epidural space runs the length of the

vertebral canal, terminating superiorly at the foramen magnum and laterally at the IV foramina, as the dura mater adheres to the periosteum surrounding each opening, and inferiorly, as the sacral hiatus is sealed by the sacrococcygeal ligament. The dura mater forms the spinal dural sac, a long tubular sheath within the vertebral canal. This sac adheres to the margin of the foramen magnum of the cranium, where it is continuous withthecranial dura mater (Konstantin *et al*., 2017).

The spinal dural sac is anchored inferiorly to the coccyx by the terminal filum. The sac is evaginated by each pair of posterior and anterior roots as they extend laterally toward their exit from the vertebral canal. Thus, tapering lateral extensions of the spinal dura surround each pair of posterior and anterior nerve roots as dural root sheaths, or sleeves. Distal to the spinal ganglia, these sheaths blend with the epineurium (outer connective tissue covering of the spinal nerves) that adheres to the periosteum lining the foramina (Moore and Dalley,2006).

The spinal arachnoid mater is a delicate, avascular membrane composed of fibrous and elastic tissue that lines the spinal dural sac and its dural root sheaths. It encloses the CSF-filled subarachnoid space containing the spinal cord, spinal nerve roots, and spinal ganglia. The arachnoid mater is not attached to the dura mater but is held against its inner surface by the pressure of the CSF (Makino *et al.*, 1996). In a lumbar spinal puncture, the needle traverses the dura and arachnoid mater simultaneously. Their opposition is the durarachnoid interface, often erroneously referred to as the subdural space. No actual space occurs naturally at this site; it is, rather, a weak cell layer (Moore and Dalley, 2006). Bleeding into this layer creates a pathological space at the arachnoid junction in which a subdural hematoma is formed.

In the cadaver, because of the absence of CSF, the arachnoid mater falls away from the inner surface of the dura mater and lies loosely on the spinal cord. The arachnoid mater is separated from the pia mater on the surface of the spinal cord by the subarachnoid space containing CSF. Delicate strands of connective tissue, the arachnoid trabeculae, span the subarachnoid space connecting the arachnoid mater and pia mater (Moore and Dalley, 2006). The spinal pia mater, the innermost covering membrane of the spinal cord, consists of flattened cells with long, equally flattened processes that closely follow all the surface features of the spinal cord (Lee *et al.*, 2014). The pia mater also directly covers the roots of the spinal nerves and the spinal blood vessels. Inferior to the medullary cone, the pia mater continues as the terminal filum.

The spinal cord is suspended in the dural sac by the terminal filum and especially by the right and left sawtooth denticulate ligaments (L. denticulus, small tooth), which run longitudinally along each side of the spinal cord (Lee *et al.*, 2014). The denticulate ligament consists of a fibrous sheet of pia mater extending midway between the posterior and the anterior nerve roots from the lateral surfaces of the spinal cord. The processes, shaped much like shark's teeth, attach to the inner surface of the arachnoid-lined dural sac. The superior process of each denticulate ligament attaches to the cranial dura mater immediately superior to the foramen magnum, and the inferior process extends from the medullary cone, passing between the T12 and the L1 nerve roots (Lee *et al.*, 2014). The subarachnoid space is located between the arachnoid mater and the pia mater and is filled with CSF. The enlargement of the subarachnoid space in the dural sac, caudal to the medullary cone and containing CSF and the cauda equina, is the lumbar cistern. It extends from the L2 vertebra to the second segment of the sacrum. Dural root sheaths, enclosing spinal nerve roots in extensions of the subarachnoid space, protrude from the sides of the lumbar cistern.

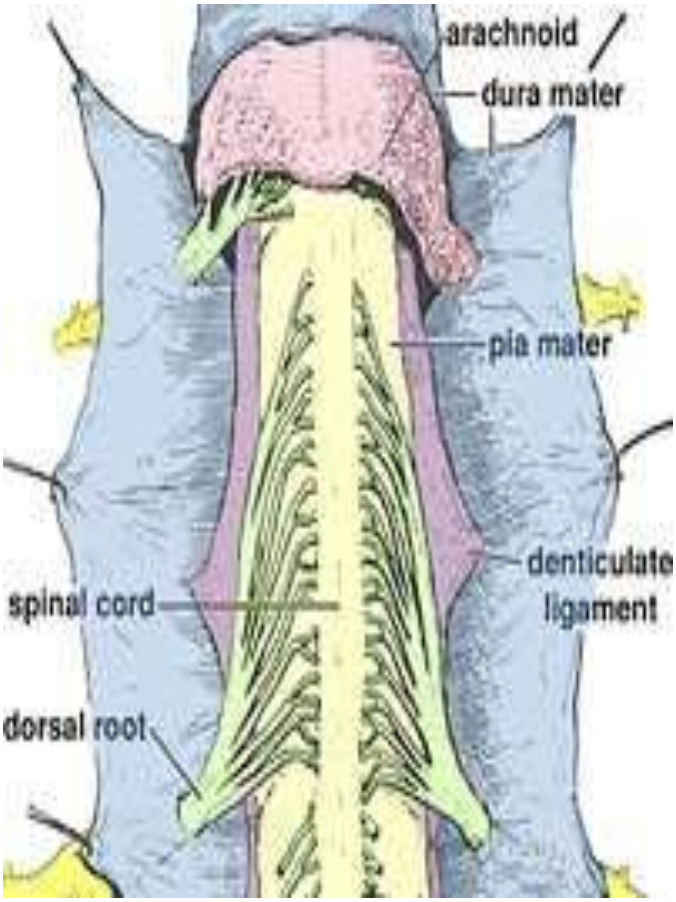


Fig 2.8: **Spinal meninges.** Three membranes (the spinal meninges) cover the spinal cord: dura mater, arachnoid mater, and pia mater.

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2.3 Histological organization of mammalian spinal cord

In most studies, the histology of the mammalians spinal cord is made up of white and gray matter. When seen in a transverse section the grey matter of the spinal cord is an H-shaped mass. Ineach half of the cord the gray matter is divided into a large ventral mass, the anterior (or the ventral) grey column and a narrow longated posterior (or dorsal) gray column. In some part of the spinal cord a lateral projection of grey matter is seen between the ventral and dorsal columns (Garcia-Ovejero, 2015, Cahoy, 2017).

The gray matter of the right and the left halves of the spinal cord is connected across the middle line by the grey commissure that is traversed by the central canal. The central canal of the spinal cord contains cerebrospinal fluid and is lined by the ependyma. The cell bodies of neuronsdiffer in size and in prominence of the Nissl substances in different regions of the spinal gray matter.

The white matter of the spinal cord is divided into right and left halves, in front by a deep anterior median fissure and behind by the posterior median septum. In each half of the spinal cord the white matter medial to the dorsal gray column forms the posterior funiculus (or the posterior white column)(Garcia-Ovejero, 2015).

The white matter of the spinal cord consists of ascending tracts of sensory fibres and descending motor tracts; passing up the spinal cord towards the brain. More and more fibres enter and leave the spinal cord so that the volume of white matter increases progressively from the sacral to cervical regions (Farhan *et al*., 2011). In the cervical segment, there is a relatively large amount of white matter. This pattern is caused by the many axons going up to the brain from all levels of the spinal cord and there are many axons traveling from the brain

down to different segments of the spinal cord. In lower segments of the spinal cord, there is less white matter because there are fewer axons travelling to and from the brain(Farhan *et al.*, 2011). Peripherally, the spinal cord has a deep ventral median fissure, but dorsally there is only a shallow dorsal midline sulcus. On each side, a dorsolateral sulcus marks the line of entry of the dorsal nerve roots. The roughly triangular area of white matter between the dorsal horns represents the ascending dorsal columns, which convey fibres for senses of vibration, proprioception and discriminatory touch to the medulla, where they synapse with second-order sensory neuronsin the gracile and cuneate nuclei (Hanna, 2017).

In the cervical region, each dorsal column is subdivided into two fascicles, the medial fasciculus gracilisconveying fibres from the lower limbs, and the lateral fasciculuscuneatus conveying fibres from the upper limbs. The ventrolateral white matter on each side is made up of ascending and descending tracts, most notably the lateral spinothalamic tract (pain and temperature), ventral spinothalamic tract (light touch), spinocerebellar tracts and corticospinal tract (motor) (Ghazi and Gholami,2016).

The cells of the anterior gray matter of the spinal cord are all multipolar, but differ essentially in that somepossess one unbranched or only sparsely-branched nerve process, while in others this gives off numerous branches. This latter set of cells can be divided into two sub-classes: (a) those in which the axis-cylinder process, in spite of it giving off branches, (b) those whose axis-cylinder process divides into delicate and very fine ramifications.Findings reports showed that the cytoarchitectonic organization of the spinal cord in rats was found to be basically similar to that of the cat. The cytoarchitectonic organization of the lower thoracic and lumbo-sacral spinal cord, the border-lines between the laminae were often found to be

ambiguous, suggestive of zones of transition rather than sharp borders (Molander *et al.*, 1989).

Bhardwaj *et al.*, (2004) carried out a Golgi study on the nucleus proprius in Wistar rat lumbar dorsal spinal horn. The dorsal horn capped with a crescentric neuronal zone, the substantial gelatinosa, recognized by the presence of densely packed small neurones. Ventral to the substantial gelatinosais a group of cells, the nucleus proprius, which contains densely packed neurones. Freire *et al.*,(2009), showed that the type I neuronsare localised mainly in the dorsal horn and around the central canal of every spinal segment, with a few scattered neuronslocated in the ventral horn of both cervical and lumbar regions. Several investigations have been carried out on the anatomy and physiology of spinal cord of Wistar rats, cats and hamsters and African giant rats.

Golgi in 1882 described two types of multipolar neuronsin the cat nervous system; the golgi type I and golgi type II neurones. He made distinction between the two types of neuronson the basis of the configuration of their axon. The axon of the golgi type I cells sends out few collateral branches, retaining its distinct identity, and becomes myelinated, divides repeatedly while that of the golgi type II cells, which usually remain unmyelinated, divide repeatedly shortly after leaving the soma, thus giving rise to a complex ramification. The differing morphologies reflect their different functional roles. Golgi described type I neuronsas having a varying axon length but that it always reaches a territory different from that in which the dendrites of the same cells arborise, in contrast, the axon of type II neuronsdivides repeatedly in the neighbourhood of its own neuronal body, that is in the same circumscribed field in which the dendrites of that cell ramify. Examples of type I neuronsare the motor neuronsof the spinal cord and brainstem, large pyramidal cells, large cells of Clarkes column, Purkinje

cells and granule cells of the cerebellar cortex. All these have axons that terminate in fields distinct from those containing the corresponding bodies and dendrites. The cell bodies and dendrites of a motor neurone lie in the spinal cord or brainstem, while the axon leaves the central nervous system and ends in the periphery. The cell and dendrites of a large pyramidal cell lie in the cerebral cortex while theyleave the cortex and terminates in other regions of the central nervous system like the brainstem or spinal cord (Inoue *et al.*,2016).

The two types of cells perform two distinct functional roles. The Golgi type I cell performs mainly integrative and projection functions. It collects information from a great number of afferents and, by means of its axons, conveys its integrated responses to more or less distant points. The Golgi type II cell, whose axon and dendrites arborise in the same circumscribed field, receives the same inputs as do the neuronson which its axon terminates, so that it has mainly local and modulatory role.

Studied have shown that the morphological and quantitative features in the adult human ventral anterior thalamic nucleus were studied in Golgi preparations. They described the type I neuronsas medium to large cells with dense dendritic trees and dendritic protrusions and short hair-like appendages and have somatic mean diameter of 30.8µm. The golgi type II neuronswere small to medium sized cells with few sparsely branching dendrites and dendritic stalked appendages with or without terminal swellings. They have somatic mean diameters of 22.2µm

It has been shown that the Grasscutter possesses good motor coordination and well-developed fore limbs and hind limbs (Kingdom, 1974). These anatomical features have been demonstrated to play a key role in the facilitation of the ability of the Grasscutter to stand only on its hind limbs especially during feeding hence have been able to developed modified

limbs. Since the anatomical bases for locomotion of the hind limbs in the rats are in the spinal cord and the nuclei involved in locomotion in the spinal cord of Grasscutter have not been identified and localised, such data, if obtained, may be of value in understanding the biology of the rodent.

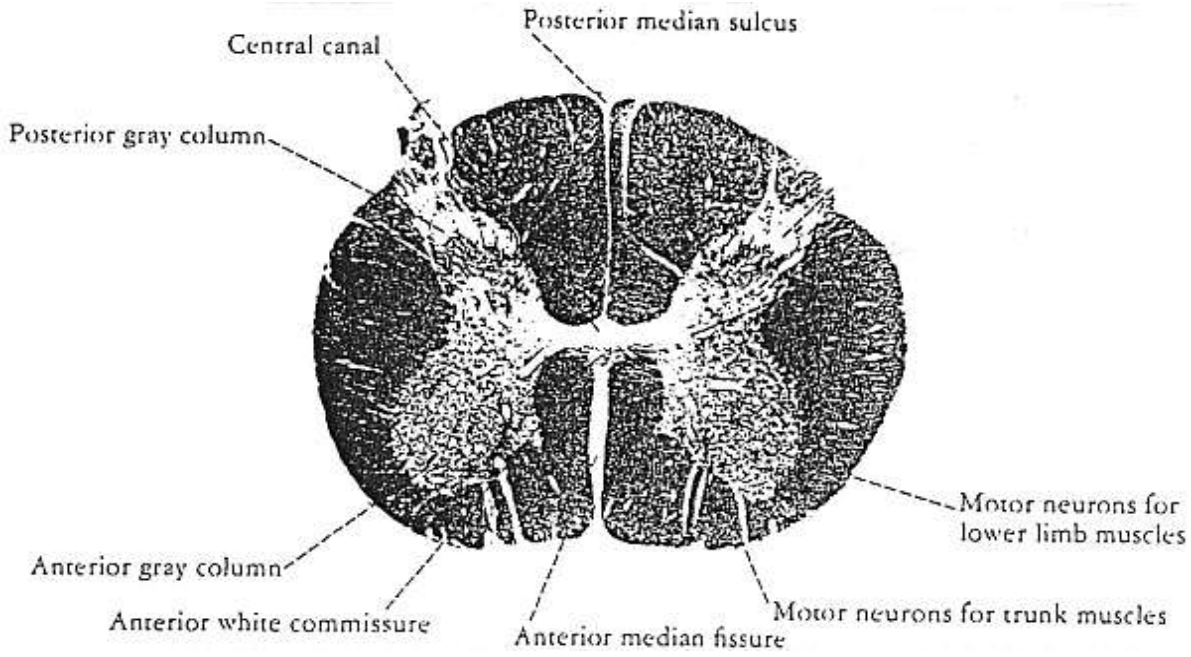


Fig 2.9: Transverse section of spinal cord at mid-lumbar level(Weigert stain for myelin).

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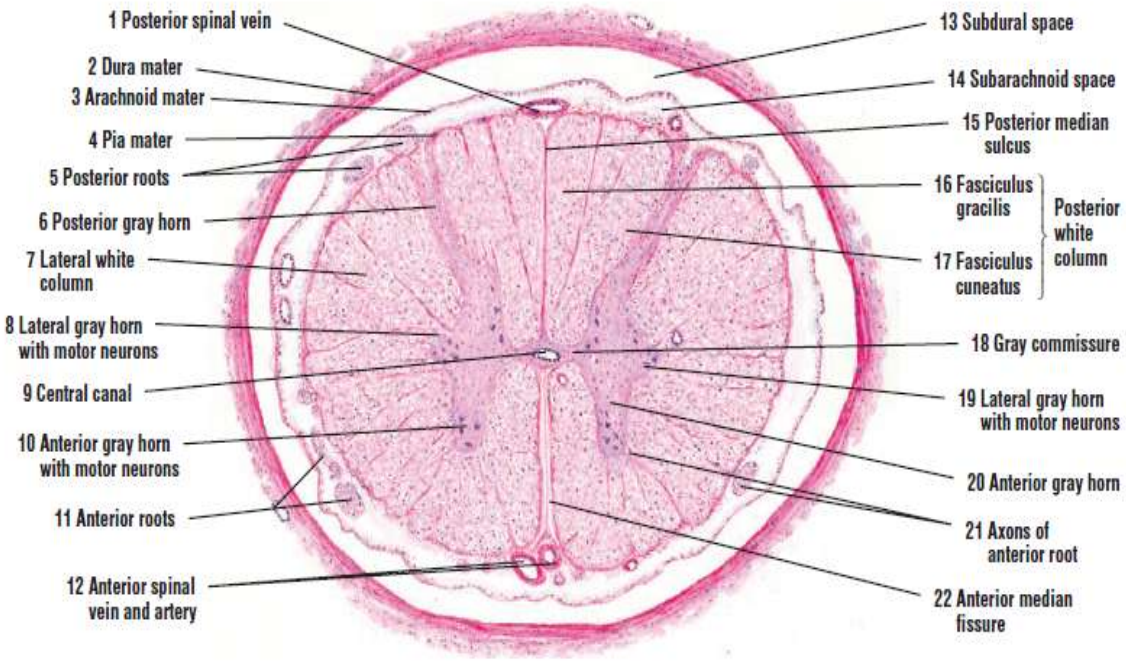


Fig 2.10: Midthoracic region (transverse section) of the spinal cord. Stain: hematoxylin and eosin Low maginification.

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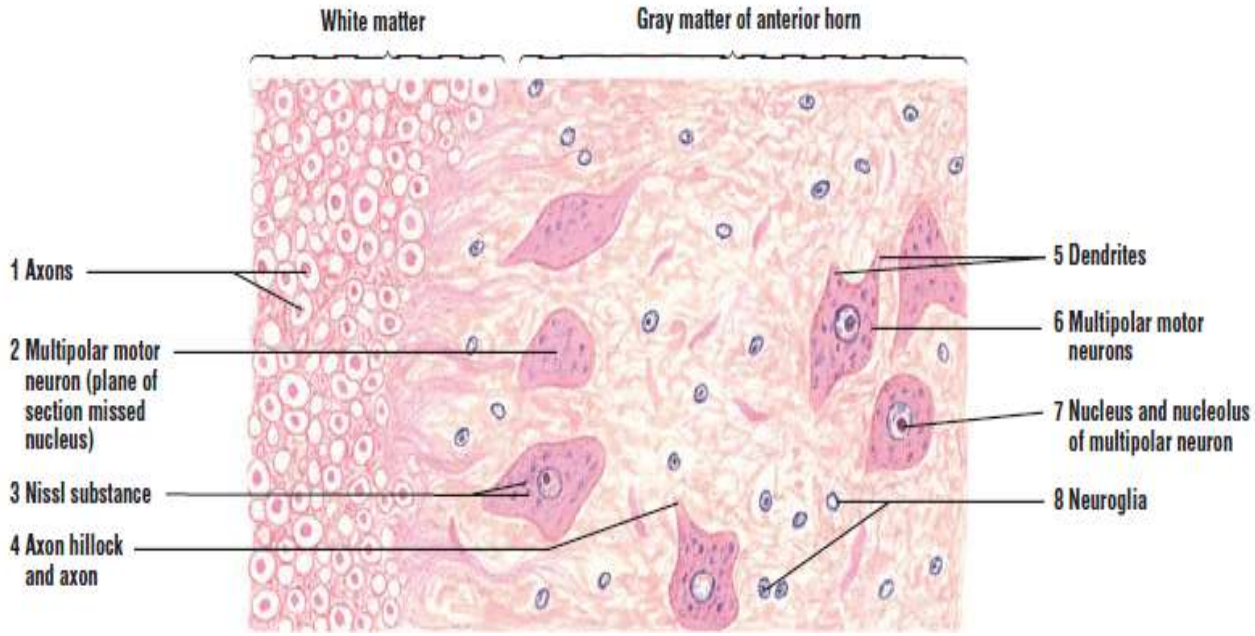


Fig 2.11: Anterior gray horn, motor neuron, and adjacent white matter of spinal cord. Stain: hematoxylinand eosin.

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2.3.1 Anatomy of neuron

A neuron (nerve cell)is an electrically excitablecell that receives, processes, and transmits information through electrical and chemical signals. These signals between neurons occur via specialized connections called synapses(Alvarez-Buylla *et al.*,2002). Neurons can connect to each other to form neural networks. Neurons are the primary components of the central nervous system, which includes the brain and spinal cord, and of the peripheral nervous system, which comprises the autonomic nervous system and the somatic nervous system(Claire *et al.,* 2000, Brunk and Terman, 2002).

There are many types of specialized neurons which are; sensory neurons,motor neuronsand interneurons. Sensory neuronresponds to one particular type of stimulus such as touch, sound, or light and all other stimuli affecting the cells of the sensory organs, and converts it into an electrical signal via transduction, which is then sent to the spinal cord or brain. Motor neurons receive signals from the brain and spinal cord to cause everything from muscle contractions and affect glandular outputs. Interneurons connect neurons to other neurons within the same region of the brain or spinal cord in neural networks (Herrero *et al*., 1993, Blakemore and Choudhury, 2006).

A typical neuron consists of a cell body (soma), dendrites, and an axon. The term neurite is used to describe either a dendrite or an axon, particularly in its undifferentiated stage. Dendrites are thin structures that arise from the cell body, often extending for hundreds of micrometers and branching multiple times, giving rise to a complex "dendritic tree". An axon (also called a nerve fiber) is a special cellular extension (process) that arises from the cell body at a site called the axon hillock and travels for a distance, as far as 1 meter in humans or even more in other species. Most neurons receive signals via the dendrites and send out signals down the axon(Zhao *et al*.,2017). Numerous axons are often bundled into

fasciclesthat make up the nerves in the peripheral nervous system (like strands of wire make up cables). Bundles of axons in the central nervous system are called tracts(Bjugn and Gundersen,1993). The cell body of a neuron frequently gives rise to multiple dendrites, but never to more than one axon, although the axon may branch hundreds of times before it terminates. At the majority of synapses, signals are sent from the axon of one neuron to a dendrite of another (Zhao, 2017). There are, however, many exceptions to these rules: for example, neurons can lack dendrites, or have no axon, and synapses can connect an axon to another axon or a dendrite to another dendrite (Anna *et al*., 2017).

All neurons are electrically excitable, due to maintenance of voltage gradients across their membranes by means of metabolically driven ion pumps, which combine with ion channels embedded in the membrane to generate intracellular-versus-extracellular concentration differences of ions such as sodium, potassium, chloride, and calcium. Changes in the cross-membrane voltage can alter the function of voltage-dependent ion channels (Gerber,2003). If the voltage changes by a large enough amounts, an all-or-none electrochemical pulse called an action potential is generated and this change in cross-membrane potential travels rapidly along the cell's axon, and activates synaptic connections with other cells when it arrives (Gerber,2003).

In most cases, neurons are generated by special types of stem cells during brain development and childhood. Neurons in the adult brain generally do not undergo cell division. Astrocytes are star-shaped glial cells that have also been observed to turn into neurons by virtue of the stem cell characteristic pluripotency(Wilson*etal*., 2012). Neurogenesis largely ceases during adulthood in most areas of the brain. However, there is strong evidence for generation of substantial numbers of new neurons in two brain areas, the hippocampus and olfactory bulb (Rutecki,1992).Neurons are highly specialized for the processing and transmission of cellular

signals. Given their diversity of functions performed in different parts of the nervous system, there is a wide variety in their shape, size, and electrochemical properties. For instance, the soma of a neuron can vary from 4 to 100 micrometers in diameter (Llinás and Rodolfo,2014).

The soma is the body of the neuron. As it contains the nucleus, most protein synthesis occurs here. The nucleus can range from 3 to 18 micrometers in diameter(Llinás and Rodolfo,2014).The dendrites of a neuron are cellular extensions with many branches. This overall shape and structure are referred to metaphorically as a dendritic tree. This is where the majority of input to the neuron occurs via the dendritic spine(Kolodin*et al.*, 2013).

The axon is a finer, cable-like projection that can extend tens, hundreds, or even tens of thousands of times the diameter of the soma in length. The axon carries nerve signals away from the soma (and also carries some types of information back to it). Many neurons have only one axon, but this axon may—and usually will—undergo extensive branching, enabling communication with many target cells(Drachman, 2005).The part of the axon where it emerges from the soma is called the axon hillock. Besides being an anatomical structure, the axon hillock is also the part of the neuron that has the greatest density of voltage-dependent sodium channels. This makes it the most easily excited part of the neuron and the spike initiation zone for the axon: in electrophysiological terms, it has the most negative action potential threshold. While the axon and axon hillock are generally involved in information outflow, this region can also receive input from other neurons (Brown*et al*., 2004).

The axon terminal contains synapses, specialized structures where neurotransmitter chemicals are released to communicate with target neurons.

The accepted view of the neuron attributes dedicated functions to its various anatomical components; however, dendrites and axons often act in ways contrary to their so-called main function (Ariane *et al*., 2017).

Axons and dendrites in the central nervous system are typically only about one micrometer thick, while some in the peripheral nervous system are much thicker. The soma is usually about 10–25 micrometers in diameter and often is not much larger than the cell nucleus it contains. The longest axon of a human motor neuron can be over a meter long, reaching from the base of the spine to the toes (López-Muñoz *et al*., 2006).

Sensory neurons can have axons that run from the toes to the posterior column of the spinal cord, over 1.5 meters in adults. Giraffes have single axons several meters in length running along the entire length of their necks. Much of what is known about axonal function comes from studying the squid giant axon, an ideal experimental preparation because of its relatively immense size (0.5–1 millimeters thick, several centimeters long) (Witcher *et al*., 2007).Fully differentiated neurons are permanently postmitotic however, research starting around 2002 shows that additional neurons throughout the brain can originate from neural stem cells through the process of neurogenesis. These are found throughout the brain, but are particularly concentrated in the subventricular zone and subgranular zone (Baker *et al.*, 1984).

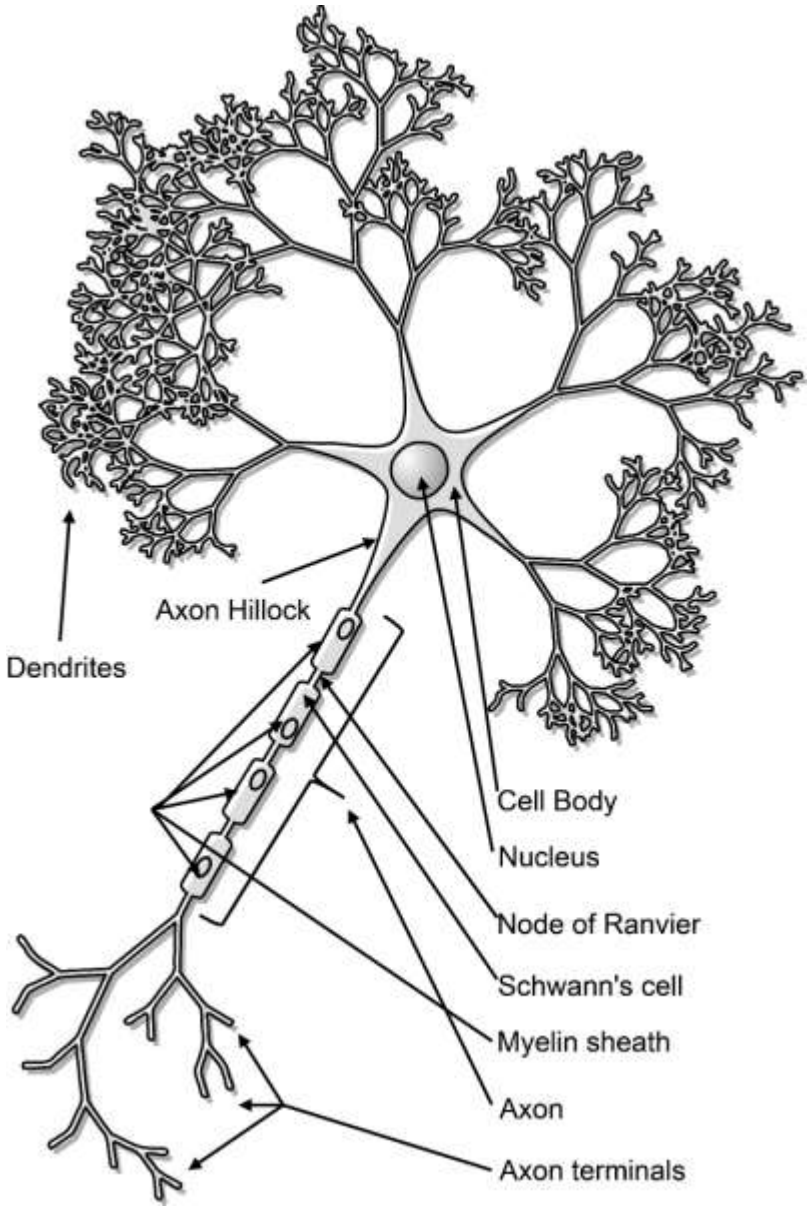


Fig 2.12: A Neuron

Source: http://www2.sunysuffolk.edu

2.3.2 Histology and internal structure of neurons

Numerous microscopic clumps called **Nissl substance** (or Nissl bodies) are seen when nerve cell bodies are stained with a basophilic (base-loving) dye. These structures consist of rough endoplasmic reticulum and associated ribosomal RNA. Named after German psychiatrist and neuropathologist Franz Nissl (1860–1919), they are involved in protein synthesis and their prominence can be explained by the fact that nerve cells are very metabolically active. Basophilic dyes such as aniline or (weakly) haematoxylin highlight negatively charged components, and so bind to the phosphate backbone of the ribosomal RNA (Sadava, 2009).

2.3.3 Nisslsubstance

Nissl substance is a composite material constructed of endoplasmic reticulum and fine granules, both of which have been revealed by electron microscopy. The first component of the Nissl substance appears to be part of the general endoplasmic reticulum of the neuron .

The reticulum extends throughout the entire cytoplasm, but is considerably more condensed within the area of the Nissl bodies than in the rest of the cell. These condensations not only determine the size and shape of each Nissl body, but also constitute a membranous framework upon which the other components are arranged. In many types of neurons, the meshes of the endoplasmic reticulum are distributed at random in three dimensions but in all neurons, and especially in the large motor neurons, the endoplasmic reticulum may display a distinctly orderly arrangement within the Nissl bodies. This orientation consists of a layering of reticular sheets, at more or less regular intervals. Each sheet is a reticulum developed predominantly in two dimensions and comprising tubules, strings of vesicles, and numerous large and flat cister-nae. Even in such highly ordered forms as the Nissl bodies of motor

neurons, the continuity of the reticulum persists, as indicated by frequent branches and anastomoses between layers (Barbeau and Rossignol, 1994).

The second component of the Nissl substance is represented by small granules disposed in patterned arrays either in close contact with the outer membranes of the endoplasmic reticulum or scattered in the intervening matrix (Barnabe-Heider, 2010). This matrix may be considered as a third component of the Nissl substance. It is evident, therefore, that although the Nissl bodies are differentiated parts of the cytoplasm, they are continuous with it by virtue of the continuity of the endoplasmic reticulum and the matrix (Kandel,1995). No interface or membrane separates them from the rest of the cytoplasm. In this respect, as well as in general architecture, they are comparable to the ergastoplasm of glandular cells. The only differences lie in (a) a different intracellular distribution, (b) a lesser degree of preferred orientation of the endoplasmic reticulum, and (c) an apparently greater concentration of fine granules within the areas of the Nissl bodies (Reece *et al.*,2011).

This arrangement is unique to neurons, and its functional significance is unknown. Most, but by no means all, proteins used throughout the neuron are synthesized in the perikaryon. During or after synthesis and processing, proteins are packaged into membrane-limited organelles, are incorporated into cytoskeletal elements, or remain as soluble constituents of the cytoplasm. After proteins have been packaged appropriately, they are transported to their sites of function (López-Muñoz *et al*., 2006).

With a few exceptions, vertebrate neurons have two discrete functional domains or compartments, theaxonal and the somatodendritic compartments, each of which encompasses a number of sub- or microdomains. The axon is perhaps the most familiar functional domain of a neuron and is classically defined as the cellular process by which a neuron makes contact with a target cell to transmit information, providing a conducting structure for transmitting

the action potential to a synapse, a specialized subdomain for transmission of a signal from neuron to target cell (neuron, muscle, etc.) Most often by release of appropriate neurotrans-mitters consequently, most axons end in a presynap-tic terminal, although a single axon may have many (hundreds or even thousands in some cases) presynap-tic specializations known as en passant synapses along its length (Llinás and Radolfo, 2014).

The axon is the first neuronal process to differentiate during development. A typical neuron has only a single axon that proceeds some distance from the cell body before branching extensively. Usually the longest process of a neuron, axons come in many sizes. In a human adult, axons range in length from a few micrometers for small interneurons to a meter or more for large motor neurons, and they may be even longer in large animals (such as giraffes, elephants, and whales). In mammals and other vertebrates, the longest axons generally extend approximately half the body length (Wilson *et al*., 2012).

Axonal diameters also are quite variable, ranging from 0.1 to 20 ^m for large myelinated fibers in vertebrates. Invertebrate axons grow to even larger diameters, with the giant axons of some squid species achieving diameters in the millimeter range. Invertebrate axons reach such large diameters because they lack the myelinating glia that speed conduction of the action potential. As a result, axonal caliber must be large to sustain the high rate of conduction needed for the reflexes that permit escape from predators and capture of prey. Although axonal caliber is closely regulated in both myelinated and nonmyelinated fibers, this parameter is critical for those organisms that are unable to produce myelin (Wilson *et. al*., 2012).

The region of the neuronal cell body where the axon originates has several specialized features. This domain, called the axon hillock, is most readily distinguished by a deficiency of Nissl substance (Wilson *et. al*., 2012). Therefore, protein synthesis cannot take place to

any appreciable degree in this region. Cytoplasm in the vicinity of the axon hillock may have a few polysomes but is dominated by the cytoskeletal and membranous organelles that are being delivered to the axon. Microtubules and neurofilaments begin to align roughly parallel to each other, helping to organize membrane-limited organelles destined for the axon. The hillock is a region where materials either are committed to the axon (cytoskeletal elements, synaptic vesicle precursors, mitochondria, etc.) or are excluded from the axon (RER and free polysomes, dendritic microtubule-associated proteins). The molecular basis for this sorting is not understood (Wilson *et. al*., 2012). Cytoplasm in the axon hillock does not appear to contain a physical "sizing" barrier (like a filter) because large organelles such as mitochondria readily enter the axon, whereas only a small number of essentially excluded structures such as polysomes are occasionally seen only in the initial segment of the axon and not in the axon proper. An exception to this general rule is during development when local protein synthesis does take place at the axon terminus or growth cone. In the mature neuron, the physiological significance of this barrier must be considerable, because axonal structures are found to accumulate in this region in many neuropathologies, including those due to degenerative diseases (such as amyotrophic lateral sclerosis) and to exposure to neurotoxic compounds (such as acrylamide) (Wilson, 2012).

The initial segment of the axon is the region of the axon adjacent to the axon hillock. Microtubules generally form characteristic fascicles, or bundles, in the initial segment of the axon. These fascicles are not seen elsewhere. The initial segment and, to some extent, the axon hillock also have a distinctive specialized plasma membrane. Initially, the plasmalemma was thought to have a thick electron-dense coating actually attached to the inner surface of the membrane, but this dense undercoating is in reality separated by 5 to 10 nm from the plasma membrane inner surface and has a complex ultrastructure. Neither the composition nor the function of this undercoating is known. Curiously, the undercoating is present in the

same regions of the initial segment as the distinctive fasciculation of microtubules, although the relationship is not understood. The plasma membrane is specialized in the initial segment and axon hillock in that it contains voltage-sensitive ion channels in large numbers, and most action potentials originate in this domain (Ishikawa*et al.*,2003).

Ultimately, axonal structure is geared toward the efficient conduction of action potentials at a rate appropriate to the function of that neuron. This can be seen from both the ultrastructure and the composition of axons. Axons are roughly cylindrical in cross section with little or no taper. As discussed later in this chapter, this diameter is maintained by regulation of the cytoskeleton. Even at branch points, daughter axons are comparable in diameter to the parent axon. This constant caliber helps ensure a consistent rate of conduction. Similarly, the organization of membrane components is regulated to this end. Voltage-gated ion channels are distributed to maximize conduction. Sodium channels are distributed more or less uniformly in small nonmyelinated axons, but are concentrated at high density in the regularly spaced unmyelinated gaps, known as nodes of Ranvier. An axon so organized will conduct an action potential or train of spikes long distances with high fidelity at a defined speed. These characteristics are essential for maintaining the precise timing and coordination seen in neuronal circuits (Reece *et al.*,2011).

Most vertebrate neurons have multiple dendrites arising from their perikarya. Unlike axons, dendrites continuously branch and taper extensively, with a reduction in caliber in daughter processes at each branching. In addition, the surface of dendrites is covered with small protrusions, or spines, which are postsynaptic specializations. Although the surface area of a dendritic arbor may be quite extensive, dendrites in general remain in the relative vicinity of the perikaryon. A dendritic arbor may be contacted by the axons of many different and distant neurons or innervated by a single axon making multiple synaptic contacts.

The base of a dendrite is continuous with the cytoplasm of the cell body. In contrast to the axon, Nissl substance extends into dendrites, and certain proteins are synthesized predominantly in dendrites. There is evidence for the selective placement of some mRNAs in dendrites as well. For example, whereas RER and polysomes extend well into the dendrites, the mRNAs that are transported and translated in dendrites are a subset of the total neuronal mRNA, deficient in some mRNA species (such as neurofilament mRNAs) and enriched in mRNAs with dendritic functions (such as microtubule-associated protein mRNAs, microtubule-associated protein. Also, certain proteinsappear to be targeted, postsyn-thesis, to the dendritic compartment as well (Araki *et al.,* 2000).

The shapes and complexity of dendritic arborizations may be remarkably plastic. Dendritesappear relatively late in development and initially have only limited numbers of branches and spines. As development and maturation of the nervous system proceed, the size and number of branches increase. The number of spines increases dramatically, and their distribution may change. This remodeling of synaptic connectivity may continue into adulthood, and environmental effects can alter this pattern significantly. Eventually, in the aging brain, there is a reduction in complexity and size of dendritic arbors, with fewer spines and thinner dendritic shafts. These changes correlate with changes in neuronal function during development and aging.

As defined by classic physiology, axons are the structural correlates for neuronal output, and dendrites constitute the domain for receiving information. A neuron without an axon or one without dendrites might therefore seem paradoxical, but such neurons do exist. Certain amacrine and horizontal cells in the vertebrate retina have no identifiable axons, although they do have dendritic processes that are morphologically distinct from axons. Such processes may have both pre- and postsynaptic specializations or they may have gap

junctions that act as direct electrical connections between two cells. Similarly, the pseudounipolar sensory neurons of dorsal root ganglia have no dendrites. In their mature form, these dorsal root ganglia sensory neurons give rise to a single axon that extends a few hundred micrometers before branching. One longbranch extends to the periphery, where it may form a sensory nerve ending in muscle spindles or skin. Large dorsal root ganglia peripheral branches are myelinated and have the morphological characteristics of an axon, but they contain neither pre- nor postsynaptic specializations. The other branch extends into the central nervous system, where it forms synaptic contacts (Reece *et al.*,2011).

The cell body of a neuron is supported by a complex mesh of structural proteins called neurofilaments, which are assembled into larger neurofibrils. Some neurons also contain pigment granules, such as neuromelanin (a brownish-black pigment that is byproduct of synthesis of catecholamines), and lipofuscin (a yellowish-brown pigment), both of which accumulate with age. Other structural proteins that are important for neuronal function are actin and the tubulin of microtubules. Actin is predominately found at the tips of axons and dendrites during neuronal development. There the actin dynamics can be modulated via an interplay with microtubule (Reece *et al.*,2011).

There are different internal structural characteristics between axons and dendrites. Typical axons almost never contain ribosomes, except some in the initial segment. Dendrites contain granular endoplasmic reticulum or ribosomes, in diminishing amounts as the distance from the cell body increases (Reece *et al.*,2011).

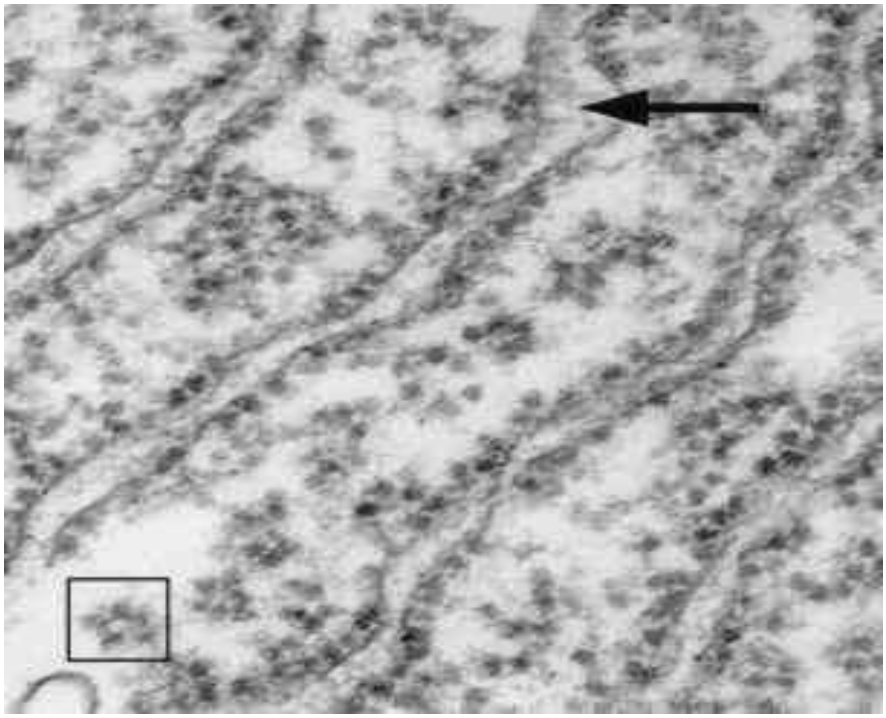


Fig 2.13: The "Nissl body" in neurons is an array of cytoplasmic free polysomal rosettes (boxed) interspersed between rows of rough endoplasmic reticulum (RER) studded with membrane-bound ribosomes.

Nascent polypeptide chains emerging from the ribosomal tunnel on the RER are inserted into the lumen (arrow), where they may be processed before transport out of the RER. Source: http://www2.sunysuffolk.edu

2.3.4 Structural classification of neurons

Most neurons can be anatomically characterized as:

- Unipolar neurons: these are neurons with only one process
- Bipolar neurons: these are neurons with one axon and one dendrite
- Multipolar neurons: these are neurons with one axon and two or more dendrites
- pseudounipolar neurons: these are neurons with one process which then serves as both an axon and a dendrite.

2.3.5 Functional classification of neurons

Functionally, neurons can be classified as afferentneurons, efferentneurons and Interneurons.Afferent neurons convey information from tissues and organs into the central nervous system and are also called sensory neurons.Efferent neurons transmit signals from the central nervous system to the effector cells and are also called motor neurons.Interneurons connect neurons within specific regions of the central nervous system(Reece *et al.*,2011).Afferent and efferent meurons also refer generally to neurons that, respectively, bring information to or send information from the brain (Reece *et al.*, 2011)

2.3.6 Action of neurons on other neurons

A neuron affects other neurons by releasing a neurotransmitter that binds to chemical receptors. The effect upon the postsynaptic neuron is determined not by the presynaptic neuron or by the neurotransmitter, but by the type of receptor that is activated. A neurotransmitter can be thought of as a key, and a receptor as a lock: the same type of key can here be used to open many different types of locks. Receptors can be classified broadly as excitatory (causing an increase in firing rate), inhibitory (causing a decrease in firing rate), or modulatory(causing long-lasting effects not directly related to firing rate (Barreiro-Iglesias *et al*., 2008).

The two most common neurotransmitters in the brain, glutamate and GABA, have actions that are largely consistent. Glutamate acts on several different types of receptors, and have effects that are excitatory at ionotropic receptors and a modulatory effect at metabotropic receptors. Similarly, GABA acts on several different types of receptors, but all of them have effects (in adult animals, at least) that are inhibitory. Because of this consistency, it is common for neuroscientists to simplify the terminology by referring to cells that release glutamate as "excitatory neurons", and cells that release GABA as "inhibitory neurons". Since over 90% of the neurons in the brain release either glutamate or GABA, these labels encompass the great majority of neurons. There are also other types of neurons that have consistent effects on their targets, for example, "excitatory" motor neurons in the spinal cord that release acetylcholine, and "inhibitory" spinal neurons that release glycine (Reece *et al.*,2011).

The distinction between excitatory and inhibitory neurotransmitters is not absolute, however. Rather, it depends on the class of chemical receptors present on the postsynaptic neuron. In principle, a single neuron, releasing a single neurotransmitter, can have excitatory effects on some targets, inhibitory effects on others, and modulatory effects on others still. For example, photoreceptor cells in the retina constantly release the neurotransmitter glutamate in the absence of light. So-called OFF bipolar cells are, like most neurons, excited by the released glutamate. However, neighboring target neurons called ON bipolar cells are instead inhibitedby glutamate, because they lack the typical ionotropicglutamate receptors and instead express a class of inhibitory metabotropic glutamate receptors (Humberto *et al.*,2015).

When light is present, the photoreceptors cease releasing glutamate, which relieves the ON bipolar cells from inhibition, activating them; this simultaneously removes the excitation from the OFF bipolar cells, silencing them (Reece *et al.*,2011).

It is possible to identify the type of inhibitory effect a presynaptic neuron will have on a postsynaptic neuron, based on the proteins the presynaptic neuron expresses. Parvalbumin-expressing neurons typically dampen the output signal of the postsynaptic neuron in the visual cortex, whereas somatostatin-expressing neurons typically block dendritic inputs to the postsynaptic neuron (Reece *et al.*,2011).

2.3.7 Discharge patterns of neurons

Neurons have intrinsic electroresponsive properties like intrinsic transmembrane voltage oscillatory patterns. Neurons can be classified according to their electrophysiological characteristics which include; tonic or regularspiking,phasic or bursting and fast spiking (Zhao, 2017).

Tonic or regularspiking are neurons which are typically constantly (or tonically) active. Example: interneurons in neurostriatum.Phasic or bursting neurons fire in bursts.Fast spiking neurons are notable for their high firing rates, for example some types of cortical inhibitory interneurons, cells in globus pallidus, retinal ganglion cells (Ershov *et al*., 2002).

2.3.8 Classification by neurotransmitter production

The following are neurons classification by neurotransmitters production Cholinergic neurons which released acetylcholine from presynaptic neurons into the synaptic cleft. It acts as a ligand for both ligand-gated ion channels and metabotropic (GPCRs) muscarinic receptors. Nicotinic receptors are pentameric ligand-gated ion channels composed of alpha and beta subunits that bind nicotine. Ligand binding opens the channel causing influx of Na^+ depolarization and increases the probability of presynaptic neurotransmitter release. Acetylcholine is synthesized from choline and acetyl coenzyme A.

GABAergic neurons—gamma aminobutyric acid. (GABA) is one of two neuroinhibitors in the central nervous system (CNS), the other being Glycine. GABA has a homologous function to ACh, gating anion channels that allow Cl^- ions to enter the post synaptic neuron. Cl^- causes hyperpolarization within the neuron, decreasing the probability of an action potential firing as the voltage becomes more negative (recall that for an action potential to fire, a positive voltage threshold must be reached). GABA is synthesized from glutamate neurotransmitters by the enzyme glutamate decarboxylase(Fedorow *et al*., 2005).

Glutamate is one of two primary excitatory amino acid neurotransmitters, the other being Aspartate. Glutamate receptors are one of four categories, three of which are ligand-gated ion channels and one of which is a G-protein coupled receptor (Barriere *et al*., 2004). Glutamate can cause excitotoxicity when blood flow to the brain is interrupted, resulting in brain damage. When blood flow is suppressed, glutamate is released from presynaptic neurons. Glutamate is synthesized from the amino acid glutamine by the enzyme glutamate synthase (Zhao, 2017).

Dopamine is connected to mood and behavior and modulates both pre and post synaptic neurotransmission. Loss of dopamine neurons in the substantial nigra has been linked to Parkinson's disease. Dopamine is synthesized from the amino acid tyrosine. Tyrosine is catalyzed into levadopa (or L-DOPA) by tyrosine hydroxylase, and levadopa is then converted into dopamine by amino acid decarboxylase (Dwarika *et al*., 2008, Fahn, 2008).

Serotonin (5-Hydroxytryptamine, 5-HT) can act as excitatory or inhibitory. Of the four 5-HT receptor classes, 3 are GPCR and 1 is ligand gated cation channel. Serotonin is synthesized from tryptophan by tryptophan hydroxylase, and then further by aromatic acid decarboxylase. A lack of 5-HT at postsynaptic neurons has been linked to depression. Drugs that block the

presynaptic serotonin transporter are used for treatment, such as Prozac and Zoloft (Diaz *et al.,* 2016).

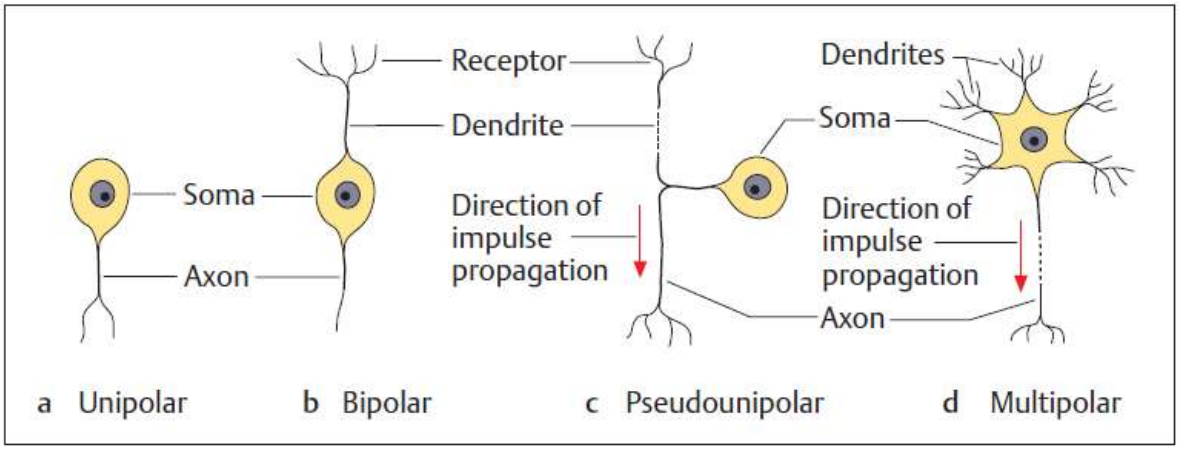


Fig: 2.14 Structural classifications of neurons. a) Unipolar; b)Bipolar; c) Pseudounipolar d) Multipolar. Source: http://www2.sunysuffolk.edu

2.3.9 Connectivity of neurons (chemical synapse)

Neurons communicate with one another via synapses, where the axon terminal or en passant bouton (a type of terminals located along the length of the axon) of one cell contacts another neuron's dendrite, soma or, less commonly, axon. Neurons such as Purkinje cells in the cerebellum can have over 1000 dendritic branches, making connections with tens of thousands of other cells; other neurons, such as the magnocellular neurons of the supraoptic nucleus, have only one or two dendrites, each of which receives thousands of synapses. Synapses can be excitatory or inhibitory and either increase or decrease activity in the target neuron, respectively. Some neurons also communicate via electrical synapses, which are direct, electrically conductive junctions between cells (Zhao, 2017).

In a chemical synapse, the process of synaptic transmission is as follows: when an action potential reaches the axon terminal, it opens voltage-gated calcium channels, allowing calcium ions to enter the terminal. Calcium causes synaptic vesicles filled with neurotransmitter molecules to fuse with the membrane, releasing their contents into the synaptic cleft. The neurotransmitters diffuse across the synaptic cleft and activate receptors on the postsynaptic neuron. High cytosolic calcium in the axon terminal also triggers mitochondrial calcium uptake, which, in turn, activates mitochondrial energy metabolism to produce ATP to support continuous neurotransmission (Barasi and Duggal 1985).

An autapse is a synapse in which a neuron's axon connects to its own dendrites. The human brain has a huge number of synapses. Each of the 10^{11} (one hundred billion) neurons has on average 7,000 synaptic connections to other neurons. It has been estimated that the brain of a three-year-old child has about 10^{15} synapses (1 quadrillion). This number declines with age,

stabilizing by adulthood. Estimates vary for an adult, ranging from 10^{14} to 5 x 10^{14} synapses (100 to 500 trillion) (Zhao, 2017).

In 1937, John Zachary Young suggested that the squid giant axon could be used to study neuronal electrical properties (Reece *et al*., 2011). Being larger than but similar in nature to human neurons, squid cells were easier to study. By inserting electrodes into the giant squid axons, accurate measurements were made of the membrane potential (Reece *et al*.,2011).

The cell membrane of the axon and soma contain voltage-gated ion channels that allow the neuron to generate and propagate an electrical signal (an action potential). These signals are generated and propagated by charge-carrying ions including sodium (Na^+), potassium (K^+), chloride (Cl^-), and calcium (Ca^{2+}) (Dohrmann, 2010).

There are several stimuli that can activate a neuron leading to electrical activity, including pressure, stretch, chemical transmitters, and changes of the electric potential across the cell membrane. Stimuli cause specific ion-channels within the cell membrane to open, leading to a flow of ions through the cell membrane, changing the membrane potential(Jaeger*et. al*., 1983).

Thin neurons and axons require less metabolic expense to produce and carry action potentials, but thicker axons convey impulses more rapidly. To minimize metabolic expense while maintaining rapid conduction, many neurons have insulating sheaths of myelin around their axons. The sheaths are formed by glial cells: oligodendrocytes in the central nervous system and Schwann cells in the peripheral nervous system. The sheath enables action potentials to travel faster than in unmyelinated axons of the same diameter, whilst using less energy. The myelin sheath in peripheral nerves normally runs along the axon in sections about 1 mm long, punctuated by unsheathed nodes of Ranvier, which contain a high density

of voltage-gated ion channels. Multiple sclerosis is a neurological disorder that results from demyelination of axons in the central nervous system (Joyce *et al*.,2014).

Some neurons do not generate action potentials, but instead generate a graded electrical signal, which in turn causes graded neurotransmitter release. Such nonspiking neurons tend to be sensory neurons or interneurons, because they cannot carry signals long distances (Reece *et al*.,2011).

2.3.10Neural coding

Neural coding is concerned with how sensory and other information is represented in the brain by neurons. The main goal of studying neural coding is to characterize the relationship between the stimulus and the individual or ensemble neuronal responses, and the relationships amongst the electrical activities of the neurons within the ensemble. It is thought that neurons can encode both digital and analog information (Reece *et. al*.,2011).

2.3.11Neuron doctrine

The neuron doctrine is the now fundamental idea that neurons are the basic structural and functional units of the nervous system. The theory was put forward by Santiago Ramón y Cajal in the late 19th century. It held that neurons are discrete cells (not connected in a meshwork), acting as metabolically distinct units (Lip *et al.,* 2017).

Later discoveries yielded a few refinements to the simplest form of the doctrine. For example, glial cells, which are not considered neurons, play an essential role in information processing Also, electrical synapses are more common than previously thought, meaning that there are direct, cytoplasmic connections between neurons. In fact, there are examples of neurons forming even tighter coupling: the squid giant axon arises from the fusion of multiple axons (Purves *et. al*.,1997).

Ramón y Cajal also postulated the Law of Dynamic Polarization, which states that a neuron receives signals at its dendrites and cell body and transmits them, as action potentials, along the axon in one direction: away from the cell body. The Law of Dynamic Polarization has important exceptions; dendrites can serve as synaptic output sites of neuron and axons can receive synaptic inputs (Purves *et. al*., 1997).

2.3.12 Neurological disorders

Below are some neurological disorders

Charcot–Marie–Tooth disease (CMT) is a heterogeneous inherited disorder of nerves (neuropathy) that is characterized by loss of muscle tissue and touch sensation, predominantly in the feet and legs but also in the hands and arms in the advanced stages of the disease. Presently incurable, this disease is one of the most common inherited neurological disorders, with 37 in 100,000 affected (López-Muñoz *et al.*,2006).

Alzheimer's disease (AD), also known simply as *Alzheimer's*, is a neurodegenerative disease characterized by progressive cognitive deterioration together with declining activities of daily living and neuropsychiatric symptoms or behavioural changes. The most striking early symptom is loss of short-term memory (amnesia), which usually manifests as minor forgetfulness that becomes steadily more pronounced with illness progression, with relative preservation of older memories. As the disorder progresses, cognitive (intellectual) impairment extends to the domains of language (aphasia), skilled movements (apraxia), and recognition (agnosia), and functions such as decision-making and planning become impaired (López-Muñoz *et al.*,2006).

Parkinson's disease (PD), also known as *Parkinson disease*, is a degenerative disorder of the central nervous system that often impairs the sufferer's motor skills and speech. Parkinson's disease belongs to a group of conditions called movement disorders. It is characterized by

muscle rigidity, tremor, a slowing of physical movement (brady kinesia), and in extreme cases, a loss of physical movement (akinesia). The primary symptoms are the results of decreased stimulation of the motor cortex by the basal ganglia, normally caused by the insufficient formation and action of dopamine, which is produced in the dopaminergic neurons of the brain. Secondary symptoms may include high level cognitive dysfunction and subtle language problems. PD is both chronic and progressive (López-Muñoz *et al.*,2006).

Myasthenia gravis is a neuromuscular disease leading to fluctuating muscle weakness and fatigability during simple activities. Weakness is typically caused by circulating antibodies that block acetylcholine receptors at the post-synaptic neuromuscular junction, inhibiting the stimulative effect of the neurotransmitter acetylcholine. Myasthenia is treated with immunosuppressants, cholinesterase inhibitors and, in selected cases, thymectomy(López-Muñoz *et al.*,2006).

2.3.13Demyelination of neurons

Demyelination is the act of demyelinating, or the loss of the myelin sheath insulating the nerves. When myelin degrades, conduction of signals along the nerve can be impaired or lost, and the nerve eventually withers. This leads to certain neurodegenerative disorders like multiple sclerosis and chronic inflammatory demyelinating polyneuropathy(López-Muñoz *et al.*,2006).

Although most injury responses include a calcium influx signaling to promote resealing of severed parts, axonal injuries initially lead to acute axonal degeneration, which is rapid separation of the proximal and distal ends within 30 minutes of injury. Degeneration follows with swelling of the axolemma, and eventually leads to bead like formation. Granular disintegration of the axonal cytoskeleton and inner organelles occurs after axolemma degradation. Early changes include accumulation of mitochondria in the paranodal regions at

the site of injury. Endoplasmic reticulum degrades and mitochondria swell up and eventually disintegrate. The disintegration is dependent on ubiquitin and calpainproteases (caused by influx of calcium ion), suggesting that axonal degeneration is an active process. Thus, the axon undergoes complete fragmentation. The process takes about roughly 24 hours in the peripheral nervous system (PNS), and longer in the CNS. The signaling pathways leading to axolemma degeneration are currently unknown (López-Muñoz *et al.*,2006).

2.4 Neuronal cell groups of the spinal cord

Viewed from the perspective of its longitudinal columnar organization, the gray matterof the spinal cord consists of a series of discontinuous cell groupings associated with their corresponding segmentally arranged spinal nerves (Gruener and Biller,2008; Freire *et. al*.,2009). At any particular spinal level (as seen in transverse section)the spinal gray matter is considered to consist of ten layers, Rexed's laminae, which are defined on the basis of neuronal size, shape, cytological featuresand density.

The laminae are numbered sequentially in a dorsoventral sequence Laminae I-IV which correspondto the head of the dorsal horn and are the main receiving areas for cutaneous primary afferent terminals and collateral branches Many complex polysynaptic reflex paths (ipsilateral,contralateral intrasegmental and intersegmental) start from this region and many longascending tract fibres which pass to higher levels arise from it.

Lamina I (lamina marginalis)is a very thin layer with an ill-defined boundary at the dorsolateral tip ofthe dorsal horn(Freire *et al*.,2009). It has a reticular appearance, reflecting its content of bundles of coarse and finenerve fibres. It contains small, intermediate and large neuronal somata, many of which arefusiform in shape. Lamina II occupies most of the head of the dorsal horn and consists of densely packed small neurons responsible for its

darkappearance in Nissl-stained sections.With myelin stains; Lamina II is characteristically distinguished from adjacent laminae by thealmost total lack of myelinated fibres. Lamina II corresponds to the substantial gelatinosa.

Lamina III consists of somata which are mostly larger, more variable and less closely packed than those in lamina II. It also contains many myelinated fibres. Some workers consider that the substantial gelatinosa contains part or all of lamina III as well as lamina II (Freire *et al.*, 2009). The ill-definednucleus proprius of the dorsal horn corresponds to some of the cell constituents of laminae IIIand IV. Lamina IV is a thick, loosely packed, heterogeneous zone permeated by fibres. Its neuronal somata vary considerably in size and shape, from small and round, through intermediate and triangular, to very large and stellate.

Laminae V and VI receive most of the terminals of proprioceptive primary afferents and profuse corticospinal projections from the motor and sensory cortex and subcortical levels, which suggests their intimate involvement in the regulation of movement. Lamina V is a thick layer which includes the neck of the dorsal horn.It is divisible into a lateral third and medial two-thirds. Both have a mixed cell population but the former contains many prominent well-staining somata interlaced by numerous bundles of transverse, dorsoventral and longitudinal fibres. Lamina VI is most prominent in the limb enlargements (Freire *et al.*, 2009). It has a densely staining medial third of small, densely packed neurons and a lateral two-thirds containing larger, more loosely packed, triangular or stellate somata. Lamina VI corresponds approximately to the base of the dorsal horn.

Laminae VII-IX show a variety of complex forms in the limb enlargements. Lamina VII includes much of the intermediate (lateral) horn. It contains prominent neurons of Clarke's

column (nucleus dorsalis, nucleus thoracis, thoracic nucleus) and intermediomedial and intermediolateral cell groupings (Humberto *et al.*,2015).

The lateral part of lamina VII has extensive ascending and descending connections with the midbrain and cerebellum (via the spinocerebellar, spinotectal, spinoreticular, tectospinal, reticulospinal and rubrospinal tracts) and is thus involved in the regulation of posture and movement. Its medial part has numerous propriospinal reflex connections with the adjacent gray matter and segments concerned both with movement and autonomic functions.

Lamina VIII spans the base of the thoracic ventral horn but is restricted to its medial aspect in limb enlargements. Its neurons display a heterogeneous mixture of sizes and shapes from small to moderately large. Lamina VIII is a mass of propriospinal interneurons (Humberto*et al*., 2015). It receives terminals from the adjacent laminae, many commissural terminals from the contralateral lamina VIII, and descending connections from the interstitiospinal, reticulospinal and vestibulospinal tracts and the medial longitudinal fasciculus. The axons from these interneurons influence motor neurons bilaterally, perhaps directly but more probably by excitation of small neurons supplying γ efferent fibres to muscle spindles.

Lamina IX is a complex array of cells consisting of α and γ motor neurons and many interneurons (Humberto *et al*., 2015). The large α motor neurons supply motor end plates of extrafusal muscle fibres in striated muscle. Recording techniques have demonstrated tonic and phasic α motor neurons. The former has a lower rate of firing and lower conduction velocity and tend to innervate type S muscle units (Humberto *et al*., 2015). The latter have higher conduction velocity and tend to supply fast twitch muscle units. The smaller γ motor neurons give rise to small-diameter efferent axons (fusimotor fibres), which innervate the

intrafusal muscle fibres in muscle spindles. There are several functionally distinct types of γ motor neurone. The 'static' and dynamic' responses of muscle spindles have separate controls mediated by static and dynamic fusimotor fibres, which are distributed variously to nuclear chain and nuclear bag fibres.Lamina X surrounds the central canal and consists of the dorsal and ventral gray commissures (Humberto *et al.*, 2015).

2.4.1 Posterior horn (Dorsal horn) of the spinal cord

The dorsal horn is a major receptive zone (zone of termination) of primary afferent fibres, which enter the spinal cord through the dorsal roots of spinal nerves. Dorsal root fibres contain numerous molecules, which are either known, or suspected, to fulfil a neurotransmitter or neuromodulator role. These include glutamate, substance, calcitonin gene-related peptide (CGRP), bombesin, vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), somatostatin, dynorphin and angiotensin II(Humberto *et al*., 2015). Dorsal root afferents carry exteroceptive, proprioceptive and interoceptive information. Laminae I-IV are the main cutaneous receptive areas; lamina V receives fine afferents from the skin, muscle and viscera; and lamina VI receives proprioceptive and some cutaneous afferents. Most, if not all, primary afferent fibres divide into ascending and descending branches on entering the cord. These then travel for variable distances in the tract of Lissauer, near the surface of the cord, and send collaterals into the subjacent gray matter. The formation, topography and division of dorsal spinal roots have all been confirmed in man(Humberto *et al*., 2015).At the dorsolateral tip of the dorsal horn, deep to the tract of Lissauer, lies a thin lamina of neurons, the lamina marginalis. Beneath this lies the substantial gelatinosa (laminae II and III), which is present at all levels, and consists mostly of small Golgi type II neurons, together with some larger neurons. It receives afferents via the dorsal roots and is the site of origin of the spinothalamic tract complex. The large cells of the nucleus proprius lie ventral to the substantial gelatinosa. These propriospinal neurons

linksegments for the mediation of intraspinal coordinationClarke's column lies at the base of the dorsal horn. At most levels, it is near the dorsal whitefuniculus and may project into it. In the human spinal cord, it can usually be identified fromthe eighth cervical to the third or fourth lumbar segments (Humberto *et al*., 2015). Neurons of Clarke's column varyin size, but most are large, especially in the lower thoracic and lumbar segments.Some sendaxons into the dorsal spinocerebellar tracts and others are interneurons.

2.4.2 Lateral horn of the spinal cord

The lateral horn is a small lateral projection of gray matter located between the dorsal and ventral horns. It is present from the eighth cervical or first thoracic segment to the second or third lumbar segment in man (Humberto*et al.*, 2015).

It contains the cell bodies of preganglionic sympathetic neurons. These develop in the embryonic cord dorsolateral to the central canal and migrate laterally, forming intermediomedial and intermediolateral cell columns. Their axons travel via ventral spinal roots and white rami communicantes to the sympathetic trunk. A similar cell group is found in the second to fourth sacral segments, but unlike the thoracolumbar lateral cell column, it does not form a visible lateral projection. It is the source of the sacral outflow of parasympathetic preganglionic nerve fibres (Humberto *et al*., 2015).

2.4.3Anteriorhorn (ventral horn) of the spinal cord

Neurons in the ventral horn vary in size. The largest cell bodies, which may exceed 25 μm in diameter, are those of α motor neurons whose axons emerge in ventral roots to innervate extrafusal fibres in striated skeletal muscles. Large numbers of smaller neurons, 15-25 μm in diameter, are also present. Some of these are γ motor neurons, which innervate intrafusal fibres of muscle spindles, and the rest are interneurons (Carp and Anderson, 1982, Humberto *et al*., 2015).

The motor neurons utilize acetylcholine as their neurotransmitter. Considered longitudinally, ventral horn neurons are arranged in elongated groups, and form a number of separate columns, which extend through several segments. These are seen most easily in transverse sections. The ventral horn is essentially divided into medial, central and lateral cell columns, which all exhibit subdivision at certain levels, usually into dorsal and ventral parts. The medial group extended throughout the spinal cord, but may be absent in the fifth lumbar and first sacral segments (Humberto *et al*., 2015).

In the thoracic and the upper four lumbar segments, it is subdivided into ventromedial and dorsomedial groups. In segments cranial and caudal to this region, the medial group has only a ventromedial moiety, except in the first cervical segment, where only the dorsomedial group exists. The central group of cells is the least extensive, and is found only in some cervical and lumbosacral segments (Humberto *et al*., 2015).

The third to seventh cervical segments contain the centrally situated phrenic nucleus - abundant experimental and clinical evidence shows that its neurons innervate the diaphragm. Neurons whose axons are thought to enter the spinal accessory nerve form an irregular accessory group in the upper five or six cervical segments at the ventral border of the ventral horn (Humberto *et al*., 2015).

The lateral group of cells in the ventral horn is subdivided into ventral, dorsal and retrodorsal groups, largely confined to the spinal segments which innervate the limbs. The nucleus of Onuf, which is thought to innervate the perineal striated muscles, is a ventrolateral group of cells in the first and second sacral segments. The motor neurons of the ventral horn are somatotopically organized. The basic arrangement is that medial cell groups innervate the axial musculature, and lateral cell groups innervate the limbs (Humberto *et al*., 2015).

The basic building block of the somatic motor neuronal populations is represented by a longitudinally disposed group of neurons, which innervate a given muscle, and in which the α and γ motor neurons are intermixed. The various groups innervating different muscles are aggregated into two major longitudinal columns, medial and lateral. In transverse section these form the medial and lateral cell groups in the ventral horn. The medial longitudinal motor column extends throughout the length of the spinal cord. Its neurons innervate epaxial and hypaxial muscle groups (Humberto *et al*., 2015).

Basically, epaxial muscles include the erector spinae group (which extend the head and vertebral column), while hypaxial muscles include prevertebral muscles of the neck, intercostal and anterior abdominal wall muscles (which flex the neck and the trunk). The epaxial muscles are innervated by branches of the dorsal primary rami of the spinal nerves, and the hypaxial muscles by branches of the ventral primary rami. In the medial column, motor neurons supplying epaxial muscles are sited ventral to those supplying hypaxial muscles. The lateral longitudinal motor column is found only in the enlargements of the spinal cord (Christie *et al.,* 2005; Joseph, 2018).

The motor neurons in this column in the cervical and lumbar enlargements innervate muscles of the upper and lower limbs, respectively. In the cervical enlargement, motor neurons which supply muscles intrinsic to the upper limb are situated dorsally in the ventral gray column, and those innervating the most distal (hand) muscles are sited further dorsally. Motor neurons of the girdle muscles lie in the ventrolateral part of the ventral horn. There is a further somatotopic organization in that the proximal muscles of the limb are supplied from motor cell groups located more rostrally in the enlargement than those supplying the distal muscles. For example, motor neurons innervating intrinsic muscles of the hand are sited in segments

cervical spinal cord (C8)and thoracic spinal cord (T1), while motor neurons of shoulder muscles are in cervical spinal cordsegmentsC5 and C6. A similar overall arrangement of motor neurons innervating lower limb muscles applies in the lumbosacral cord. The main afferent connections to motor neurons are direct monosynaptic connections from proprioceptive dorsal root afferents in the same or nearby segments; connections from axonal collaterals of dorsal horn and other and direct mono symaptic connections from the vestibulospinal and corticospinal tracts (Humberto *et al*., 2015).

2.4.4Spinal white matter

The spinal white matter surrounds the central core of gray matter. It contains nerve fibres, neuroglia and blood vessels. Most of the nerve fibres run longitudinally. They are arranged in three large masses, the dorsal, lateral and ventral funiculi, on either side of the cord. Fibres of related function and those with common origins or destinations are grouped to form ascending and descending tracts within the funiculi (Humberto *et al*., 2015).

Narrow dorsal and ventral white commissures run between the two halves of the cord. In the following account, the tracts are considered fewer than three main headings: ascending; descending; and propriospinal. Ascending tracts contain primary afferent fibres, which enter by dorsal roots, and fibres derived from intrinsic spinal neurons, which carry afferent impulses to supraspinal levels(Lima *et al*.,2010).

Descending tracts contain long fibres, which descend from various supraspinal sources to synapse with spinal neurons. Propriospinal tracts, both ascending and descending, contain the axons of neurons which are localized entirely to the spinal cord, and link nearby and distant spinal segments. They mediate intrasegmental and intersegmental coordination. Fibres in the white matter vary in calibre. Many are small and lightly, or non-, myelinated (Chang *et al*., 2007).

Most regions contain a wide spectrum of fibre diameters, from 1 μm or less to 10 μm. Some tracts typically contain only small fibres, e.g. the dorsolateral tract, fasciculus gracilis and central part of the lateral funiculus. The fasciculus cuneatus, anterior funiculus and peripheral zone of the lateral funiculus all contain many large-diameter fibres. Whilst the ascending and descending tracts are to a large extent discrete and regularly located, significant overlap between adjacent tracts occurs (Lima*et al*.,2010). The following account of spinaltracts is concerned with the human cord, and reference to findings in animals is only madewhere adequate clinicopathological data are unavailable in man.

2.4.5 Ascending tracts

Dorsal columns

The dorsal funiculus on each side of the cord consists of two large ascending tracts, the fasciculus gracilis and fasciculus cuneatus, separated by a posterointermediate septum. They are also known as the dorsal columns. The dorsal columns contain a high proportion of myelinated fibres carrying proprioception (position sense and kinaesthesia) and exteroceptive (touch-pressure) information, including vibratory sensation, to higher levels (Laureen*et al*.,2017).

These fibres come from several sources, namely: long primary afferent fibres which enter the cord in the dorsal roots of spinal nerves and ascend to the dorsal column nuclei in the medulla oblongata; shorter primary afferent fibres projecting to neurons of Clarke's column and other spinal neurons; and axons from secondary neurons of the spinal cord ascending to the dorsal column nucleilevels. The dorsal columns also contain axons of propriospinal neurons(Laureen *et al*., 2017).

Thefasciculus gracilis begins at the caudal end of the spinal cord. It contains long ascending bramches of primary afferents which enter the branches of primary afferents enter the spinal cord through ipsilateral dorsal spinal roots and ascending axons of secondary neurons in laminae IV to VI of the ipsilateral dorsal horn levels (Laureen*et al*.,2017).

As the fibres ascend, they are joined by axons of successive dorsal roots. Fibres entering in coccygeal and lower sacral regions are shifted medially by successive additions of fibres entering at higher levels (Laureen*et al*., 2017). The fasciculus gracilis lies medial to the fasciculus cuneatus in the upper spinal cord. At upper cervical levels the fasciculus gracilis contains a larger proportion of afferents from cutaneous receptors than from deep proprioceptors because many of the latter leave the fasciculus at lower segments to synapse in

Clarke's column. Indeed, proprioception from the lower limb mostlyreaches the thalamus by relaying in Clarke's column (Laureen *et al*.,2017).

Axons of the fasciculus gracilis, from both primary and secondary neurons, terminate in the nucleus gracilis of the dorsal medulla. The fasciculus cuneatus begins at midthoracic level and lies lateral to the fasciculus gracilis(Laureen*et al*.,2017).It is composed mostly of primary afferent fibres of the upper thoracic and cervical dorsal roots. At upper cervical levels it contains a large population of afferents from both deep and cutaneous receptors of the upper limb. In addition, some of its axons arise from secondary neurons in laminae IV-VI of the ipsilateral dorsal horn. Many axons (both primary and secondary) that ascend in the fasciculus cuneatus terminate in the nucleus cuneatus of the dorsal medulla. Some also end in the lateral (external or accessory) cuneate nucleus, whose neurons project to the cerebellum via the cuneocerebellar pathway (Laureen*et al.*, 2017).

Many ascending fibres of the fasciculus gracilis and fasciculus cuneatus terminate by synapsing on neurons of the dorsal column nuclei (nucleus gracilis and nucleus cuneatus, respectively) in the medulla oblongata. (The connections of the dorsal column nuclei are described further with the medulla oblongata. Axons arising from neurons in the dorsal column nuclei arch ventromedially round the central gray matter of the medulla as internal arcuate fibres and decussate in the sensory or lemniscal decussation(Laureen*et al*.,2017). As the medial lemniscus, they ascend to the ventral posterolateral nucleus of the thalamus, from where neurons project to the somatosensory cortex in the postcentral gyrus of the parietal --lobe (areas 3, 1 and 2) (Boulenguez and Vinay, 2009).

Some neurons of the dorsal column nuclei form posterior external arcuate fibres, which enter the cerebellum. The high degree of somatotopic organization that is present in the dorsal

columns is preserved as the pathways ascend through the dorsal column nuclei and thalamus to reach the primary somatosensory cortex. In the dorsal column nuclei the lower limb is represented in the nucleus gracilis, the upper limb in the nucleus cuneatus, and the trunk lies between. Fibres are also segregated by modality in the dorsal columns. Fibres from hair receptors are most superficial, while those from tactile and vibratory receptors lie in deeper layers (Boulenguez and Vinay, 2009).

Spinocerebellar tracts

There are two principal spinocerebellar tracts, dorsal or posterior and ventral or anterior. They occupy the periphery of the lateral aspect of the spinal white matter and carry proprioceptive and cutaneous information to the cerebellum for the coordination of movement. Both tracts contain large-diameter myelinated fibres, but there are more in the posterior tract (Moore, 2007). Finer-calibre fibres are associated with the anterior tract. The dorsal spinocerebellar tract lies lateralto the lateral corticospinal tract. It begins about the level of the second or third lumbar segment and enlarges as it ascends. Axons of the tract originate ipsilaterally from the larger neurons of Clarke's column, in lamina VII throughout spinal segments T1-L2. Clarke's column receives input from collaterals of long ascending primary afferents of the doral columns and terminals of shorter ascending primary afferents of the dorsal columns (Moore, 2007). Many of these afferent fibres ascend from segments caudal to L2. In the medulla, the dorsal spinocerebellar tract passes through the inferior cerebellar peduncle to terminate ipsilaterally in the rostral and caudal parts of the cerebellar vermis. The ventral spinocerebellar tract lies immediately ventral to the dorsal spinocerebellar tract. The cells of origin are in laminae V-VII of the lumbosacral cord and the tract carries information from the lower limb. Axons forming the tract mostly decussate - a few remain ipsilateral. The tract begins in the upper lumbar region and ascends through the medulla oblongata to reach the upper pontine level, from where it descends in the dorsal part

of the superior cerebellar peduncle to terminate, mainly contralaterally, in the anterior cerebellar vermis (Moore, 2007).

The spinocerebellar tracts are laminated, such that fibres from lower segments are superficial. Both tracts convey proprioceptive and exteroceptive information, but they are functionally different. Neurons of Clarke's column are excited monosynaptically by Ia and Ib primary afferent fibres (from muscle spindles and tendon organs, respectively) and also by group II muscle afferents, and cutaneous touch and pressure afferents. The proprioceptive impulses often arise from a single muscle or from synergistic muscles acting at a common joint. Thus, the dorsal spinocerebellar tract transmits modality-specific and space-specific information that is used in the fine coordination of individual limb muscles (Moore, 2007). On the other hand, the cells of the ventral tract are activated monosynaptically by Ib afferents and transmit information from large receptive fields that include different segments of a limb. The ventral tract lacks subdivisions for different modalities and transmits information for the coordinated movement and posture of the entire lower limb. Since Clarke's column diminishes rostrally and does not extend above the lowest cervical segment, it follows that the dorsal spinocerebellar tract carries information from the trunk and lower limb (Moore, 2007). Proprioceptive and exteroceptive information from the upper limb trunk and lower limb. Proprioceptive and exteroceptive information from the upper limb travels in primary afferent fibres of the fasciculus cuneatus. These fibres end somatotopically in the accessory (external or lateral) cuneate nucleus and the adjoining part of the cuneate nucleus situated in the medulla oblongata. Cells of these nuclei give rise to the posterior external arcuate fibres that form the cuneocerebellar tract, which enters the cerebellum via the ipsilateral inferior cerebellar peduncle. The accessory cuneate nucleus and the lateral part of the cuneate nucleus are considered to be homologous to the cells of Clarke's column. The cuneocerebellar tract is

therefore functionally allied to the dorsal spinocerebellar tract, and is its upper limb equivalent. Axons of all the spinocerebellar tracts and the cuneocerebellar tract form part of the 'mossy- fibre system'. They end in the cerebellar cortex in a highly organized, somatotopical and functional pattern (Skagerberg*et al*., 2015).

Spinothalamic tracts

The spinothalamic tracts consist of second-order neurons which convey pain, temperature, coarse (non-discriminative) touch and pressure information to the somatosensory region of the thalamus. Axons arise from neurons in diverse laminae in all segments of the cord. Tract fibres decussate in the ventral white commissure. Pain and temperature fibres do so promptly, within about one segment of their origin, whilst fibres carrying other modalities may ascend for several segments before crossing (Moore, 2007).

Spinothalamic fibres mostly ascend in the white matter ventrolateral to the ventral horn, partly intermingled with ascending spinoreticular fibres and descending reticulospinal fibres. Some authorities describe two spinothalamic tracts (lateral and ventral) with more-or-less distinct anatomical locations and functions. However, it should be noted that physiological studies in animals support the notion that these tracts are best considered as a structural and functional continuum. The lateral spinothalamic tract is sited in the lateral funiculus, lying medial to the ventral spinocerebellar tract (Mobbs and Patrick,2009).

Clinical vidence indicates that it subserves pain and temperature sensations. The ventral spinothalamic tract lies in the anterior funiculus medial to the point of exit of the ventral nerve roots and dorsal to the vestibulospinal tract, which it overlaps. On the basis of clinical evidence, it subserves coarse tactile and pressure modalities (Moore, 2007).

A dorsolateral spinothalamic tract has been described in animals, arising mainly from lamina I neurons whose axons cross to ascend in the contralateral dorsolateral funiculus. These

neurons respond maximally to noxious, mechanical and thermal cutaneous stimuli. That such a projection exists in man is suggested by examples of clinical pain relief following dorsolateral cordotomy.

On reaching the lower brain stem, spinothalamic tract axons separate. Axons in the ventral tract join the medial lemniscus. Axons in the lateral tract continue as the spinal lemniscus. There is clear somatotopic organization of the fibres in the spinothalamic tracts throughout their extent. Fibres crossing at any cord level join the deep aspect of those that have already crossed, which means that both tracts are segmentally laminated (Moore, 2007). In the midbrain, fibres in the spinal lemniscus conveying pain and temperature sensation from the lower limb extend dorsally, while those from the trunk and upper limb are more ventrally placed. Both lemnisci ascend to end in the thalamus. The major spinothalamic projections in man are to the ventral posterolateral nucleus, and also to the centrolateral intralaminar nucleus of the thalamus (Moore, 2007).

2.4.6 Descending pathways

Descending pathways to the spinal cord originate primarily in the cerebral cortex and in numerous sites within the brain stem (Fritschy and Grzanna, 1990).They are concerned with the control of movement, muscle tone and posture; the modulation of spinal reflex mechanisms; and the transmission of afferent information to higher levels. They also mediate control over spinal autonomic neurons (Moore, 2007).

Corticospinal tracts

Corticospinal fibers arise from neurons of the cerebral cortex. They project, in a somatotopically organized fashion, to neurons that are mostly located in the contralateral side of the spinal cord. The majority of corticospinal fibres arise from cells situated in the upper

two-thirds of the precentral motor cortex (area 4), and from the premotor cortex (area 6). A small contribution of fibres stems from cells of the postcentral gyrus (somatosensory cortex, areas 3, 1, and 2) and the adjacent parietal cortex (area 5). In the monkey, 30% of corticospinal fibres arise from area 4, 30% from area 6, and 40% from the parietal regions (Ramer *et al*., 2014).

Cells of origin of corticospinal fibres vary in size in the different cortical areas and are clustered into groups or strips. The largest cells (giant pyramidal neurons or Betz cells) are in the precentral cortex. Corticospinal fibres descend at first through the subcortical white matter and enter the posterior limb of the internal capsule (Ramer *et al*., 2014). They then pass through the ventral part of the midbrain in the cerebral peduncle or crus cerebri. As they continue caudally through the pons, they are separated from its ventral surface by transversely running pontocerebellar fibres. In the medulla oblongata, they form a discrete bundle, the pyramid, which forms a prominent longitudinal column on the ventral surface of the medulla (Ramer *et al*., 2014).

The corticospinal tracts are therefore also referred to as the pyramidal tracts. However, this term is often used to are therefore also referred to as the pyramidal tracts. However, this term is often used to embrace not only corticospinal fibres, but also corticobulbar fibres, which diverge above this level and end in association with cranial motor nuclei. Each pyramid contains about a million axons of varying diameter. The majority (70%) are myelinated: most (90%) have a diameter of 1-4 μm; 9% have diameters of 5-10 μm; and less than 2% have diameters of 11-22 μm. The largest diameter axons arise from the giant Betz cells Just rostral to the level of the spinomedullary junction, c.75-90% of the corticospinal fibres in the pyramid cross the median plane in the pyramidal decussation (decussation of the pyramids)

and continue caudally as the lateral corticospinal tract (Ramer *et al*., 2014; Yokoyama*et al*., 1999).

The rest of the fibres continue uncrossed as the ventral corticospinal tract. The lateral tract also contains some uncrossed corticospinal fibres. The lateral corticospinal tract descends in the lateral funiculus throughout most of the length of the spinal cord. It occupies an oval area, ventrolateral to the dorsal horn and medial to the dorsal spinocerebellar tract. In the lumbar and sacral regions, wherethe dorsal spinocerebellar tract is absent; the lateral corticospinal tract reaches the dorsolateral surface of the cord. As it descends, the lateral corticospinal tract progressively diminishes in size until about the fourth sacral spinal segment. Its axons terminate on ipsilateral spinal neurons. The smaller ventral corticospinal tract descends in the ventral funiculus. It lies close to the ventral median fissure, and is separated from it by the sulcomarginal fasciculus. The ventral corticospinal tract diminishes as it descends and usually disappears completely at midthoracic cord levels (Ramer *et al*., 2014).

It may either be absent or, very rarely, contain almost all the corticospinal fibres. Near their termination, most fibres of the tract cross the median plane in the ventral white commissure to synapse with contralateral neurons. The vast majority of corticospinal fibres, irrespective of the tract in which they descend, therefore terminate in the spinal cord on the side contralateral to their cortical origin. Knowledge of the detailed termination of corticospinal fibres is based largely upon animal studies, but is supplemented by data from postmortem studies on human brains using anterograde degeneration methods. Most corticospinal fibres are believed to terminate contralaterally on interneurons in the lateral parts of laminae IV-VI and both lateral and medial parts of lamina VII. Some are also distributed to lamina VIII bilaterally. Terminals are also associated with contralateral motor neuronal cell groups in

lamina IX, in the dorsolateral group and the lateral parts of both central and ventrolateral groups. Corticospinal fibres from the frontal cortex, including motor and premotor areas 4 and 6, terminate mostly on interneurons in laminae V-VIII, with the densest concentration laterally in lamina VI(Ramer *et al*., 2014). They influence α and γ motor neurons of lamina IX via these interneurons. Because the widespread dendrites of multipolar neurons in lamina IX penetrate lamina VII, direct monosynaptic axodendritic contacts also occur on large α motor neurons. Direct termination on motor neurons is most abundant in the spinal enlargements. Experimental evidence shows that precentral corticospinal axons influence the activities of both α and γ motor neurons, facilitating flexor muscles and inhibiting extensors, which are the opposite effects to those mediated by lateral vestibulospinal fibres. Evidence from animal studies shows that direct projections from the precentral cortical areas to spinal motor neurons are concerned with highly fractionated, precision movements of the limbs. Accordingly, in primates, precentral corticospinal fibres are mainly distributed to motor neurons supplying the distal limb muscles. Corticospinal projections may use glutamate or aspartate, often co-localized, as excitatory neurotransmitters. Corticospinal fibres from parietal sources end mainly in the contralateral dorsal horn, in the lateral parts of laminae IV-VI and lamina VII (Johnson *et al*., 2011).

Phylogenetically these fibres represent the oldest part of the corticospinal system. Axons from the sensory cortex terminate chiefly in laminae IV and V. They are concerned with the supraspinal modulation of the transmission of afferent impulses to higher centres, including the motor cortex. Experimental studies in primates indicate that isolated transection of corticospinal fibres at the level of the pyramid (pyramidotomy) results in flaccid paralysis or paresis of the contralateral limbs and loss of independent hand and finger movements. Destruction of corticospinal fibres at the level of the internal capsule, commonly caused by a

cerebral vascular accident or 'stroke', results in a contralateral hemiplegia. The paralysis is initially flaccid, but later becomes spastic, and is most marked in the distal muscles of the extremities, especially those concerned with individual movements of the fingers and hand. Associated signs on the paralysed side are: hyperactive deep tendon reflexes; hypertonicity; the loss of superficial abdominal and cremasteric reflexes; and the appearance of dorsiflexion of the toes (Babinski's sign) in response to stroking the sole of the foot. The latter is usually interpreted as pathognomonic of corticospinal damage, but it is not always present in patients with confirmed corticospinal lesions. Moreover, Babinski's sign is normally present in human infants up to about 2 years of age - its subsequent disappearance may reflect the completion (Ramer *et al*., 2014).

Rubrospinal tract

The rubrospinal tract arises from neurons in the caudal magnocellular part of the red nucleus (an ovoid mass of cells situated centrally in the midbrain tegmentum. This part of the nucleus contains some 150-200 large neurons, interspersed with smaller neurons (Ramer *et al*., 2014). The origin, localization, termination and functions of rubrospinal connections are poorly defined in man, and the tract appears to be rudimentary. Rubrospinal fibres cross in the ventral tegmental decussation and descend in the lateral funiculus of the cord, where they lie ventral to, and intermingled with, fibres of the lateral corticospinal tract. In animals the tract descends as far as lumbosacral levels, whereas in man it appears to project only to the upper three cervical cord segments. Rubrospinal fibres are distributed to the lateral parts of laminae V-VI and the dorsal part of lamina VII of the spinal gray matter. The terminal zones of the tract correspond to those of corticospinal fibres from the motor cortex. Animal studies demonstrate that the effects of rubrospinal fibres on α and γ motor neurons are similar to those of corticospinal fibres (Ramer *et al*., 2014).

Tectospinal tract

The tectospinal tract arises from neurons in the intermediate and deep layers of the superior colliculus of the midbrain. It crosses ventral to the periaqueductal gray matter in the dorsal tegmental decussation and descends in the medial part of the ventral funiculus of the spinal cord(Ramer *et al*., 2014). Fibres of the tract project only to the upper cervical cord segments, ending in laminae VI-VIII. They make polysynaptic connections with motor neurons serving muscles in the neck, facilitating those that innervate contralateral muscles and inhibiting those that innervate ipsilateral ones(Ramer *et al*., 2014). In animals, turning of the head to the contralateral side results from unilateral, electrical stimulation of the superior colliculus, and is mainly affected through the tectospinal tract(Ramer *et al*., 2014).

Vestibulospinal tracts

The large vestibular nuclear complex lies in the lateral part of the floor of the fourth ventricle around the pontomedullary junction of the brain stem. It gives rise to the lateral and ventral vestibulospinal tracts, which are functionally and topographically distinct. The lateral vestibulospinal tract arises from small and large neurons of the lateral vestibular nucleus (Deiters' nucleus) (Ramer *et al*., 2014).

It descends ipsilaterally, initially in the periphery of the ventrolateral spinal white matter but subsequently shifting into the medial part of the ventral funiculus at lower spinal levels. Fibres of this tract are somatotopically organized (Moore, 2007). Thus, fibres projecting to the cervical, thoracic and lumbosacral segments of the cord arise from neurons in the rostroventral, central and dorsocaudal parts, respectively, of the lateral vestibular nucleus. Lateral vestibulospinal fibres end ipsilaterally, mostly in the medial part of the ventral horn in lamina VIII and the medial part of lamina VII. Axons of the lateral vestibulospinal tract excite, through mono-and polysynaptic connections, motor neurons of extensor muscles of

the neck, back and limbs; γ motor neurons are also probably facilitated. Lateral vestibulospinal tract axons also inhibit, disynaptically, motor neurons of flexor limb muscles, via inhibitory interneurons (Moore, 2007).

The medial vestibulospinal tract arises mainly from neurons in the medial vestibular nucleus, but some are also located in the inferior and lateral vestibular nuclei. The medial vestibulospinal tract descends in the medial longitudinal fasciculus into the ventral funiculus of the spinal cord where it lies close to the midline in the so-called sulcomarginal fasciculus. Unlike the lateral tract it contains both crossed and uncrossed fibers and does not extend beyond the midthoracic cord. Fibers of the medial tract project mainly to the cervical cord level (Moore, 2007).

Reticulospinal tracts

The reticulospinal tracts pass from the brain stem reticular formation to the spinal cord. Detailed knowledge of their origins and connections has been obtained mainly from studies in animals (Moore, 2007). The medial reticulospinal tract originates from the medial tegmental fields of the pons and medulla. The main sources are the oral and caudal pontine reticular nuclei and the gigantocellular reticular nucleus in the medulla. Pontine fibres descend, mainly ipsilaterally, in the ventral funiculus of the cord (Moore, 2007).

Medullary fibres descend, both ipsilaterally and contralaterally, in the ventral funiculus and the ventral part of the lateral funiculus (Moore, 2007). These fibres have many types of collateral and two-thirds of the reticulospinal neurons that reach the cervical cord also descend to lumbosacral levels. The terminals of reticulospinal fibres are distributed to lamina VIII, and the central and medial partsof lamina VII(Moore, 2007). The medullary reticulospinal terminals are more widely distributed, ending additionally in the lateral parts of

laminae VI and VII and also directly on motor neurons(Joseph, 2018).

Both α and γ motor neurons are influenced by reticulospinal fibres, through polysynaptic and monosynaptic connections (Joseph, 2018). Physiological evidence shows that reticulospinal fibres from pontine sources excite motor neurons of axial and limb muscles, while medullary fibres excite, or inhibit motor neurons of cervical muscles and excite motor neurons of axial muscles (Joseph, 2018).

Functionally, the medial reticulospinal tract is concerned with posture, the steering of head and trunk movements in response to external stimuli, and crude, stereotyped movements of the limbs. The lateral reticulospinal tract lies in the lateral funiculus of the spinal cord, closely associated with the rubrospinal and lateral corticospinal tracts. Its fibres arise from neurons of the ventrolateral tegmental field of the pons. The fibres cross in the rostral medulla oblongata and project, with a high degree of collateralization, throughout the length of the spinal cord. Axons of this tract terminate in laminae I, V and VI, and also bilaterally in the lateral cervical nucleus. Evidence suggests that this pathway is involved in the control of pain perception and in motor functions (Joseph, 2018).

Interstitiospinal tract

The interstitiospinal tract arises from neurons in the interstitial nucleus (of Cajal) and the immediate surrounding area, and descends via the medial longitudinal fasciculus into the ventral funiculus of the spinal cord. Its fibres project, mainly ipsilaterally, as far as lumbosacral levels, and are mostly distributed to the dorsal part of lamina VIII and the dorsally adjoining part of lamina VII. They establish some monosynaptic connections with motor neurons supplying neck muscles, but their main connections are disynaptic with motor neurons supplying limb muscles (Joseph, 2018).

Solitariospinal tract

The solitariospinal tract is a small group of mostly crossed fibres which arises from neurons in the ventrolateral part of the nucleus solitarius of the medulla. Descending in the ventral funiculus and ventral part of the lateral funiculus of the cord, these axons terminate on phrenic motor neurons supplying the diaphragm and thoracic motor neurons which innervate intercostal muscles. A pathway with somewhat similar course and terminations to that of the solitariospinal tract originates from the nucleus retroambiguus. Both pathways subserve respiratory activities by driving inspiratory muscles, and some descending axons from the nucleus retroambiguus facilitate expiratory motor neurons. There is clinical evidence that bilateral ventrolateral cordotomy at high cervical levels abolishes rhythmic ventilatory movements (Moore, 2007).

Hypothalamospinal fibres

Hypothalamospinal fibres exist in animals. They arise from the paraventricular nucleus and other areas of the hypothalamus and descend ipsilaterally, mainly in the dorsolateral region of the cord, to be distributed to sympathetic and parasympathetic preganglionic neurons in the intermediolateral column (Moore, 2007). Fibres from the paraventricular nucleus show oxytocin and vasopressin immunoreactivity. They are also distributed to laminae I and X. Descending fibres from the dopaminergic cell group situated in the caudal hypothalamus innervate sympathetic preganglionic neurons and neurons in the dorsal horn. That similar pathways exist in man may be inferred from ipsilateral sympathetic deficits (e.g. Horner's syndrome), which follow lesions of the hypothalamus, the lateral tegmental brain stem, or the lateral funiculus of the cord (Moore, 2007).

Monoaminergic spinal pathways

Monoaminergic cell groups utilize dopamine, adrenaline (epinephrine), noradrenaline (norepinephrine) and 5-HT (5-hydroxytryptamine, serotonin) as neurotransmitters. They occur widely throughout the brain stem and in the hypothalamus. They project rostrally to many forebrain areas and caudally to the spinal cord and appear to be concerned with the modulation of sensory transmission, and the control of autonomic and somatic motor neuronal activities (Moore, 2007).

The projections to the spinal cord arise from several sources. Coeruleospinal projections originate from noradrenergic cell groups A4 and A6 in the locus coeruleus complex in the pons and descend via the ventrolateral white matter to innervate all cord segments bilaterally. They end in the dorsal gray matter (laminae IV-VI) and the intermediate and ventral horns (Moore, 2007).

They also project extensively to preganglionic parasympathetic neurons in the sacral cord. Descending noradrenergic fibres, which arise from the lateral tegmental cell groups A5 and A7 of the pons, travel in the dorsolateral white matter. They are distributed to laminae I-III, and particularly to the intermediate gray horn (Mohamad *et al.*, 2014). Descending fibres from adrenergic cell groups C1 and C3 of the medulla oblongata have been traced into the anterior funiculus of the cord and are extensively distributed column.

Dopaminergic fibres projecting to the spinal cord travel in the hypothalamospinal pathway. The raphe nuclei pallidus, obscurus and magnus in the brain stem give rise to two serotoninergic descending bundles. The lateral raphe spinal bundle, from B3 neurons, is concerned with the control of nociception. It descends close to the lateral corticospinal tract and ends in the dorsal horn (laminae I, II and V). The ventral bundle, composed mainly of axons from B1 neurons, travels in the medial part of the ventral white column and ends in the

ventral horn (laminae VIII and IX). It facilitates extensor and flexor motor neurons. Some descending serotoninergic fibres project to sympathetic preganglionic neurons and are concerned with the central control of cardiovascular function (Joseph, 2018).

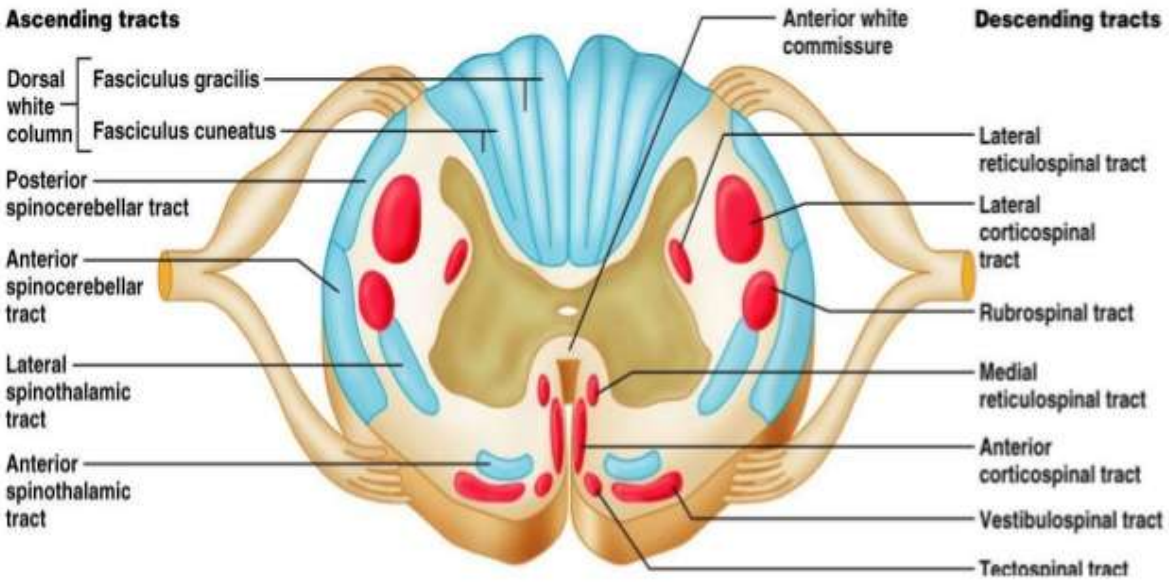


Fig 2.15 Ascending and desebding tracts of the human spinal cord.

Image source: anatomy2medicine.com

Fig 2.15: Ascending and desebding tracts of the human spinal cord.

Image source: anatomy2medicine.com

2.5 Dopamine

Dopamine is an organic chemical of the catecholamine and phenethylamine families that plays several important roles in the brain and the body. It is an amine synthesized by removing a carboxyl group from a molecule of its precursor chemicalL-DOPA, which is synthesized in the CNS and kidneys (Karoum*et al.*, 1981, Berridge*et al.*,2009).

Dopamine is also synthesized in plants and most animals. In the CNS, dopamine functions as a neurotransmitter—a chemical released by neurons to send signals to other nerve cells. The brain includes several distinct dopamine pathways, one of which plays a major role in reward-motivated behavior(Commissiong,1979). Most types of rewards increase the level of dopamine in the brain, and many addictivedrugs increase dopamine neuronal activity.
Other brain dopamine pathways are involved in motor control and in controlling the release of various hormones (Commissiong, 1979, Jonathan *et al.*,2005).

These pathways and cell groups form a dopamine system which is neuromodulatory. Outside the central nervous system, dopamine functions primarily as a local chemical messenger. In

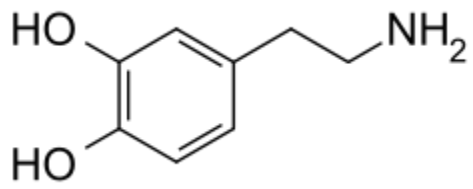
blood vessels, it inhibits norepinephrine release and acts as a vasodilator (at normal concentrations); in the kidneys, it increases sodium excretion and urine output; in the pancreas, it reduces insulin production; in the digestive system, it reduces gastrointestinal motility and protects intestinal mucosa; and in the immune system, it reduces the activity of lymphocytes. With the exception of the blood vessels, dopamine in each of these peripheral systems is synthesized locally and exerts its effects near the cells that release it. Several important diseases of the nervous system are associated with dysfunctions of the dopamine system, and some of the key medications used to treat them work by altering the effects of dopamine.

Parkinson's disease, a degenerative condition causing tremor and motor impairment, is caused by a loss of dopamine-secreting neurons in an area of the midbraincalled the substantial nigra. There is evidence that schizophrenia involves altered levels of dopamine activity, and most antipsychotic drugs used to treat this are dopamine antagonists which reduce dopamine activity. Similar dopamine antagonist drugs are also some of the most effective anti-nausea agents. Restless legs syndrome and attention deficit hyperactivity disorder are associated with decreased dopamine activity (Beaulieu and Gainetdinov, 2011).

Dopaminergicstimulants can be addictive in high doses, but some are used at lower doses to treat deficit hyperactivity disorder. Dopamine itself is available as a manufactured medication for intravenous injection: although it cannot reach the brain from the bloodstream, its peripheral effects make it useful in the treatment of heart failure or shock, especially in newborn babies.A dopamine molecule consists of a catechol structure (a benzene ring with two hydroxyl side groups) with one amine group attached via an ethyl chain (Kitti*et. al.*, 2012). As such, dopamine is the simplest possible catecholamine, a family that also includes the neurotransmittersnorepinephrine and epinephrine.The presence of a benzene ring with

this amine attachment makes it a substituted phenethylamine, a family that includes numerous psychoactive drugs. Like most amines, dopamine is an organic base(Ben-Jonathan *et al*.,2001, Johnson *et al*., 2010).

As a base, it is generally protonated in acidic environments (in an acid-base reaction). The protonated form is highly water-soluble and relatively stable, but can become oxidized if exposed to oxygen or other oxidants (Kitti*et al*., 2012). In basic environments, dopamine is not protonated.In this free base form, it is less water-soluble and also more highly reactive Because of the increased stability and water-solubility of the protonated form, dopamine is supplied for chemical or pharmaceutical use as dopamine hydrochloride that is, the hydrochloridesalt that is created when dopamine is combined with hydrochloric acid. In dry form, dopamine hydrochloride is a fine colorless powder (Bhatt-Mehta and Nahata, 1989).



Dopamine structure

Phenethylamine structure

Catechol structure(Kitti *et. al*., 2012)

2.5.1 Synthesis of dopamine

Dopamine is synthesized in a restricted set of cell types, mainly neurons and cells in the medulla of the adrenal glands(Kaasinen*et al.*,2000, Seeman,2009*).* The primary and minor metabolic pathways respectively are:

Primary: L-Phenylalanine → L-Tyrosine → L-DOPA → Dopamine

Minor: L-Phenylalanine → L-Tyrosine → *p*-Tyramine → Dopamine

Minor: L-Phenylalanine → *m*-Tyrosine → *m*-TyramineDopamine(Björklund and Dunnett,2007).

The direct precursor of dopamine, L-DOPA, can be synthesized indirectly from the essential amino acidphenylalanine or directly from the non-essential amino acid tyrosine(Musacchio,2013). These amino acids are found in nearly every protein and so are readily available in food, with tyrosine being the most common. Although dopamine is also found in many types of food, it is incapable of crossing the blood–brain barrier that surrounds and protects the brain (Musacchio,2013*).* It must therefore be synthesized inside the brain to perform its neuronal activity(Musacchio, 2013). L-Phenylalanine is converted into L-tyrosine by the enzymephenylalanine hydroxylase, with molecular oxygen (O_2) and tetrahydrobiopterin as cofactors. L-Tyrosine is converted into L-DOPA by the enzyme tyrosine hydroxylase, with tetrahydrobiopterin, O_2, and iron (Fe^{2+}) as cofactors (Seeman,2009). L-DOPA is converted into dopamine by the enzyme aromatic L-amino acid decarboxylase (also known as DOPA decarboxylase), with pyridoxal phosphate as the cofactor(Kadiri *et al.*,2011*).*

Dopamine itself is used as precursor in the synthesis of the neurotransmitters norepinephrine and epinephrine(Kitti*etal.*,2012). Dopamine is converted into norepinephrine by the enzyme

dopamine β-hydroxylase, with O_2 and L-ascorbic acid as cofactors (Fleetwood-Walker *et al.*,2014).Norepinephrine is converted into epinephrine by the enzyme phenylethanolamine *N*-methyltransferase with *S*-adenosyl-L-methionine as the cofactor. Some of the cofactors also require their own synthesis (Kitti*et al*., 2012). Deficiency in any required amino acid or cofactor can impair the synthesis of dopamine, norepinephrine, and epinephrine. Dopamine is broken down into inactive metabolites by a set of enzymes—monoamine oxidase, catechol-*O*-methyl transferase, and aldehyde dehydrogenase, acting in sequence (Bjugn and Gundersen,1993).

Both isoforms of monoamine oxidase, monoamineoxidase-A and monoamineoxidase-B, effectively metabolize dopamine.Different breakdown pathways exist but the main end-product is homovanillic acid, which has no known biological activity (Eisenhofer *et al.*, 2004).From the bloodstream, homovanillic acid is filtered out by the kidneys and then excreted in the urine (Björklund and Skagerberg,1979). Although dopamine is normally broken down by an oxidoreductase enzyme, it is also susceptible to oxidation by direct reaction with oxygen, yielding quinones plus various free radicals as products (Karthik and Lisbon, 2006).

The rate of oxidation can be increased by the presence of ferric iron or other factors. Quinones and free radicals produced by autoxidation of dopamine can poison cells, and there is evidence that this mechanism may contribute to the cell loss that occurs in Parkinson's disease and other conditions. Dopamine exerts its effects by binding to and activating cell surface receptors(Miyazaki and Asanuma, 2008).

In humans, dopamine has a high binding affinity at dopamine receptors and trace amine-associated receptor (Miyazaki and Asanuma, 2008). In mammals, five subtypes of dopamine receptors have been identified, labeled from D1 to D5. All of them function as metabotropic,

G protein-coupled receptors, meaning that they exert their effects via a complex second messenger system(Romanelli *et al.*,2009).These receptors can be divided into two families, known as D1-like and D2-like.For receptors located on neurons in the nervous system, the ultimate effect of D1-like activation (D1 and D5) can be excitation (via opening of sodium channels) or inhibition (via opening of potassium channels); the ultimate effect of D2-like activation (D2, D3, and D4) is usually inhibition of the target neuron.

Consequently, it is incorrect to describe dopamine itself as either excitatory or inhibitory: its effect on a target neuron depends on which types of receptors are present on the membrane of that neuron and on the internal responses of that neuron to the second messenger cAMP(Romanelli *et. al.,* 2009). D1 receptors are the most numerous dopamine receptors in the human nervous system; D2 receptors are next; D3, D4, and D5 receptors are present at significantly lower levels (Eiden *et al*.,2004).

Inside the brain, dopamine functions as a neurotransmitter and neuromodulator, and is controlled by a set of mechanisms common to all monoamine neurotransmitters. After synthesis, dopamine is transported from the cytosol into synaptic vesicles by a solute carrier—a vesicular monoamine transporter. Dopamine is stored in these vesicles until it is ejected into the synaptic cleft. In most cases, the release of dopamine occurs through a process called exocytosis which is caused by action potentials, but it can also be caused by the activity of an intracellular trace amine-associated receptor (Eiden *et al.,*2004).

Trace amine-associated receptor 1 is a high-affinity receptor for dopamine, trace amines, and certain substituted amphetamines that is located along membranes in the intracellular milieu of the presynaptic cell; activation of the receptor can regulate dopamine signaling by inducing dopamine reuptake inhibition and efflux as well as by inhibiting neuronal firing

through a diverse set of mechanisms. Once in the synapse, dopamine binds to and activates dopamine receptors (Eiden *et al.,* 2004). These can be postsynaptic dopamine receptors, which are located on dendrites (the postsynaptic neuron), or presynaptic autoreceptors (e.g., the $D_2$and presynaptic D_3 receptors), which are located on the membrane of an axon terminal (the presynaptic neuron). After the postsynaptic neuron elicits an action potential, dopamine molecules quickly become unbound from their receptors. They are then absorbed back into the presynaptic cell, via reuptake mediated either by the dopamine transporter or by the plasma membrane monoamine transporter (Oh *et al.,* 2009).

Once dopamine is back in the cytosol, dopamine can either be broken down by a monoamine oxidase or repackaged into vesicles byvesticular monoamine transporter, making it available for future release. In the brain the level of extracellular dopamine is modulated by two mechanisms: phasic and tonic transmission(Rice *et al*., 2011). Phasic dopamine release, like most neurotransmitter release in the nervous system, is driven directly by action potentials in the dopamine-containing cells (Rice *et al*., 2011).Tonic dopamine transmission occurs when small amounts of dopamine are released without being preceded by presynaptic action potentials. Tonic transmission is regulated by a variety of factors, including the activity of other neurons and neurotransmitter reuptake (Rice *et al.,* 2011).

As part of the reward pathway, dopamine is manufactured in nerve cell bodies located within the ventral tegmental area and is released in the nucleus accumbens and the prefrontal cortex. The motor functions of dopamine are linked to a separate pathway, with cell bodies in the substantial nigra that manufacture and release dopamine into the dorsal striatum.Inside the brain, dopamine plays important roles in executive functions, motor control, motivation, arousal, reinforcement, and reward, as well as lower-level functions including lactation,

sexual gratification, and nausea. The dopaminergic cell groups and pathways make up the dopamine system which is neuromodulatory (Blessing and Chalmers, 1979).

Dopaminergic neurons (dopamine-producing nerve cells) are comparatively few in number—a total of around 400,000 in the human brain (Björklund and Dunnett,2007)and their cell bodies are confined in groups to a few relatively small brain areas (Björklund and Dunnett,2007). However their axons project to many other brain areas, and they exert powerful effects on their targets (Björklund and Dunnett,2007).

These dopaminergic cell groups were first mapped in 1964 by Annica Dahlström and Kjell Fuxe, who assigned them labels starting with the letter "A" (for "aminergic"). In their scheme, areas A1 through A7 contain the neurotransmitter norepinephrine, whereas A8 through A14 contain dopamine. The dopaminergic areas they identified are the substantial nigra (groups 8 and 9); the ventral tegmental area (group 10); the posterior hypothalamus (group 11); the arcuate nucleus (group 12); the zona incerta (group 13) and the periventricular nucleus (group 14).

The substantial nigra is a small midbrain area that forms a component of the basal ganglia. This has two parts—an input area called the pars compacta and an output area the pars reticulata. The dopaminergic neurons are found mainly in the pars compacta (cell group A8) and nearby (group A9) (Malenka *et al*., 2009).

In humans, the projection of dopaminergic neurons from the substantial nigra pars compacta to the dorsal striatum, termed the nigrostriatal pathway, plays a significant role in the control of motor function and in learning new motor skills(Malenka *et al*.,2009).

These neurons are especially vulnerable to damage, and when a large number of them die, the result is a parkinsonian syndrome.

The ventral tegmental area is another midbrain area. The most prominent group of ventral tegmental area dopaminergic neurons projects to the prefrontal cortex via the mesocortical pathway and another smaller group projects to the nucleus accumbens via the mesolimbic pathway. Together, these two pathways are collectively termed the *mesocorticolimbic projection*(Malenka*et al.*,2009).The ventral tegmental area also sends dopaminergic projections to the amygdala, cingulate gyrus, hippocampus, and olfactory bulb (Malenka *et al.*,2009). Mesocorticolimbic neurons play a central role in reward and other aspects of motivation.The posterior hypothalamus has dopamine neurons that project to the spinal cord, but their function is not well established (Bouthenet *et al*., 2016).

There is some evidence that pathology in this area plays a role in restless legs syndrome, a condition in which people have difficulty sleeping due to an overwhelming compulsion to constantly move parts of the body, especially the legs (Paulus and Schomburg,2006). The arcuate nucleus and the periventricular nucleus of the hypothalamus have dopamine neurons that form an important projection the tuberoinfundibular pathwaywhich goes to the pituitary gland, where it influences the secretion of the hormone prolactin. Dopamine is the primary neuroendocrine inhibitor of the secretion of prolactin from the anterior pituitary gland. Dopamine produced by neurons in the arcuate nucleus is secreted into the hypophyseal portal system of the median eminence, which supplies the pituitary gland(Paulus and Schomburg, 2006).The prolactin cells that produce prolactin, in the absence of dopamine, secrete prolactin continuously; dopamine inhibits this secretion (Bregman, 1987).

In the context of regulating prolactin secretion, dopamine is occasionally called prolactin-inhibiting factor, prolactin-inhibiting hormone, or prolactostatin (Ben-Jonathan and Hnasko,2001).The zona incerta, grouped between the arcuate and periventricular nuclei, projects to several areas of the hypothalamus, and participates in the control of gonadotropin-releasing hormone, which is necessary to activate the development of the male and female reproductive systems, following puberty (Ben-Jonathan and Hnasko,2001).An additional group of dopamine-secreting neurons is found in the retina of the eye.These neurons are amacrine cells, meaning that they have no axons. They release dopamine into the extracellular medium, and are specifically active during daylight hours, becoming silent at night (Commissiong, 1983 Bas *et al*.,2009).

This retinal dopamine acts to enhance the activity of cone cells in the retina while suppressing rod cells—the result is to increase sensitivity to color and contrast during bright light conditions, at the cost of reduced sensitivity when the light is dim.The largest and most important sources of dopamine in the vertebrate brain are the substantial nigra and ventral tegmental area.These structures are closely related to each other and functionally similar in many respects. Both are components of the basal ganglia, a complex network of structures located mainly at the base of the forebrain. The largest component of the basal ganglia is the striatum(Benoit-Marand*et al*.,2001, Bruinstroop *et al.*, 2012).

The substantial nigra sends a dopaminergic projection to the dorsal striatum, while the ventral tegmental area sends a similar type of dopaminergic projection to the ventral striatum. Dopamine contributes to the action selection process in at least two important ways. First, it sets the "threshold" for initiating actions(Chakravarthy *et al.*,2010). The higher the level of dopamine activity, the lower the impetus required to evoke a given behaviour (Chakravarthy

et al., 2010). As a consequence, high levels of dopamine lead to high levels of motor activity and impulsive behavior; low levels of dopamine lead to torpor and slowed reactions (Chakravarthy *et al.*,2010). The second important effect of dopamine is as a "teaching" signal. When an action is followed by an increase in dopamine activity, the basal ganglia circuit is altered in a way that makes the same response easier to evoke when similar situations arise in the future (Chakravarthy *et al.*,2010). This is a form of operant conditioning, in which dopamine plays the role of a reward signal.Within the brain, dopamine functions partly as a "global reward signal", where an initial phasic dopamine response to a rewarding stimulus encodes information about the salience, value, and context of a reward (Schultz, 2015).

In the context of reward-related learning, dopamine also functions as a reward prediction error signal, that is, the degree to which the value of a reward is unexpected (Schultz, 2015).According to this hypothesis of Wolfram Schultz, rewards that are expected do not produce a second phasic dopamine response in certain dopaminergic cells, but rewards that are unexpected, or greater than expected, produce a short-lasting increase in synaptic dopamine, whereas the omission of an expected reward actually causes dopamine release to drop below its background level (Schultz, 2015).The "prediction error" hypothesis has drawn particular interest from computational neuroscientists, because an influential computational-learning method known as temporal difference learning makes heavy use of a signal that encodes prediction error.This confluence of theory and data has led to a fertile interaction between neuroscientists and computer scientists interested in machine learning(Schultz, 2015).

Evidence from microelectrode recordings from the brains of animals shows that dopamine neurons in the ventral tegmental area and substantial nigra are strongly activated by a wide variety of rewarding events(Schultz, 2015). These reward-responsive dopamine neurons in the ventral tegmental areaand substantial nigra are crucial for reward-related cognition and serve as the central component of the reward system. The function of dopamine varies in each axonal projection from the ventral tegmental areaand substantial nigra for example, the ventral tegmental area–nucleus accumbens shell projection assigns incentive salience ("want") to rewarding stimuli and its associated cues, theventral tegmental area –orbitofrontal cortex projection updates the value of different goals in accordance with their incentive salience, the ventral tegmental area–amygdala and ventral tegmental area–hippocampus projections mediate the consolidation of reward-related memories, and both the ventral tegmental area–nucleus accumbens core and substantial nigra–dorsal striatum pathways are involved in learning motor responses that facilitate the acquisition of rewarding stimuli(Jarcho *et al.,* 2012,Jiang*et al*.,2015). Some activity within theventral tegmental area dopaminergic projections appears to be associated with reward prediction as well. While dopamine has a central role in mediating "wanting" associated with the appetitive or approach behavioural responses to rewarding stimuli, detailed studies have shown that dopamine cannot simply be equated with hedonic "liking" or pleasure, as reflected in the consummatory behavioral response (Bucher *et al*, 2003, Bush and Allman, 2003).

Dopamine neurotransmission is involved in some but not all aspects of pleasure-related cognition, since pleasure centers have been identified both within the dopamine system (i.e., nucleus accumbens shell) and outside the dopamine system (i.e., ventral pallidum and parabrachial nucleus)(Berridge *et al.*, 2009). For example, direct electrical stimulation of dopamine pathways, using electrodes implanted in the brain, is experienced as pleasurable,

and many types of animals are willing to work to obtain it. Antipsychotic drugs used to treat psychosis reduce dopamine levels and tend to cause anhedonia, a diminished ability to experience pleasure. Many types of pleasurable experiences—such as sex, enjoying food, or playing video games—increase dopamine release. All addictive drugs directly or indirectly affect dopamine neurotransmission in the nucleus accumbens;these drugs increase drug "wanting", leading to compulsive drug use, when repeatedly taken in high doses, presumably through the sensitization of incentive-salience. Moreover, animals in which the ventral tegmental dopamine system has been rendered inactive do not seek food, and will starve to death if left to themselves, but if food is placed in their mouths, they will consume it and show expressions indicative of pleasure (Christine and Aminoff 2004).

Dopamine has been investigated relative to its possible role in the pathophysiology of spinal cord injury (Bergson*et al*., 1995, Fisher*et al.*, 2017). Dopamine was found to be decreased by a factor of four at the site of spinal cord impact within one hour after trauma. Other investigators have noted no significant alteration in dopamine concentration in the contused spinal cord. Studies havereported a significant elevation in dopamine concentration following experimental spinal cord injury. The latter authors (also observed that the use of haloperidol, predominantly a dopaminergic blocker, lent a high degree of clinicopathological protection to the injured canine spinal cord, and hypothesised that the traumarelated dopamine elevation may induce intramedullary vasoconstriction through an alpha-adrenergic mechanism which is blocked by this agent**(**Abramets and Samoilovich, 1991).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The materials used for the study include grasscutters, weighing balance, measuring ruler, vernier caliper, Digital electronic balance (Mettler balance P1210, Mettler instrument AG, Switzerland: sensitive, normal saline, distilled water, Bouin's fluid, metal cages, organ bottles, dissecting kit, cotton wool, bone cutters, chloroform, Hand Gloves, Towel, Microscope, dissecting board, Plane sheets, electronic scale, Analytical balance, Cover slips, Pack of slides.

3.2 Animal Procurement and Management

Twelve (12) adult Grasscutters (*thryonomys swinderianus*) made up of six males and six females were utilized for the study. The photograph of the animal is shown in plate 3.1a. The animals were procured from Tamqua Farm in Zaria, Kaduna State and were transported to the animal houseof the Department of Human Anatomy, Ahmadu Bello University, Zaria using metal cages. The animals were healthy looking and the body weight of each Grasscutter was obtained using weighing balance. Animals were fed with sugar cane and elephant grass and water was provided ad-*libitum*.

3.3 Methods

3.3.1Animal sacrifice

Physical observations ofthe grasscuters were done after which the animals were anaesthetized by chloroform inhalation and were dissected. After which the vertebra containing the spinal cords were dissected out cleanedand photographed in situ using digital camera. Spinal cords were exposed by dissection using bone cutter to open through the vertebral columns. Pictures of the dissections are shown in Plate I. By the process of splitting the vertebral columns the spinal cords

3.4 Study Design

The spinalcord of the twelve (12) adult Grasscutters was used for the Anatomical, histological, histochemical and immunohistochemical studies.

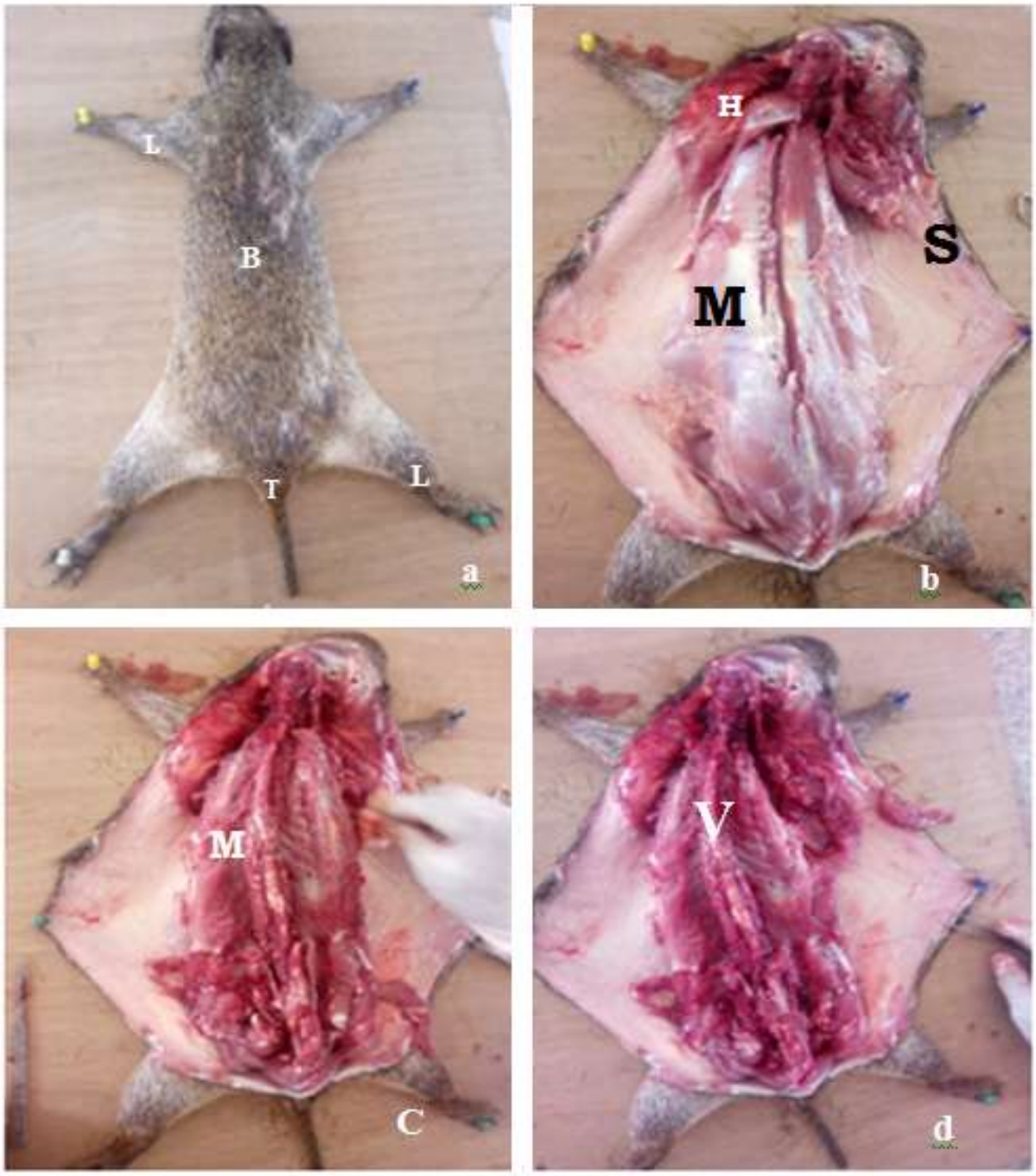
3.5 Morphological studies

The skin and epaxial muscles were dissected to expose the vertebral spine and the spinal cord were harvested as shown in Plate I (a,b,c and d). This was according to the method of Olude *et al*.,(2015). Spinal cord segments were identified and transected per animal based on the methods described by Farag *et al*., (2012), Olude *et al*., (2015). The altlantooccipital junction was identify and the spinal cord was dissected.The removed spinal cords of both male and female grasscutters were observed for gross features namely the spinal cord enlargements, the shapes, the sizes, the surfaces and the borders and were photographed. Definitions of gross anatomical structures based on the standard information on rodent anatomy by Olude *et al*., (2015).

3.6 Morphometric Studies

Live weight of each grasscutterand the weight of the spinal cords was obtained using the Mettle balance P1210 (Mettle instrument AG, Switzerland) with a sensitivity of 0.1g anddigital measuring balance with sensitivity of 0.01gaccording to the method of Ko *et al.*,(2004) and Bahar *et al.*,(2013). The weights of the live grasscutter and spinal cord were recorded in grams.

The total length of spinal cords and the width of the various segments which include cervical,thoracic,lumbar and sacral segments were measured using the meter ruler (made in China) and vernier calliper (made in China). The sensitivity of meter rulerwas 0.1cm and 0.01cm for vernier calliper. These instruments were used according to the method of Ko *et al.*(2004).



PlateI: Dissection of the back and vertebral column of the Grasscutter to expose the spinal cord. In (a) the Grasscutter was being dissected through the skin while in (b) and C the muscles were dissectedto expose the verterbral column. In (d)the vertebral column containing the spinal cord of the Grasscutter was exposed. The head (H),body (B),tail (T) and limbs (L),muscle (M),skin (S) and vertebral column (V) of grasscutter are shown.

3.7 Histological Studies

The harvested spinal cords were fixed in Bouin's fluid and the tissues were taken to the tissue preparatory section of histology laboratory of the Deparment of Human Anatomy, Ahmadu Bello Univesity, Zaria for processing and staining.

3.7.1 Histological processing method

The spinal cord tissues were removed and immediately fixed in Bouin’s fluid. The various segments of the spinal cord of Grasscutter (cervical, thoracic, lumbar and sacral segments) were fixed in different tissue sample containers for 24 hours.

After fixation, the tissues were transferred into automatic tissue processor where they were processed through a process of dehydration in ascending grades of alcohol (ethanol) 70%, 80%, 95% and absolute alcohol (100%) for two changes each. Each change lasted for one hour.

The tissues were then cleared in xylene and embedded in paraffin wax. Serial sections were made using a rotary microtome.

The tissue sections were deparaffinised, rehydrated and stained using routine haematoxylin and eosin staining method (H&E).

The stained sections were examined under the binocular light Olympus microscope (HM-LUX, Leitz Wetzlar,Germany) at different magnifications. The binocular light Olympus microscope (HM-LUX Leitz Wetzlar, Germany) was fitted with a digital camera and a computer with imaging software (AmScope MT version 3.0.0.5 USA) according to the manufacturer instructionHistoloical slides were studied and photomicrographs of the sectionswere taken with different magnifications to show the different parts of spinal cord.

3.8 Histochemical Studies

For histochemical studies, the spinal cord tissue sections were stained with Crestyl fast Violet to demonstrate Nissl bodies and Golgi methodto demonstrate different neurons with their axons and dentrites.

3.8.1 Histochemical method for crestyl fast violet stain for spinal cord tissue to demonstrate Nissl bodies

After fixating the tissues in Bouin's fluid, the spinal cord tissues were removed from the fixative and passed through a process of dehydration in ascending grades of alcohol (ethanol) namely; 70%, 80%, 95% and absolute alcohol (100%) for two changes each. Each of the changes lasted for two hours. The tissues were then cleared in xylene and embedded in paraffin wax. Serial sections were made using a rotary microtome. The tissue sections were deparaffinised and rehydratedin two changes of absolute alcohol forthree minutes each change andthe tissues were stained in 0.1% Cresyl violet for fifteen minutes . The tissues were quickly rinsed in tap water to remove excess stain. After rising the tissues in tap water, the spinal cord tissues were washed in 70% ethanolto remove the stain.

The sections were immersed for two minutes in differentiation solution and staining was checked on the microscope.

Spinal cord tissues were dehydrated through changes of absolute ethanol andcleared in xylene. After whichthe tissues were mounted and were allowed to dry. The stained spinal cord sections were examined for Nissl bodies under the light microscope fitted with a digital camera and connected to a laptop.

The stained sections were examined under the binocular light Olympus microscope (HM-LUX Leitz Wetzlar Germany) at different magnification. The binocular light Olympus microscope (HM-LUX,Leitz Wetzlar, Germany) was fitted with a digital camera and a computer with imaging soft ware (AmScope MT version 3.0.0.5 USA) according to the

manufacturer instructions. Histological slides were studied for Nissl bodies (Nissl substances) and photomicrographs of the tissues were taken at different magnifications

3.8.2 Histochemical method for golgi stain of spinal cord tissuesto demonstrate different neurons with their axons and dentrites

Spinal cord tissues used for Golgi study were fixed in Bouin's fluid. The tissues were removed from the fixative and cleared in xylene and embedded in paraffin wax. After which the tissue blocks were placed in 3% potassium bichromate for seven days. Light was avoided and a fresh solution of 3% potassium bichromate was used for each new day.

After the seven days tissue blocks were transferred into 2% silver nitrate solution for three days at room temperature. The silver nitrate solution contained 2g of silver nitrate and 100ml of distilled water. Light was also avoided. Serial sections were cut at 5um thick spread in distilled water and mounted. Tissues were air dried for ten minutes. Then dehydrated through 95% Alcohol, 100% alcohol, cleared in xylene and cover slipes were used to cover the tissues. The stained sections were examined under the light microscope fitted with a digital camera connected to a laptop. Histological sections werestudied for neuronal cells types and distribution in the binocular light Olympus microscope at different magnifications and photomicrographs of the histological sections were taken.

3.9 Immunohistochemical Studies

For immunohistochemical studies; sections were treated with Alpha-1 antitryrosine hydroxylase for the identification and localization of dopaminergic neurons in the spinal cord of Grasscutter.

3.9.1 Immunohistochemical protocol for the localization and identification of dopaminergic neurons in the spinal cord tissue of Grasscutter

Cut sections of the spinal cord tissue were taken to distilled water. Endogenous peroxidase activity was blocked in 3% hydrogen peroxide.Sections were washed in distilled water. Epitopes were retrieved in Citrate Buffer (PH 6) for twenty minutes. Sections were washed in distilled water and were incubated in Antitrypsin diluted 1/100 for sixty minutes and were washed in phosphate buffered saline (PH 7.4). Sections were incubated in Mouse and Rabbit horseradish peroxidases for 30minutes. Sections were then washed in phosphate buffered saline (PH 7.4).

The tissue sections were incubated in 20ul of diminobenzidine hydrochloride in 1ml of substrate buffer for 5minutes and were washed in distilled water. Sections were counterstained in Hematoxylin for 1minute. Sections were then washed in distilled water. Sections were dried and mounted with DPX.

The stained sections were examined for the presence of dopminergic neurons under the binocular light Olympus microscope (HM-LUXLeitz Wetzlar Germany) at different magnifications. The binocular light Olympus microscope (HM-LUX Leitz Wetzlar Germany) was fitted with a digital camera and connected to a computer with imaging soft ware (AmScope MT version 3.0.0.5 USA) according to the manufacturer's instruction histological slides were studied and photomicrographs of the slides were made at magnification of X250 and X 400.

3.10 Histometry

The histometry was done using the light microscope with a ×40 objective (×400 magnification) and computer running image processing (AmScope MT version 3.0.0.5, USA) for microscopy. Digital micrographs which were microscopic images of the spinal cord

(CFV) stain) were captured and calibration of software for measurement of microscopic features on the micrographs of spinal cord which were stained with crestyl fast violet stain was conducted.

The process of calibrations was done to measure the perimeters and the areas neurons in the gray matter of the spinal cord of Greasscutter. The calibrations involved the following steps; firstly, a micrometer slide that is containing the spinal cord tissue which comes as 1 mm graduated in 0.01 mm was captured at the same magnification and quality (pixels). Then, the micrograph of the spinal cord was photographed at the same magnification. . Secondly, the line tool was selected and was carefully measured from start of one major line of the micrographs of spinal cord of Grasscutter from end to end. Recorded length in pixels; in this study was 649 pixels/100um, 6.49 pixel/um. Next, the polygon tool was used to measure the area and perimeter of the desired neurons of the various spinal cord segments of Grasscutter. Values of the measurements were computed and presented automatically by the software. Plate II below is showing the summary of the process of thehistometry that was used in this study.

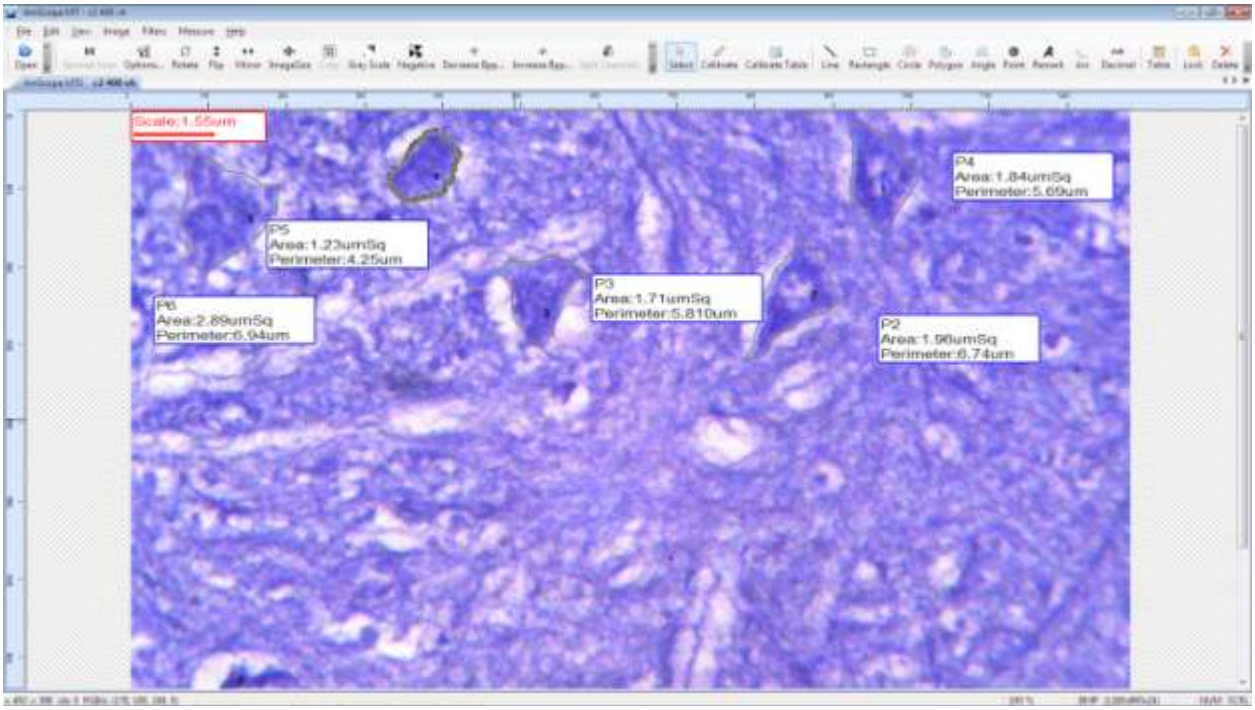


Plate II:Histometric studiesusing a crestyl fast violet stained micrograph (digital microscopic images) with a computer running imaging software (AmScope MT Version 3.0.0.5, USA)

3.11 Data Analysis

Data on weights, lengths,widths and areas of neurons were expressed as mean ± standard error of mean and statistical analysis of the data was done using the Student *t*-test for differences between males and females' animals. Student *t*-test was also done for differences between neuronal sizes of the anterior and posterior horns of the various segments of the spinal cord of Grasscutter. Correlation analysis was done between weight, length and width of the spinal cord with the body weight of the animals for both male and female animals. P-value less than 0.05 was taken to be significant ($P<0.05$). Statistical analysis of the data was done using SPSSversion 20.0.

CHAPTER FOUR

4.0 RESULTS

4.1 Physical Observations

The body of the Grasscutters were heavily built with bristle furs and coat. The head was broad with short flattened muzzle and with small eyes and circular ears. The animals have shorttails which were covered with small hairs, the furs reflect the colour of the animal, with brownish colour from the base to the middle of the fur, while the upper fur appears light yellow to reddish. The combination of these colours gave the animal brownish colour as shown in Plate I.

4.2.1 Gross morphological studies of the spinal cord

The spinal cord of the Grasscutter was observed to be elongated, whitish cylindrical part of the central nervous system, occupying the vertebral canal (Plate IV and Plate V). The spinal cord extends from the first cervical vertebra (C_1) tothe second sacral vertebra (S_2). The result of the study showed that the spinal cord is made up of spinal cord segments as shown in PlateIV.

The spinal cord was continuous cranially with the medulla oblongata, and narrowest caudally to the conus medullaris at the level of S_2 (PlateV) From the apex of conus medullaris, a connective tissue filament, the filum terminale, was observed to descend and was attached to the first coccygeal vertebral segment (PlateIV). The result showed abundle of spinal nerves extending inferiorly from lumbosacral enlargementand conus medullarisurrounding the filum terminaleto form cauda equine (PlateV).

The result showed the spinal cord of the Grasscutter has two enlargements which were identified as cervical and lumbosacral enlargements (PlateV). Cervical enlargementwas shown to innervate the fore limbs and begins at the junction between the second cervical

(C_2) and the third cervical (C_3) vertebral discs and ended between the seventh cervical (C_7) and the first thoracic (T_1) vertebrae while the result showed that lumbosacral enlargement which innervates the hind limbs, begins at the junction of the first lumbar (L_1) and second lumbar (L_2) and ended at the level of S_2 vertebrae as shown in Plates IV,V and VI.

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Plate III: Physical features of Grasscutter showing the head (H) broad with short flattened muzzle and with small eyes and circular ears,fore limbs (FL), hindlimbs (HL) and a short tail (T).



Plate IV: The Grasscutterspinal cord (SC) exposed by splitting of vertebral bones in its anatomical position

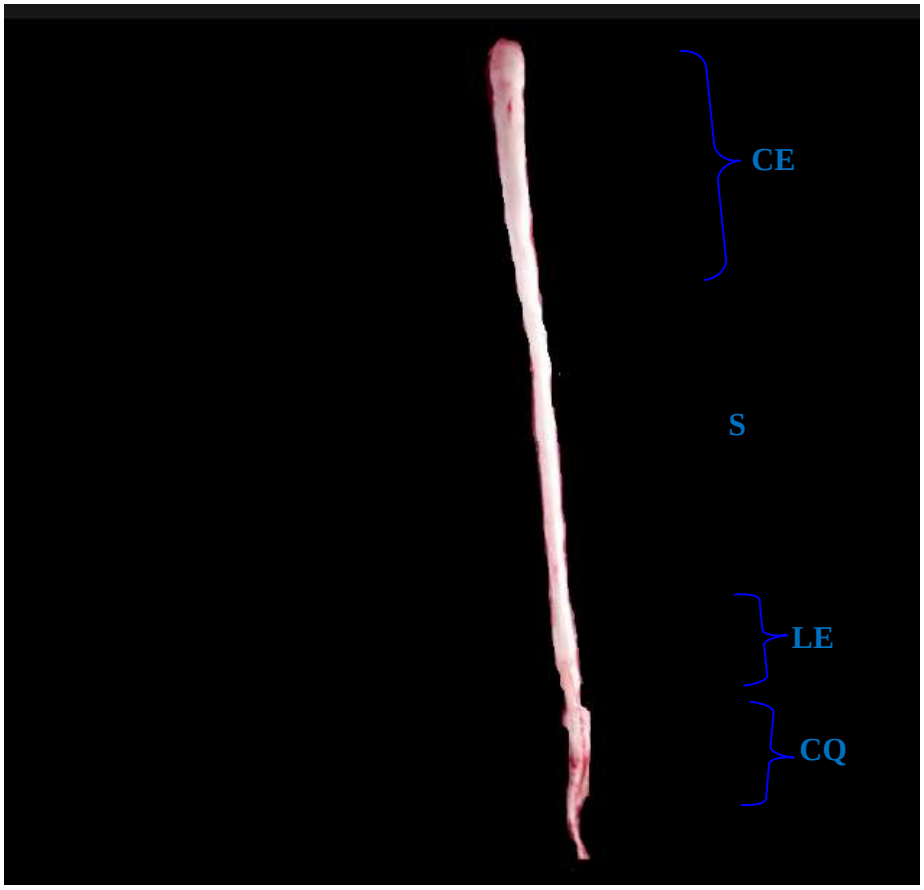


Plate V: The Grasscutter spinal cord (S) showing cervical enlargements (CE), lumbosacral enlargement (LE) and caudal equina (CQ) .



Plate VI: The Grasscutter spinal cord (S) with the length of the spinal cord.

4.2.2 Morphometricstudies of the spinal cord

The mean bodyweight of the twelve Grasscutters was observed to be 4200 ± 4.50g. The mean body weight of male and female Grasscutters were observed to be 4700.27 ±3.50g and 3633.40 ± 3.80g respectively. The mean body weight showed a significant difference between the male and the female Grasscutters whichshowed sexual dimorphism with the male body weight significantly higher than that of the female Grasscutter($P<0.05$).

The result showed that the mean length and the weight of spinal cord of male Grasscutter were observed to be 20.30 ± 1.50cm and 2.60 ± 0.02g respectively while the mean length and weight of spinal cord of female Grasscutter were observed to be 19.40 ± 1.04cm and 2.02 ± 0.01g respectively. The result in Table 4.1 showed that the mean values for the length and weight of spinal cord of both male and female Grasscutters do not show sexual dimorphism ($P<0.05$).The organ weight ratio of the spinal cord of the male and female Grasscutters were observed to be 0.20 and 0.15 respectively (Table 4.1).

The result in Table 4.2 using Pearson correlation analysis showed that there was a positive correlation between the body weights and spinal cord parameters in both sexes of the Grasscutters.

Table 4.1: Body and Spinal Cord measurements of the Grasscutter

Parameters	Male	Female
Weight of animal (g)	4700.27 ± 0.01*	3633.40 ± 0.80
Weight of spinal cord (g)	2.60 ± 0.02	3.02±0.01
Tail length (cm)	10.10 ± 0.56	11..20 ±0.98
Length of spinal cord (cm)	22.30 ± 0.02	24.40 ±1.04
Width of cervical segment of spinal cord (cm)	0.60 ± 0.02	0.71 ± 0.01*
Width of thoracic segment of spinal cord (cm)	0.30 ± 0.02	.35 ± 0.01*
Width of lumbar segment of spinal cord (cm	0.8 ± 0.01	0.92 ± 0.01*
Width of sacral segment of spinal cord (cm	0.60 ± 0.02	0.65 ± 0.02

Analysis of data was done using student's t-test between male and female Grasscutters. Data were expressed as mean ± standard error of mean (± SEM) of values collected, where * is significantly different (P<0.05) between the male and the female Grasscutter.

Table 4.2: Correlation between organ (Spinal cord) parameters and body weight ofGrasscutter

	Spinal cord Length cm	Spinal cord weight (g)
GC (Female) Body weight (g)	**0.98***	**0.89***
GC (Male) Body weight (g)	**0.99***	**0.85***

Pearson Correlation Analysis of data

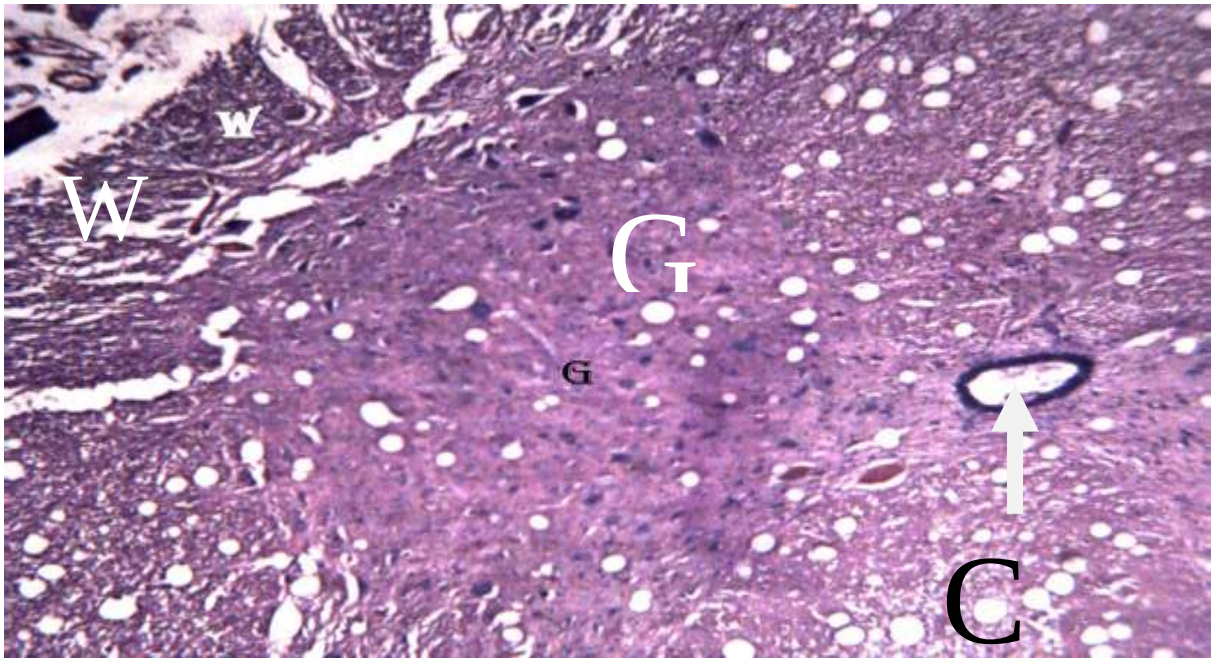
***=P<0.05 Grasscutter (GC) body weight correlates with the spinal cord length and spinal cord weight in both male and female Grass**

4.3 Histological studies

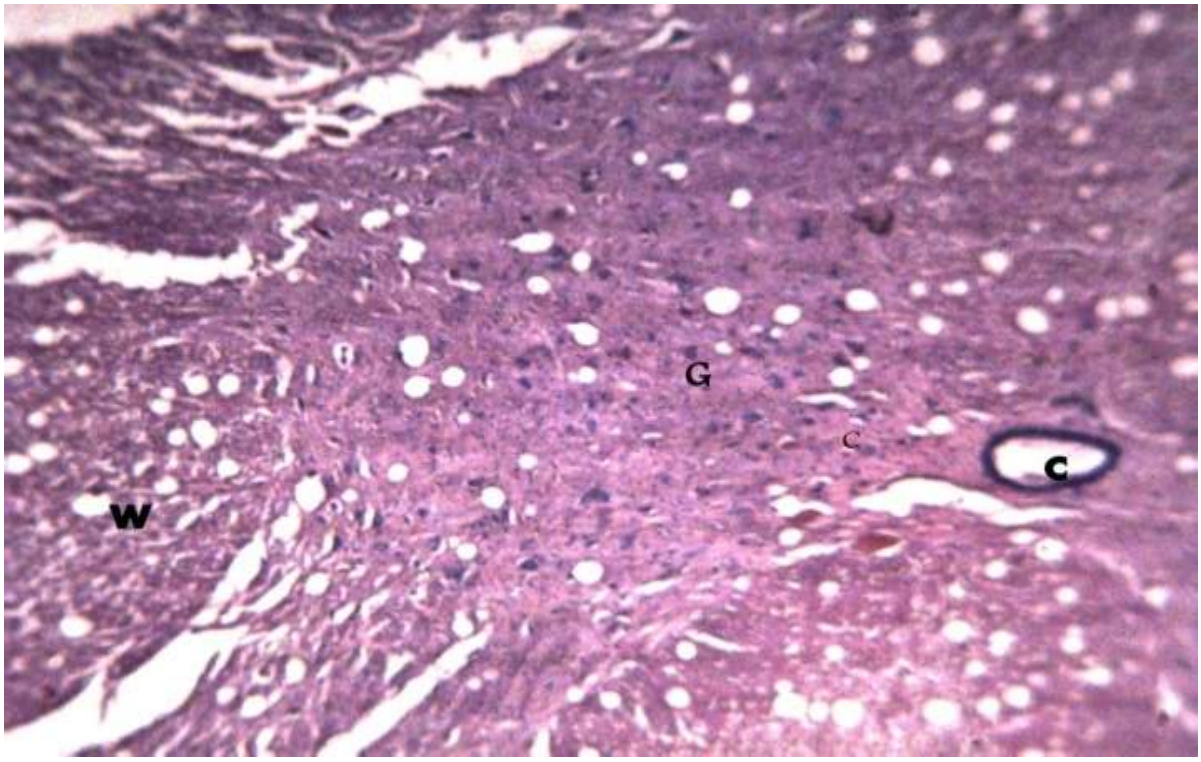
The result shows the hematoxylin and eosin stained sections of the transverse sections of the spinal cord of Grasscutterwere made up of outer white matter and an inner dark gray matter (Plate VII).

The cervical region of the spinal cord showed gray matter and white matterwith the central canal in the gray matter of the region (PlateVII,Plate VIII and Plate IX).

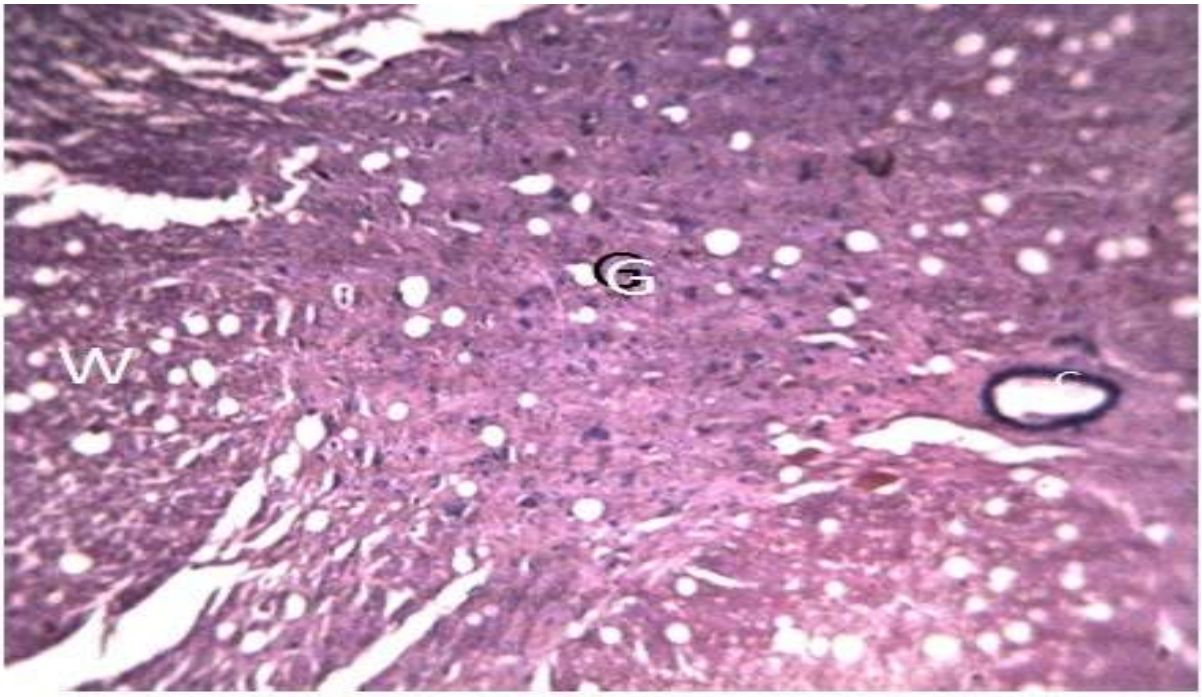
The transverse sections of thoracic spinal cord segment with inner dark gray matter were observed to be divided into ventraland dorsal horns as shown in Plates XI, XII and XIII.At sacral level, the gray matter and white matter were present and the central canal was observed in the the gray matter of the sacral region (Plate XVIII).



PlateVII: A transverse section of the cervical spinal cord of Grasscutter at the level of C_2, with gray matter (G), central canal (C), white matter (W) H&E stain, X50



PlateVIII: A transverse section of the cervical spinal cord of Grasscutter at the level ofC_3. with gray matter (G) and white matter(W) of the spinal cord and central canal (C)of spinal cord. H&E stain (Mag X50).



PlateIX: A transverse section of the cervical spinal cord of Grasscutter at the level of $C_4$with the gray (G) and white matter (W) of the spinal cord the central canal (C). H&E stain (Mag X50).

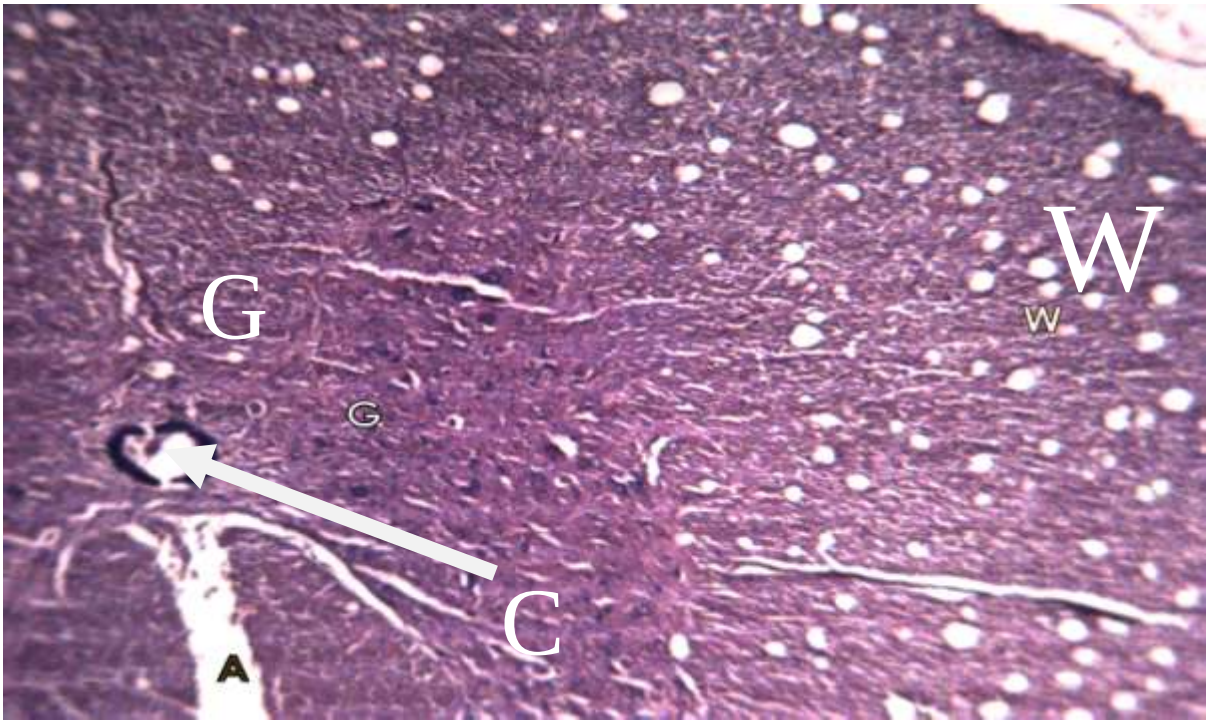
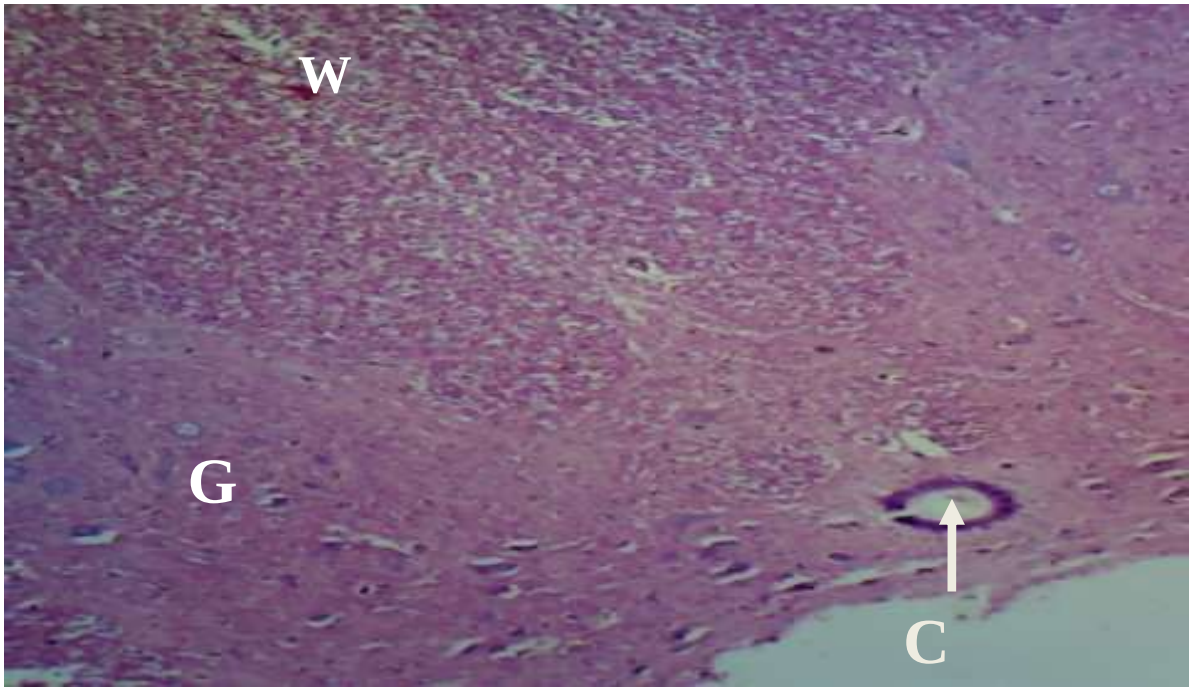
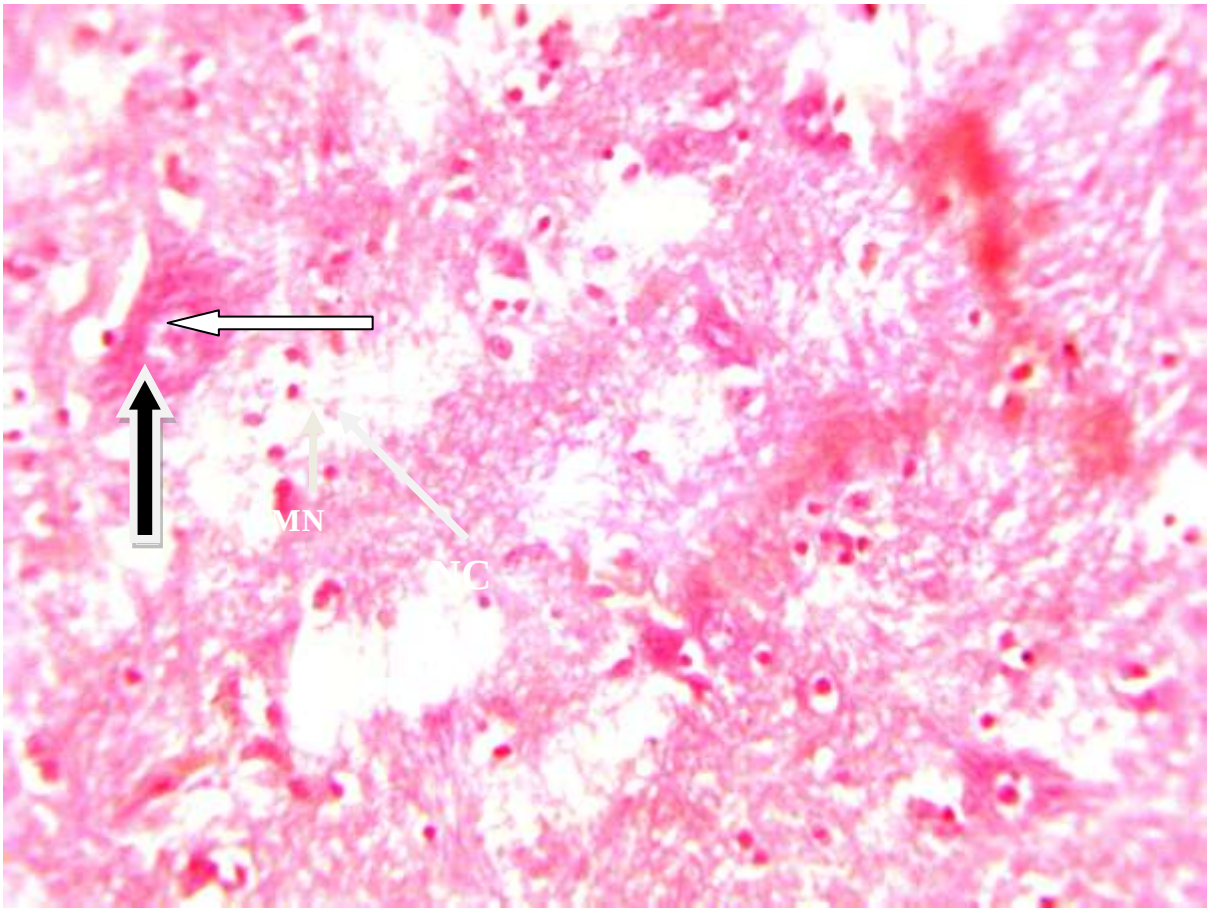


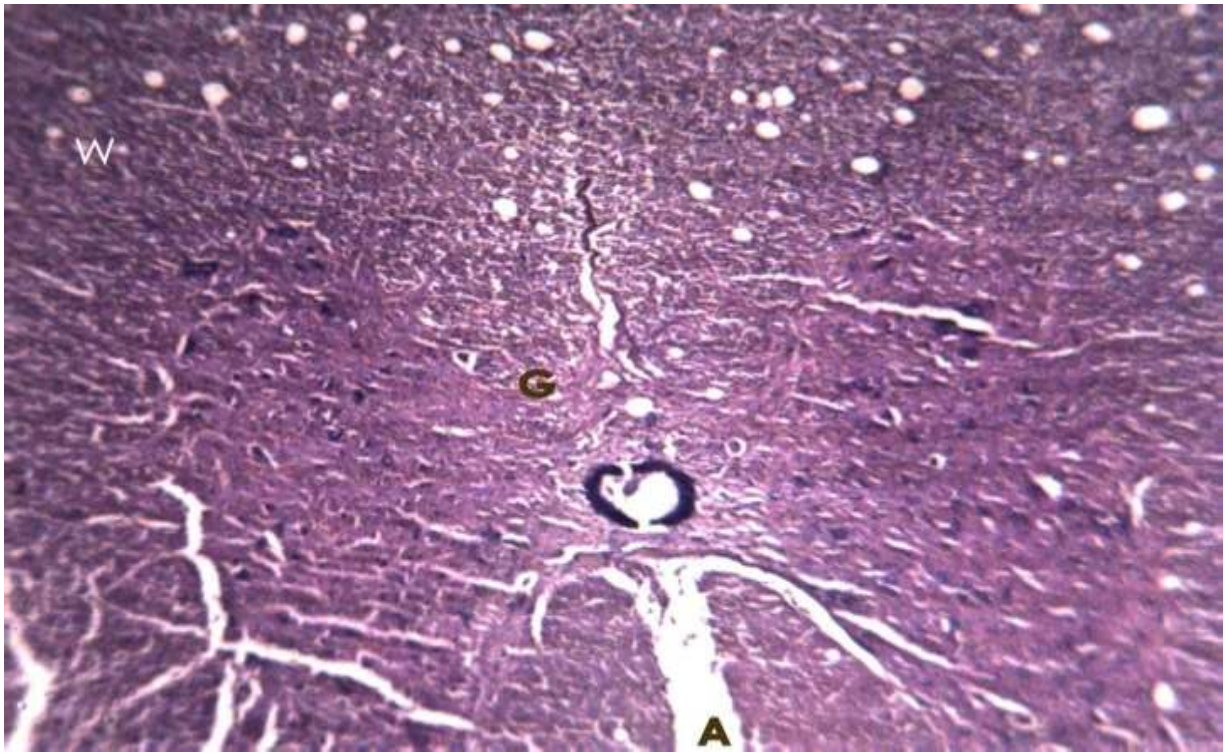
Plate X: A transverse section of the thoracic spinal cord of Grasscutter at the level of$T_1$with the gray matter (G) and white matter (W), the central canal of the spinal cord (C) and Anterior median fissure (A). H&E stain (Mag X100).



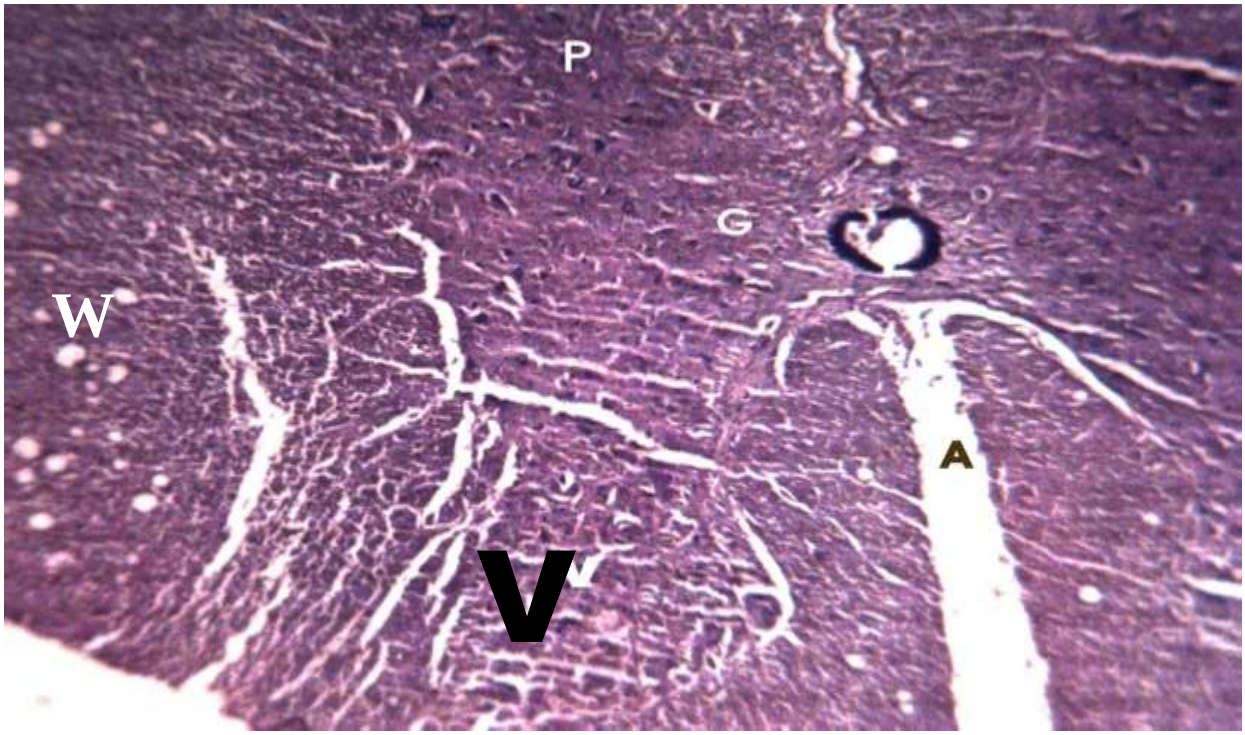
PlateXI: A transverse section of the thoracic spinal cord of Grasscutter at the level of T_4, with the white matter (W) gray matter (G) and central canal (C). H&E stain (Mag X50).



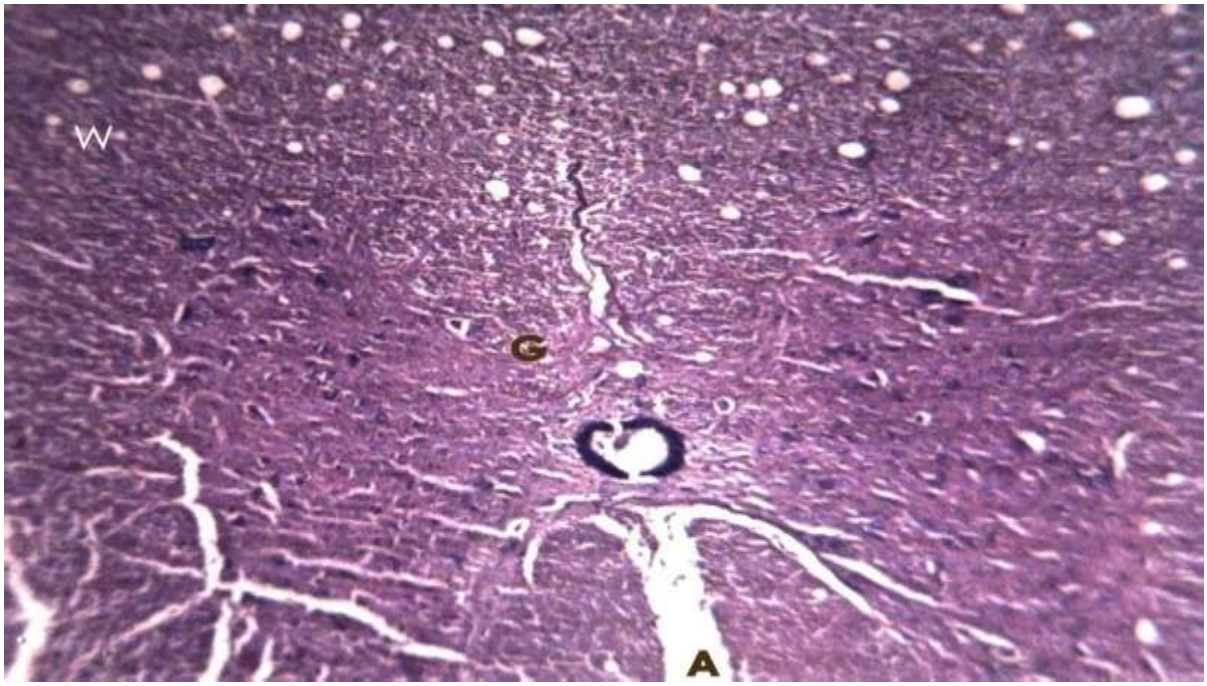
PlateXII: A transverse section of the thoracic spinal cord of Grasscutter at the level of $T_4$with ventral horn (Anterior horn). Mutipolar motor neurons (black arrow) in the ventral horn of the spinal cord of Grasscutter with nucleous (white arrow). H&E stain (Mag X400).



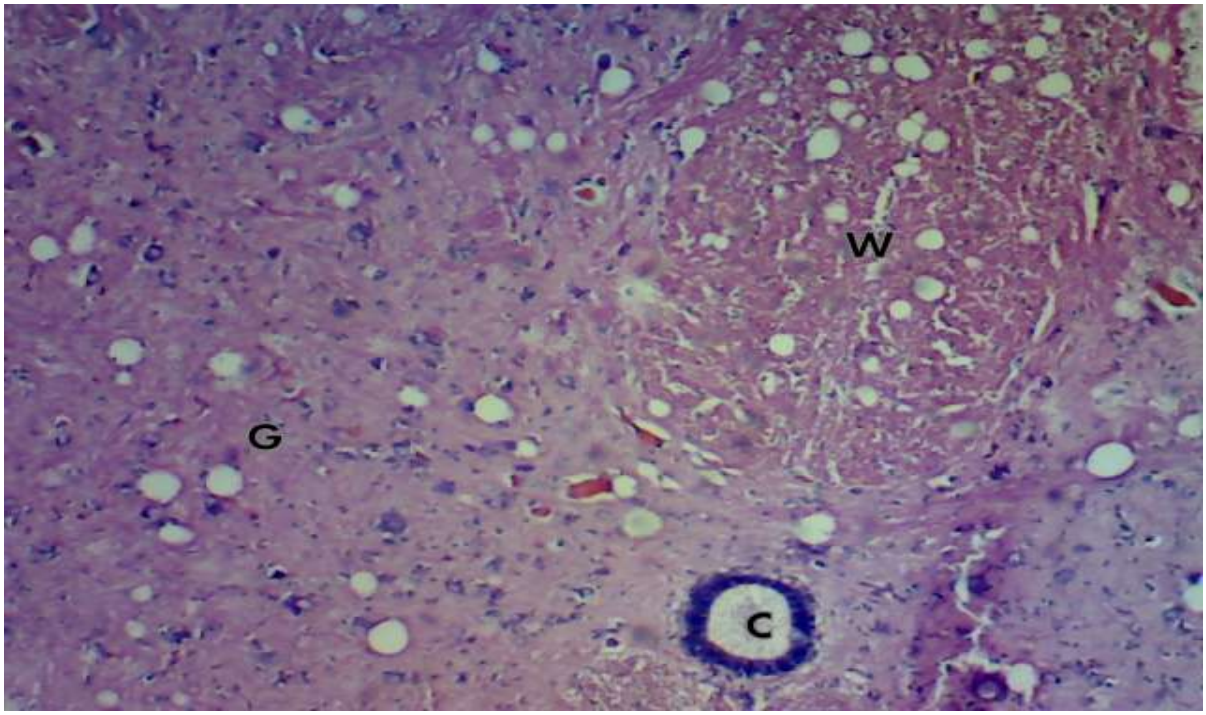
PlateXIII: A transverse section of the thoracic spinal cord of Grasscutter at the level ofT_1 with the gray matter (G) located deep to the white matter (W) the central canal (C), Anterior median fissure (A). H&E stain (Mag X50).



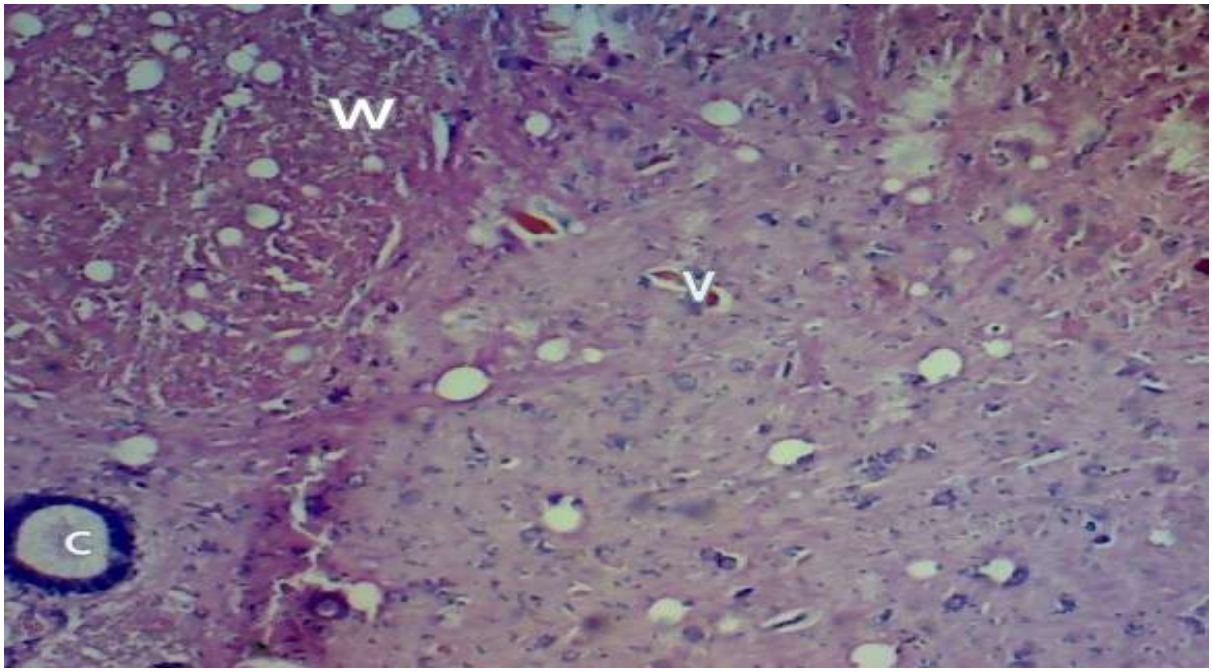
PlateXIV: A transverse section of the thoracic spinal cord of Grasscutter at the level of $T_2$with the gray matter (G) white matter (W) the central canal (C) has different sharp from the cervical spinal cord region, Anterior horn (V), Posterior horn (P) and Anterior median fissure (A). H&E stain (Mag X50).



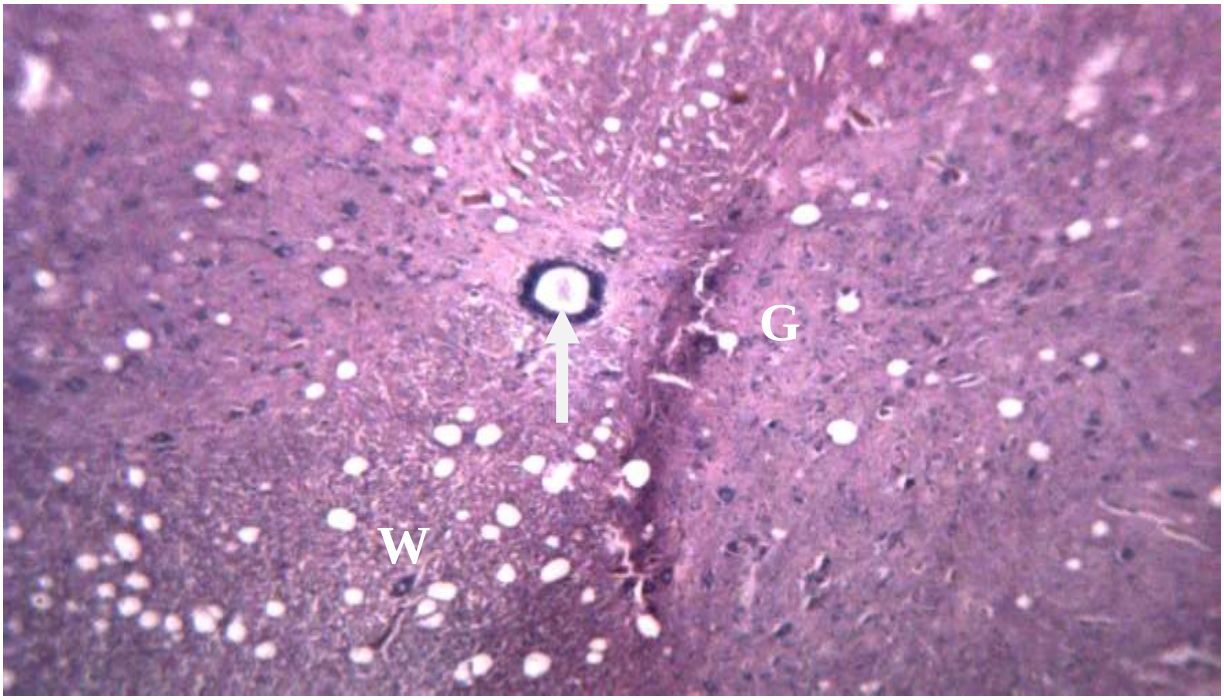
PlateXV: A transverse section of the lumbar spinal cord of Grasscutter at the level of $L_2$with the gray matter (G) located deep to the white matter (W) the central canal (C) and anterior median fissure (A). H&E stain (Mag X50).



PlateXVI: A transverse section of the sacral spinal cord of Grasscutter at the level of $S_1$with the gray matter (G) the white matter (W) the shape of central canal (C) which is round. H&E stain (Mag X100).



PlateXVII: A transverse section of the sacral spinal cord of Grasscutter at the level of $S_1$with the gray matter(G) the white matter(W). the central canal, (C), the anterior horn (V). H&E stain (Mag X100).



PlateXVIII: A transverse section of the sacral spinal cord of Grasscutter at the level of $S_1$with gray matter (G) the white matter (W) the arrow showing central canal. H&E stain (Mag X100).

4.4 Histochemical Studies

4.4.1 Golgi study of the spinal cord of Grasscutter

The result of Golgi method of silver impregnation of nerve cells and their processes showed homogenous black deposits of silver nitrate in transverse sections of spinal cord segments in Grasscutter were observed to be divided into white matter and gray matter. The gray matterwas divided into ventral and dorsal horns as shown in Plates XIX, XX, XXI, XXII, XXII, XXIV, and XXV.

Neurons in the spinal cord of the Grasscutter showed positive Golgi reaction in which different sizes and shapes of neurons were identified in the gray horn of the spinal cord.

The result revealed that the gray matter was divided into dorsal horn, ventral horn and an intermediate zone throughout the entire spinal cord length.

Different shapes of neuronal cells were seen with axons and dendrites (Plate XXVI, plate XXXII, plate XLII). In the posterior horn of the cervical spinal cord several small sized neurons axons were seen (PlateXXVII) and complex pattern of dendritic connections in the cervical enlargement as shown in PlateXXVI and Plate XXX. The posterior horn of the thoracic spinal cord of the Grasscutter was also observed to have small sized neuronsas shown in Plate XXXVI. In the posterior horn of the lumbar spinalcord, neurons were seen withelaborate dendritic connections especially in the enlarged area (PlateXLII and PlateXLIII). Neuronal soma was small and dendrites were many as seen in the cervical enlargement.Posterior horns of the sacral spinal segments showed few scattered cells with poor connections. The anterior gray horn of the spinal cord of Grasscutter was presented with features which were quite different from the posterior gray column. In the cervical spinal cord segments, the result showed several multipolar neurons. Neurons showed several conections with other neurons (Plate XXXI, PlateXLIII) while the dendrites of the neurons

showed extensive branching especially in the enlarged segments. Some large neurons were seen basically in the anterior horns of the spinal cord of the Grasscutter. These neurons are large and of various sizes and shapes. Some large neurons are like pyramidal cells of the cerebral cortex (PlateXLIII)

In the thoracic and lumbar regions, the anterior gray column of the spinal cord of the Grasscutter showed motor neurons of various shapes and large sizes which were poor connections while the lateral horns were shown to contain muiltipolar neurons. The enlarged segments contain neurons which are closely packed and showed a lot of connections while anterior horns of the sacral spinal cord segments showed few neurons with very poor cellular network compared with both cervical and lumbar segments (PlateXLVI).

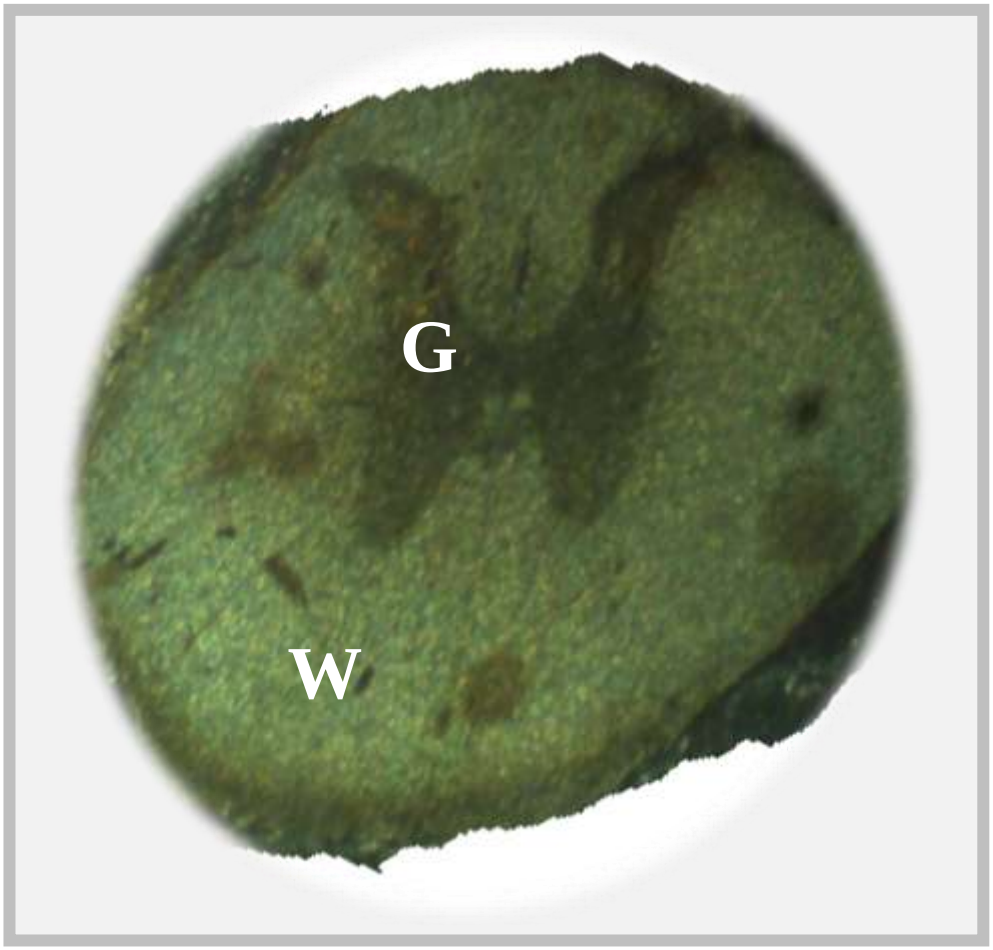
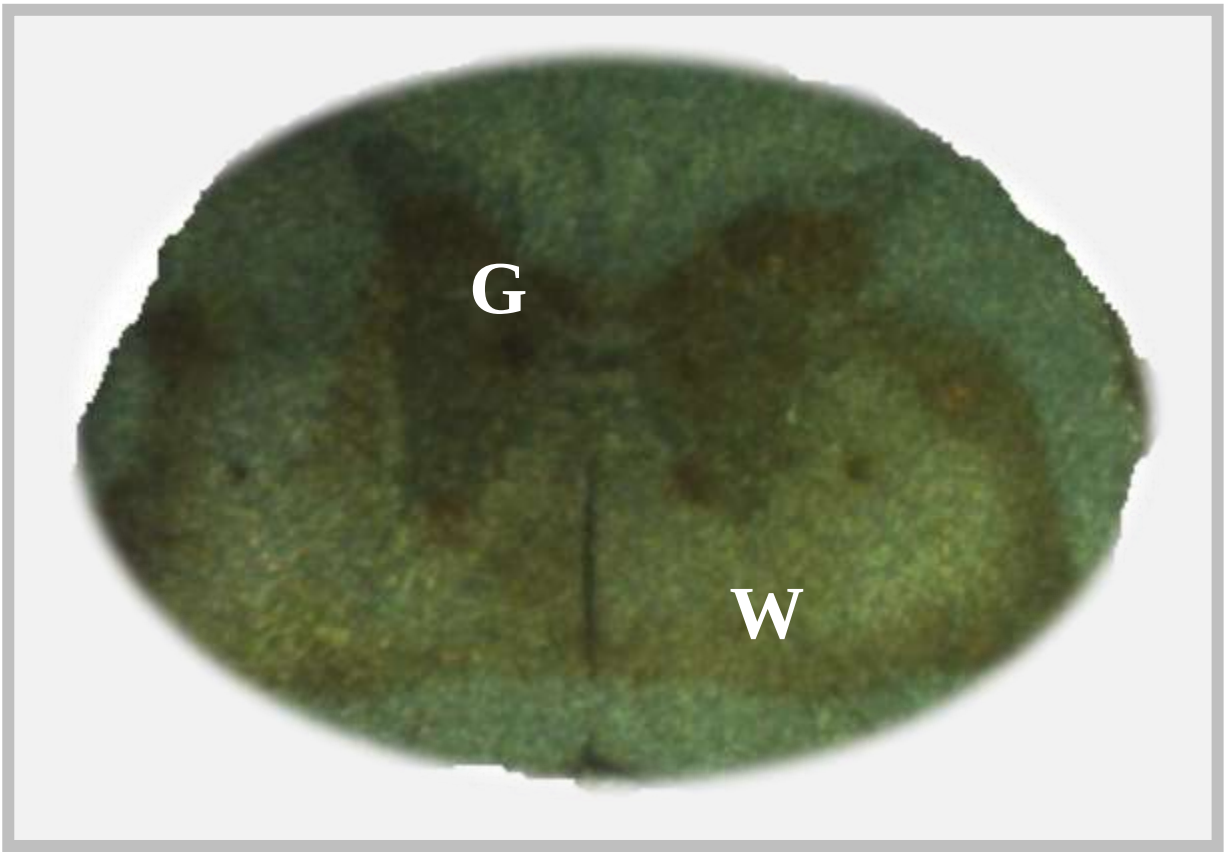
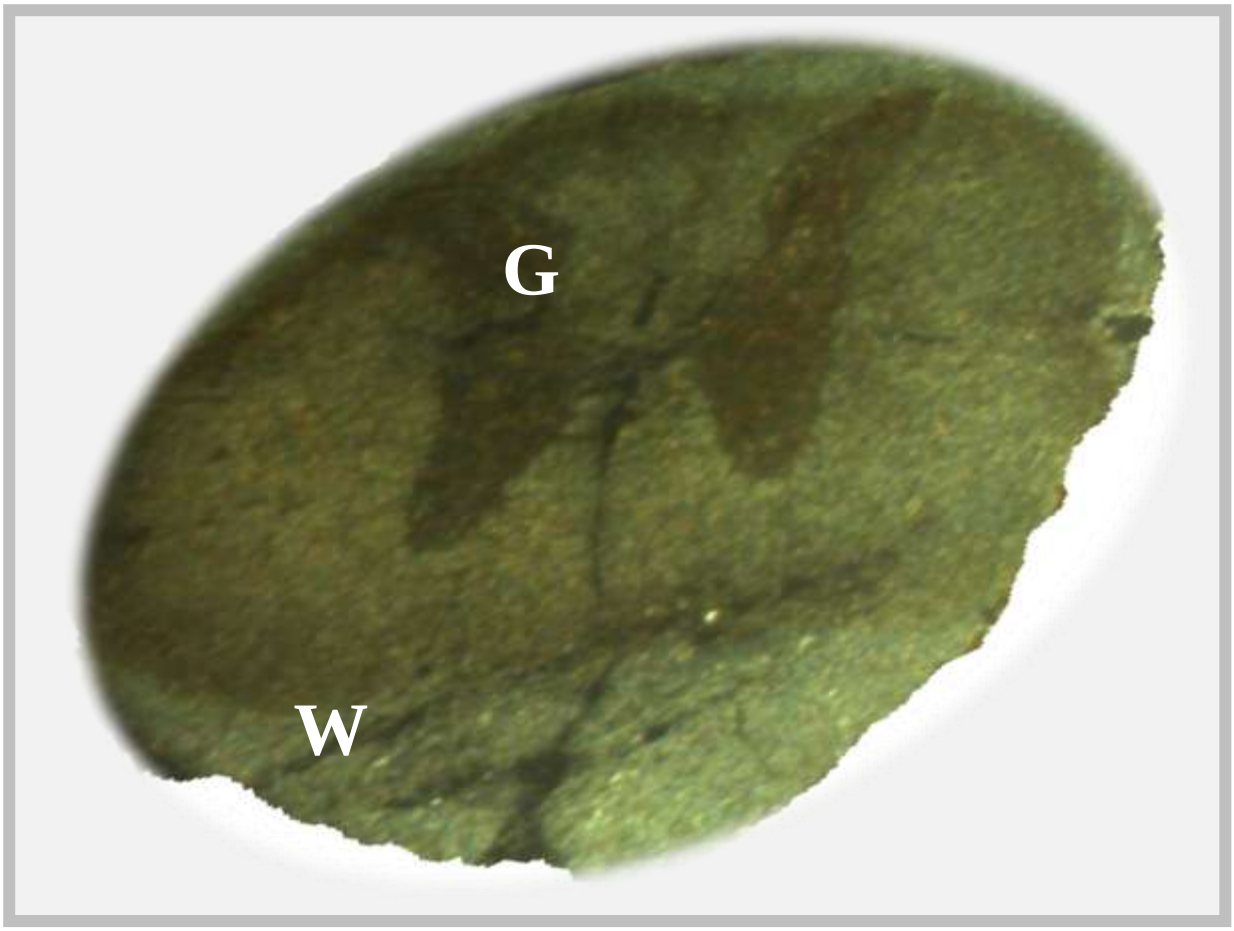


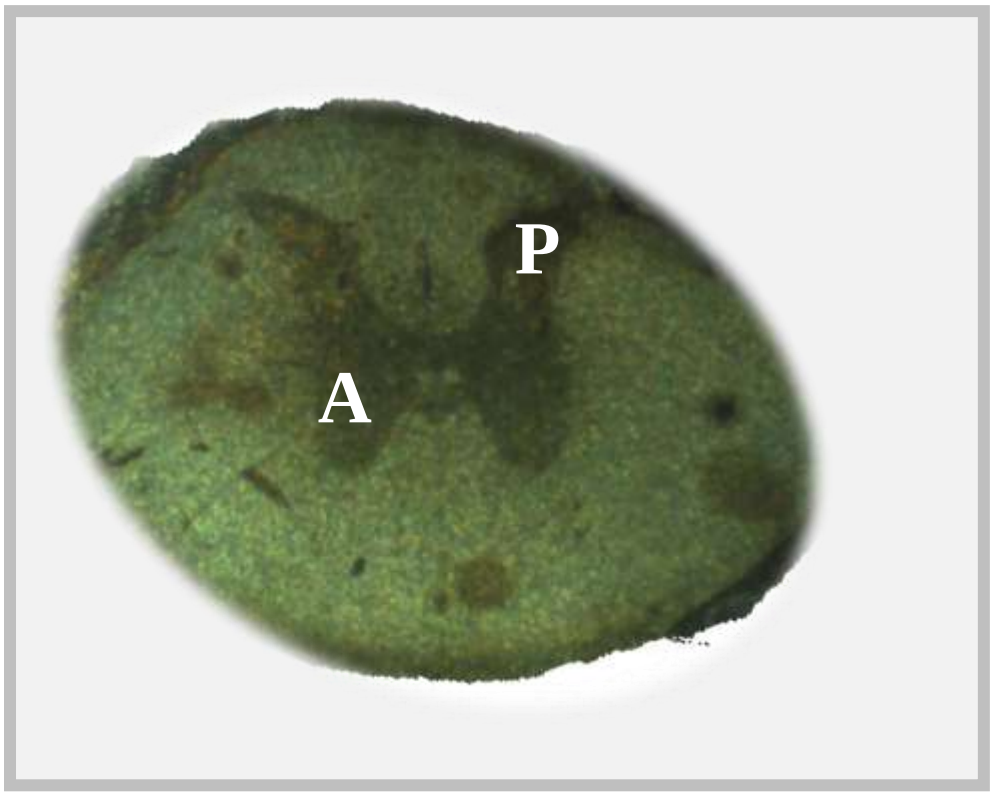
Plate XIX; A transverse section of the thoracic spinal cord of Grasscutter at the level of $T_{2.}$ withthe white matter (W) and the gray matter (G). Golgi stain (Mag X10).



PlateXX; A transverse section of the cervical spinal cord of Grasscutter at the level of $C_{4,}$ with white matter (W) and the gray matter (G). Golgi stain (Mag X10).



PlateXXI: A transverse section of the thoracic spinal cord of Grasscutter at the level of T_2 with white matter (W) and the gray matter (G). Golgi stain (Mag X10).



PlateXXII:A transverse section of the thoracic spinal cord of Grasscutter at the level of T_4showing gray matter with anterior horn (A) and posterior horn (P). Golgi stain (Mag X10).

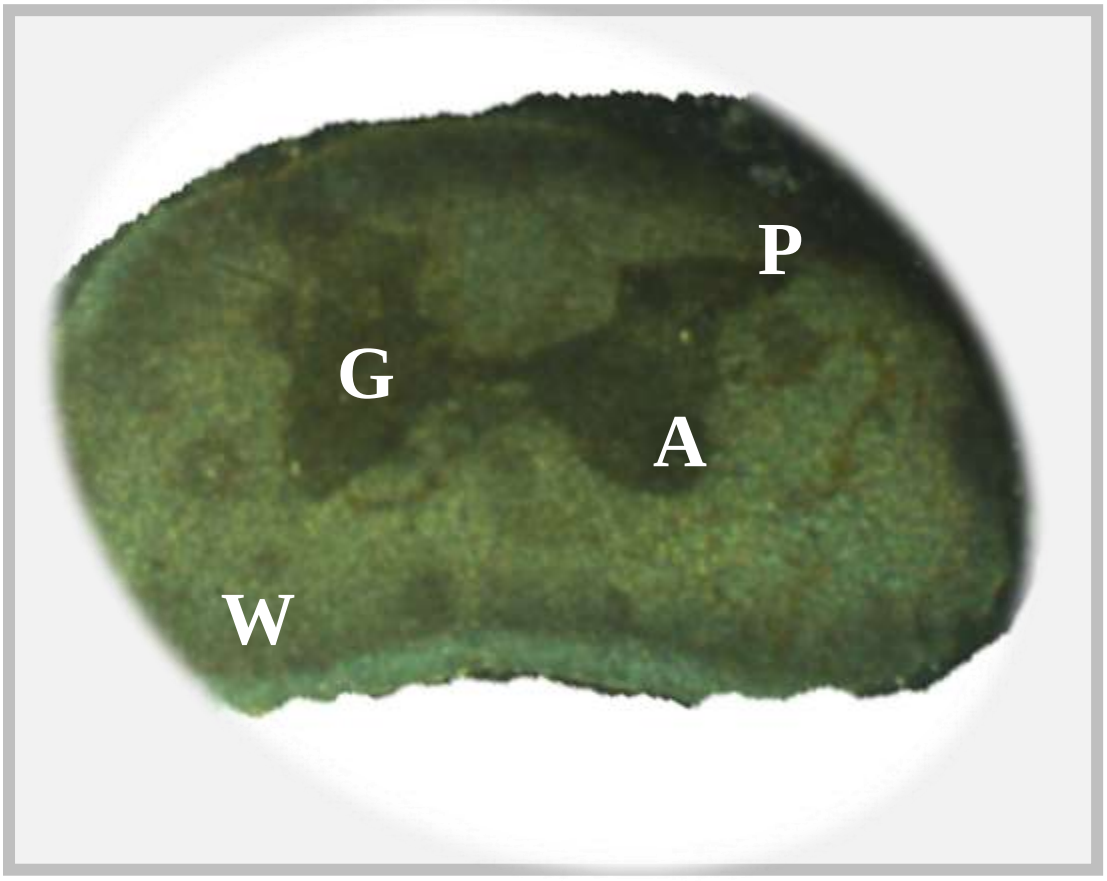
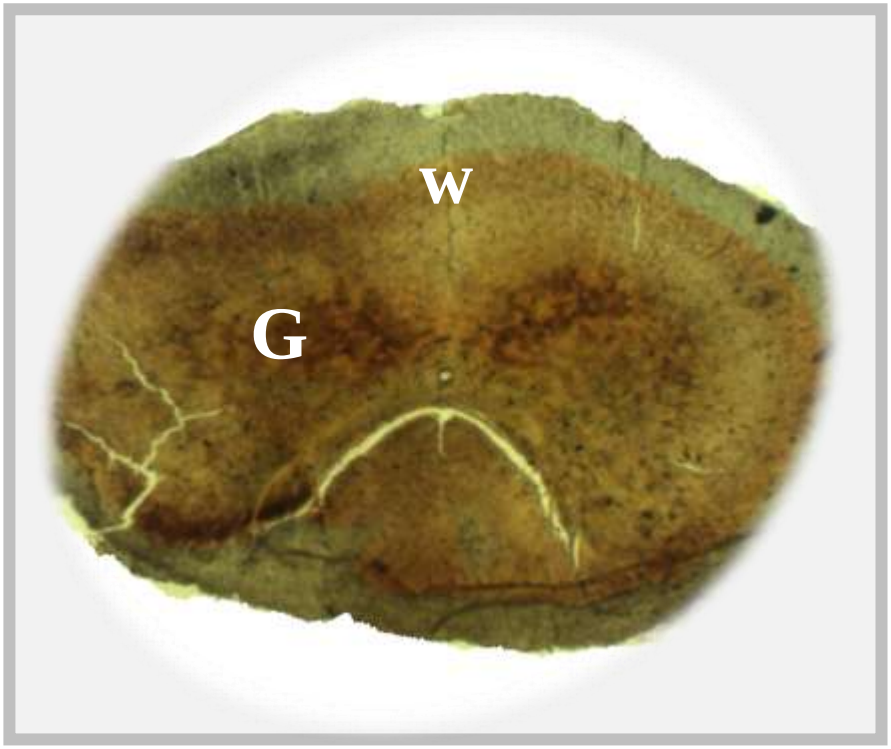
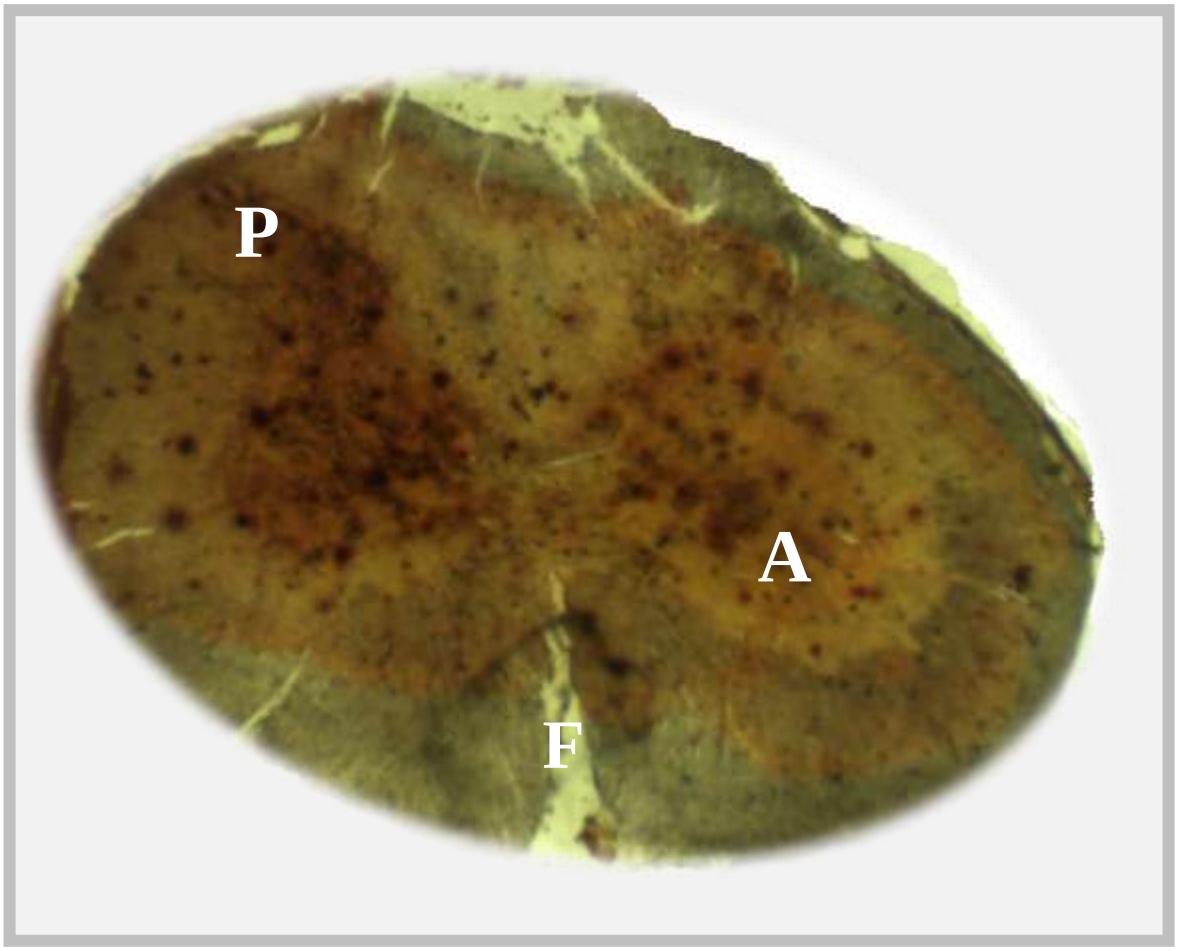


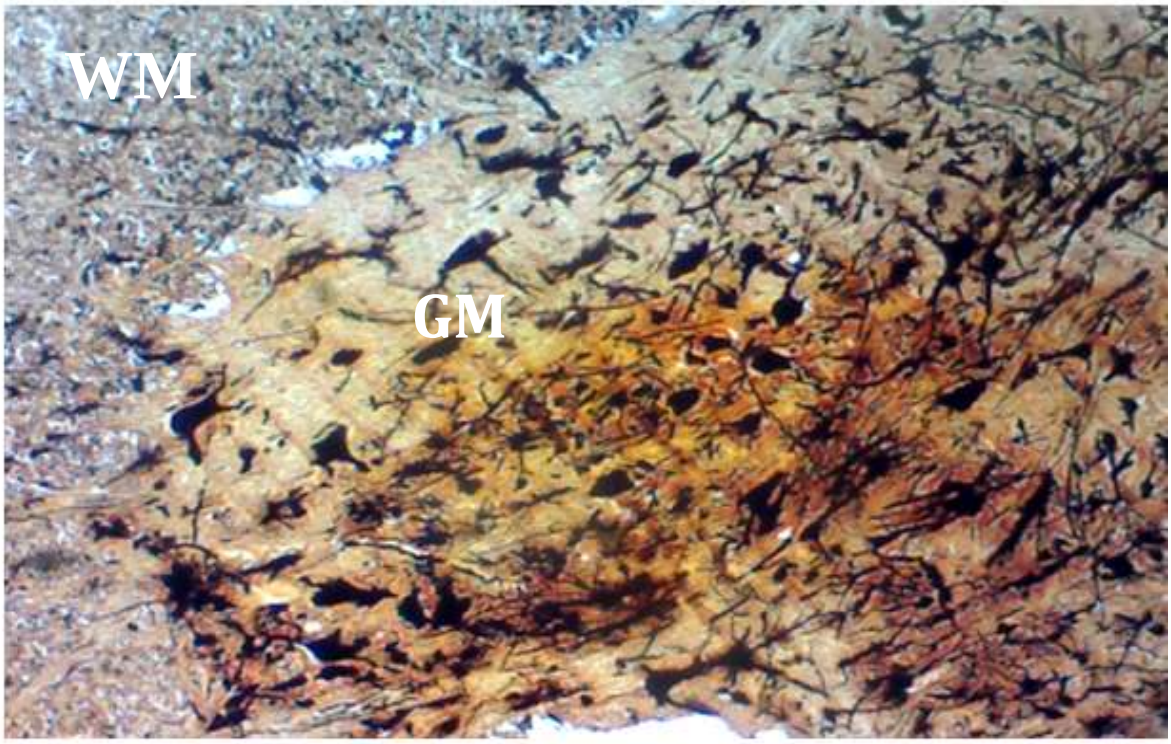
Plate XXIII:A transverse section of the lumbar spinal cord of Grasscutter at the level of $L_2$with white matter (W) and gray matter (G) with anterior horn (A) and posterior horn (P). Golgi stain (Mag X10).



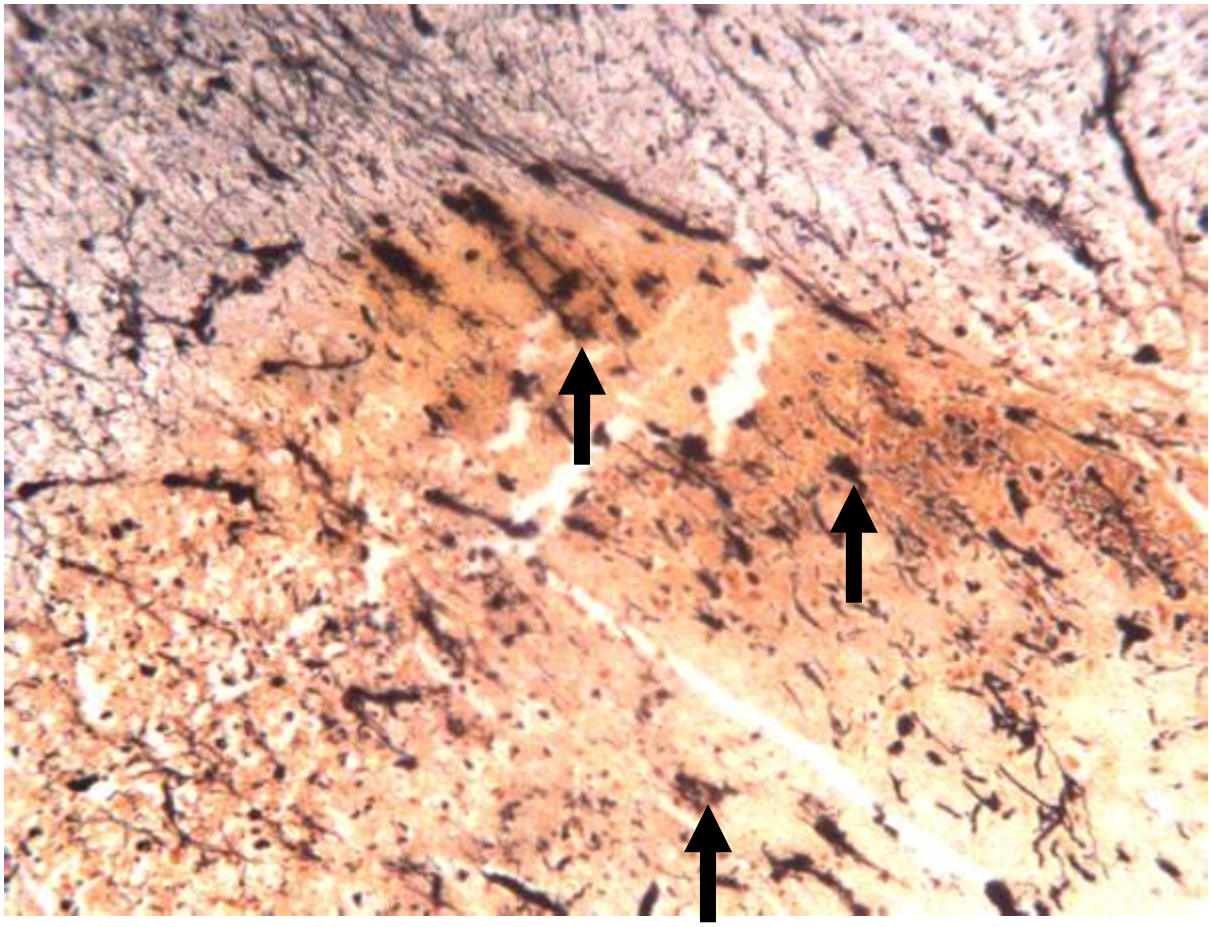
PlateXXIV:A transverse section of the lumbar spinal cord of Grasscutter at the level of L_4, with white matter (W) and gray matter (G). Golgi stain (Mag X10).



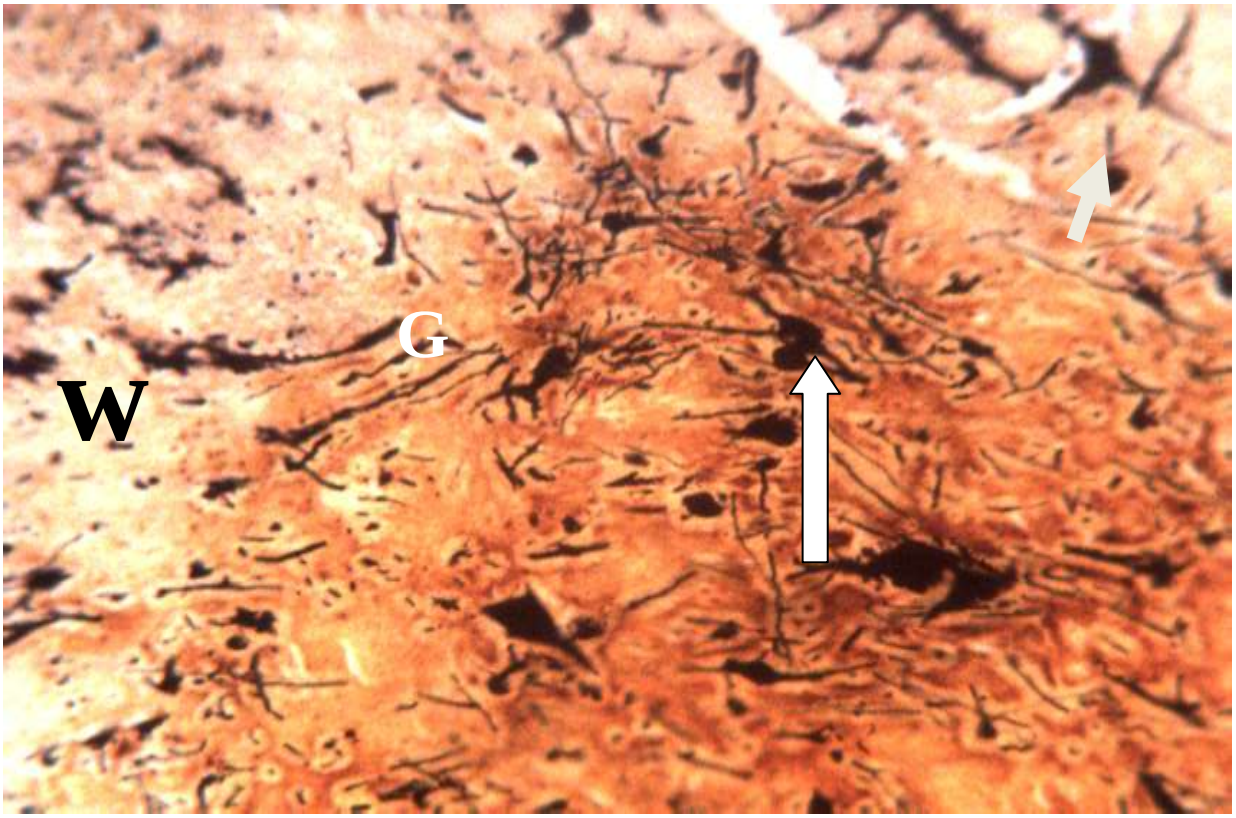
PlateXXV: A transverse section of the sacral spinal cord of the Grasscutter at the level of $S_1$with anterior horn (A) and posterior horn (P) of the gray matter, anterior median fissure (F).Golgi stain (Mag X10).



PlateXXVI: A Transverse section of the cervical spinal cord of the Grasscutter at the level of $C_2$with elaborate connections the dendrite of neurons in the gray matter (GM) of the spinal cord can be seen and the white matter is labelled WM. Golgi stain (Mag X100).



PlateXXVII: A Transverse section of the cervical spinal cord of Grasscutter C_2, with the presenceof numerous small neurons (black arrows) in the gray matterof the region. Golgi stain (Mag X100).



PlateXXVIII: A transverse section of the cervical spinal cord of Grasscutterat the level of C_2 showing posterior horn of the spinal cord with its two sections gray matter (G) and white natter (W).The arrows are with neurons in the gray matter of the spinal cord segment of Grasscutter. Golgi stain (Mag X250).

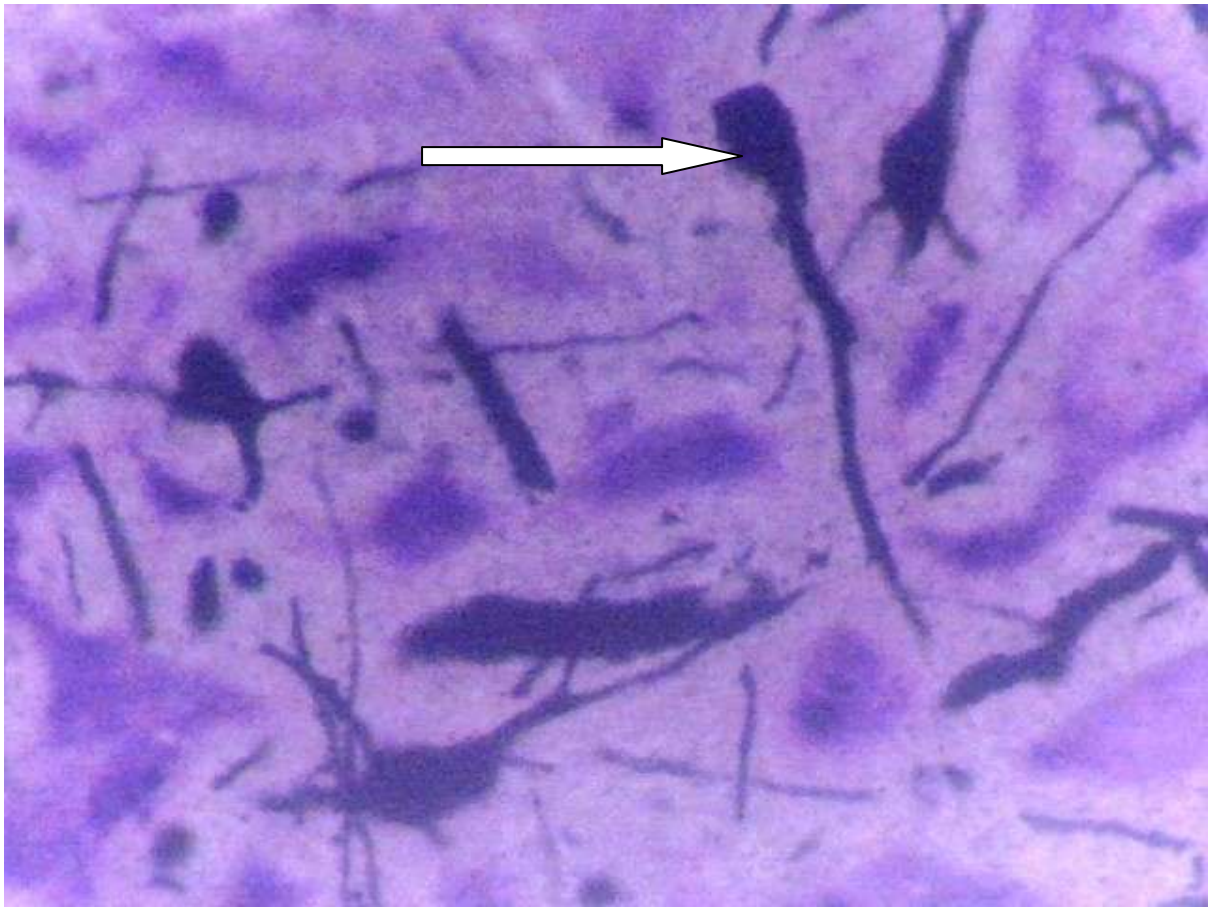


Plate XXIX: A transverse section of the cervical spinal cord of Grasscutter at the level of $C_2$with posterior horn of the spinal cord.The arrows are showing unipolar neuron with long axon in the gray matter of the spinal cord segment of Grasscutter Golgi stain (Mag X400).

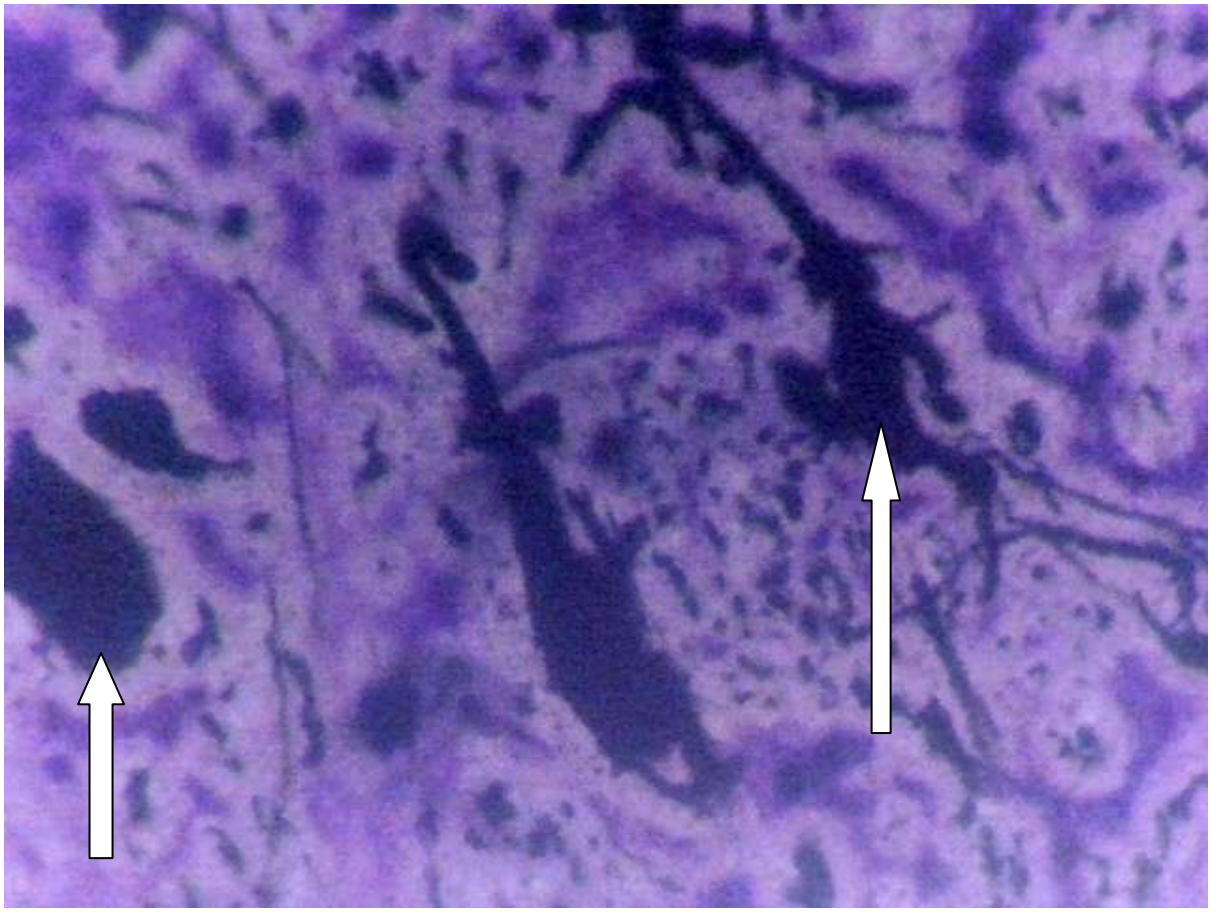


Plate XXX: A transverse section of the cervical spinal cord of Grasscutter at the level of C_2withanterior horn of the spinal cord. The arrows are showing large motor neurons in the gray matter of the spinal cord segment of Grasscutter. Golgi stain (Mag X400).

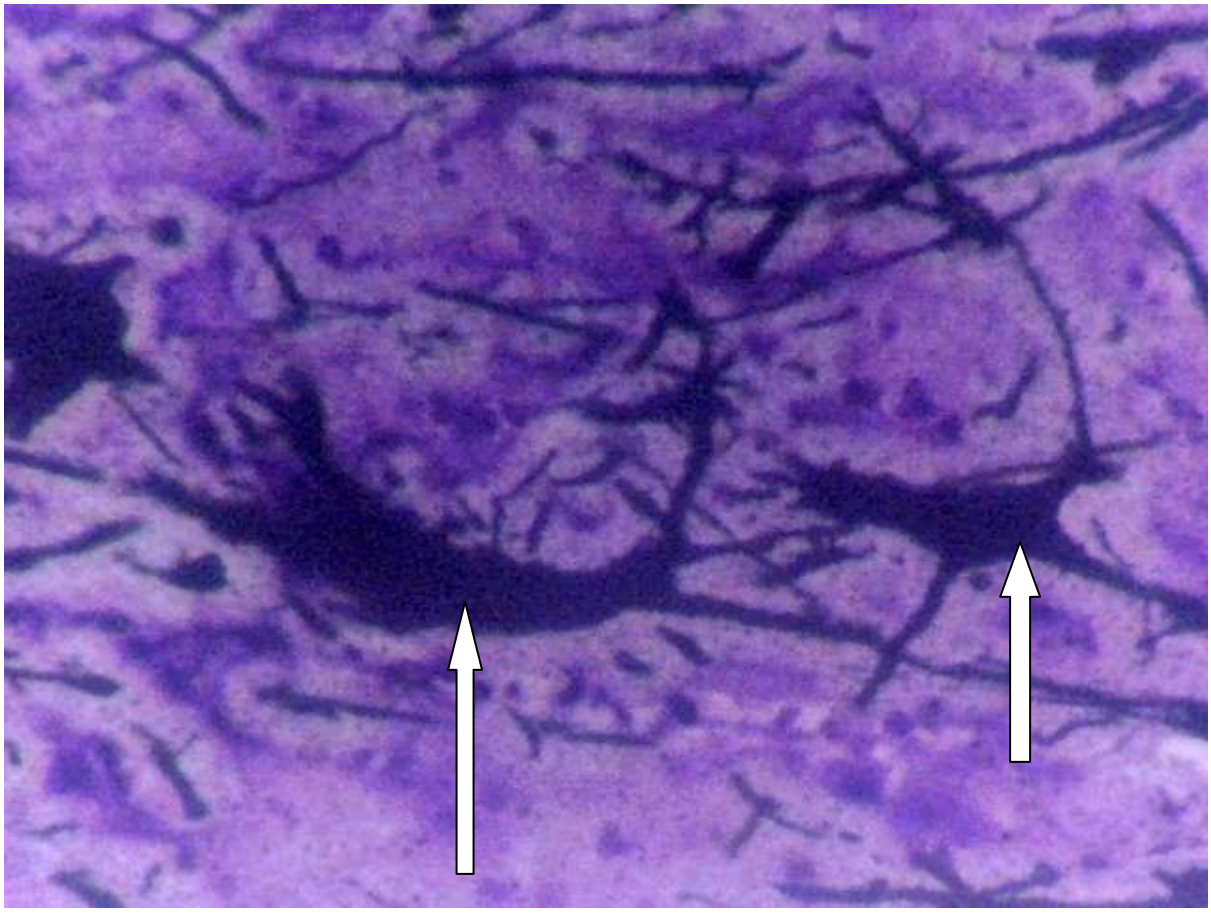


Plate XXXI: A transverse section of the cervical spinal cord of Grasscutter at the level of $C_4$with anterior horn of the spinal cord. The arrows are showing large motor neurons in the gray matter of the spinal cord segment of Grasscutter. Golgi stain (Mag X400).

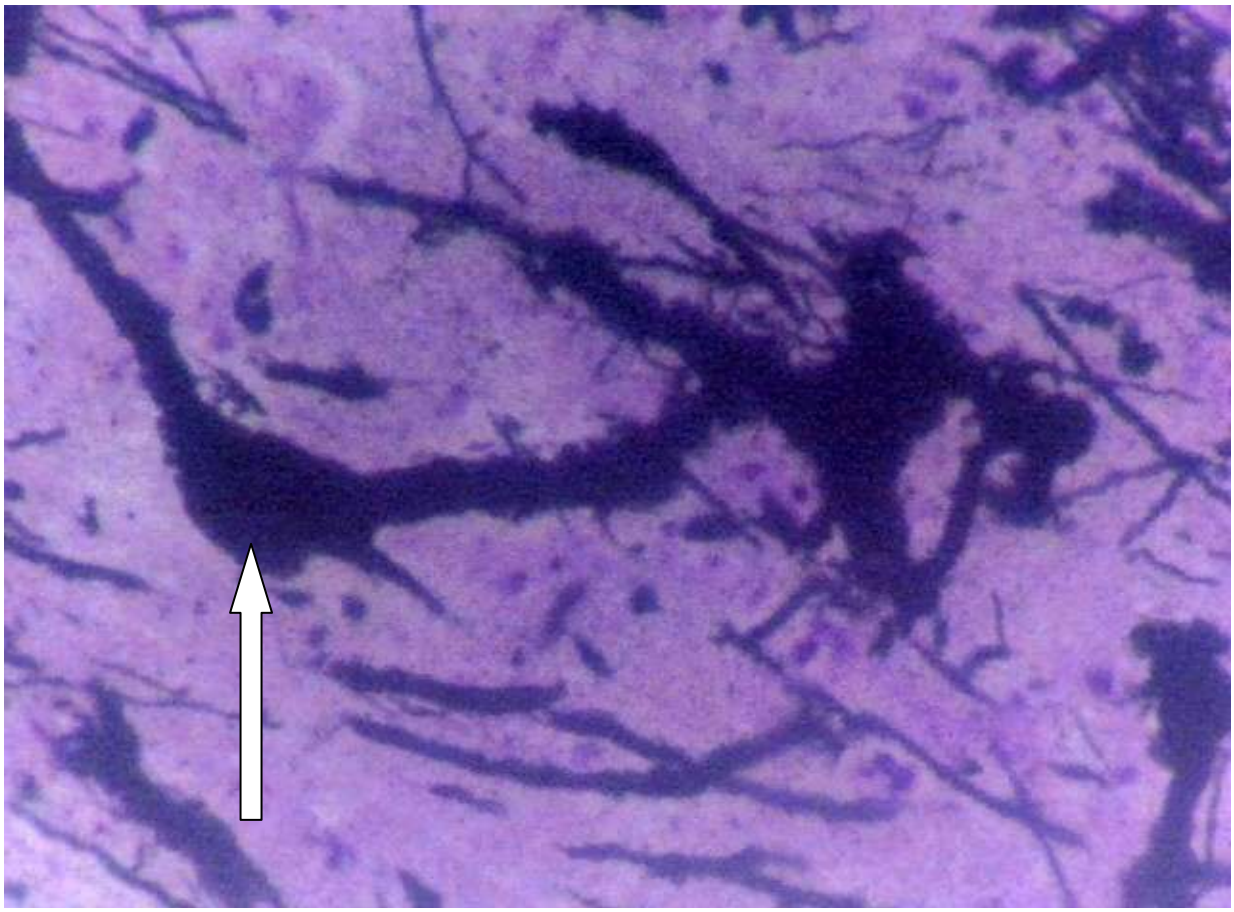


Plate XXXII:A transverse section of the cervical spinal cord of Grasscutter at the level of $C_4$with posterior horn of the spinal cord. The arrows are showing neuron with long axon in the gray matter of the spinal cord segment of Grasscutter. Golgi stain (Mag X400).

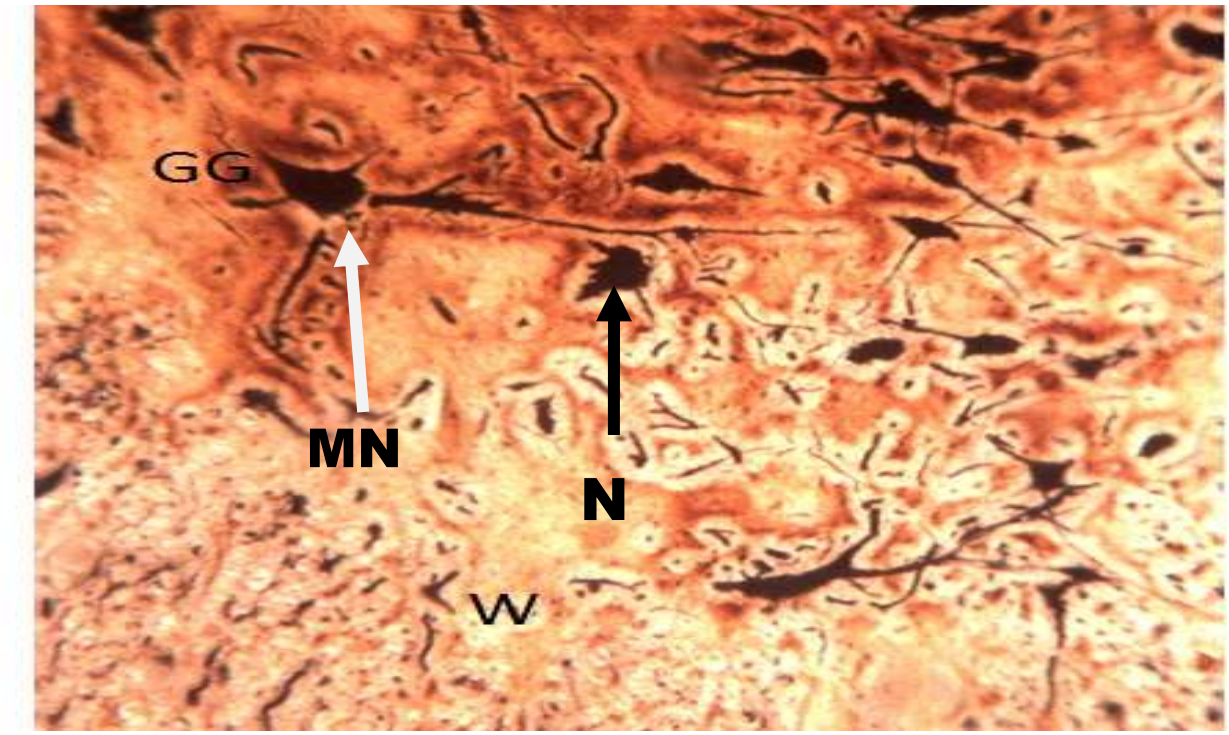
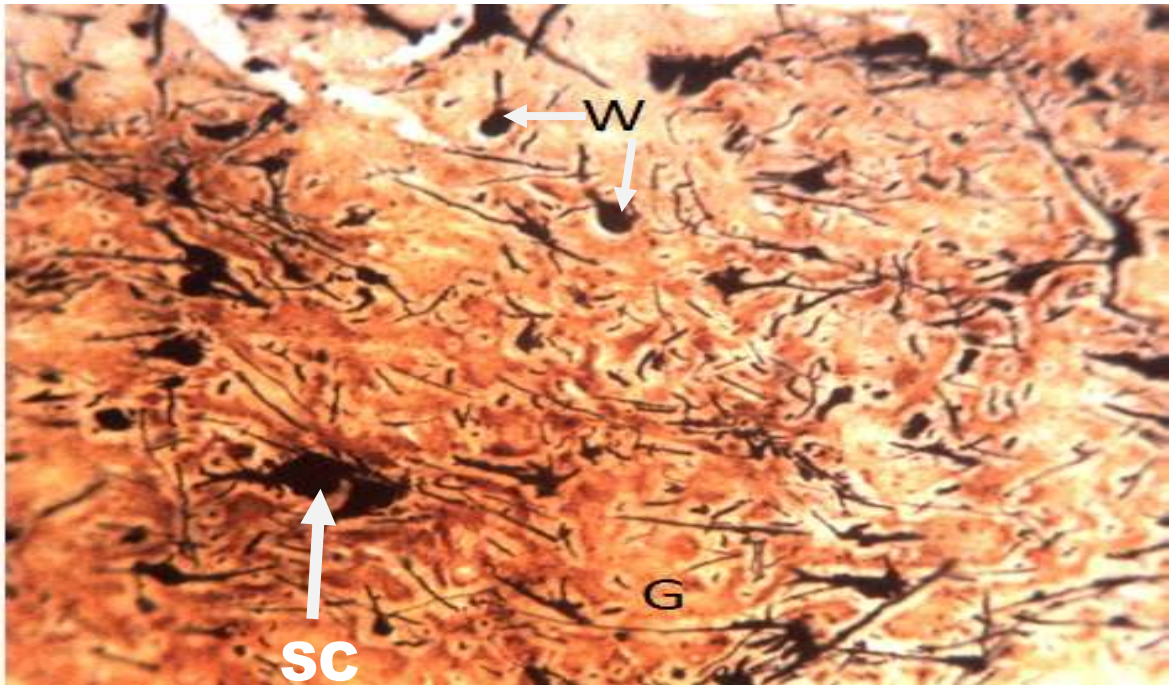


Plate XXXIII: A Transverse section of the cervical spinal cord of Grasscutter at the level of $C_2$with anterior horn gray matter (G) and white matter (W).Ramificationof the dendrite of large motor mutipolar neurons (MN), large neuron (N), of various shapes and sizes were present in the gray matter. Golgi stain (Mag X250).



PlateXXXIV:A Transverse section ofthe cervical spinal cord of Grasscutter at the level of $C_4$with Posterior horn of the gray matter (G). The elaborate aborization of the dendrites of neurons in the gray matter of the posterior horn can be seen. Neurons of various shapes and sizes can be seen including Stellate cells (SC), unipolar beurons, (W). Golgi stain (Mag X250).

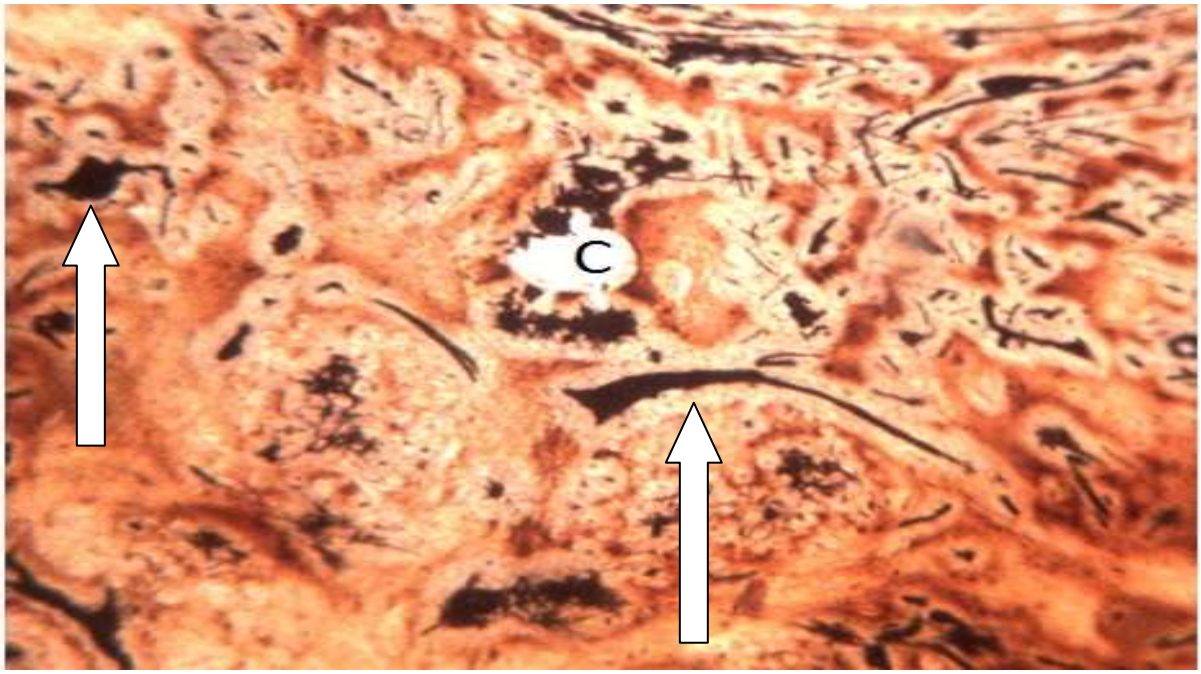


Plate XXXV: A Transverse section of the cervical spinal cord of Grasscutter at the level of C_4 withGray commissure containing central canal (C) and the arrows are showing neurons. Golgi stain (Mag X50).

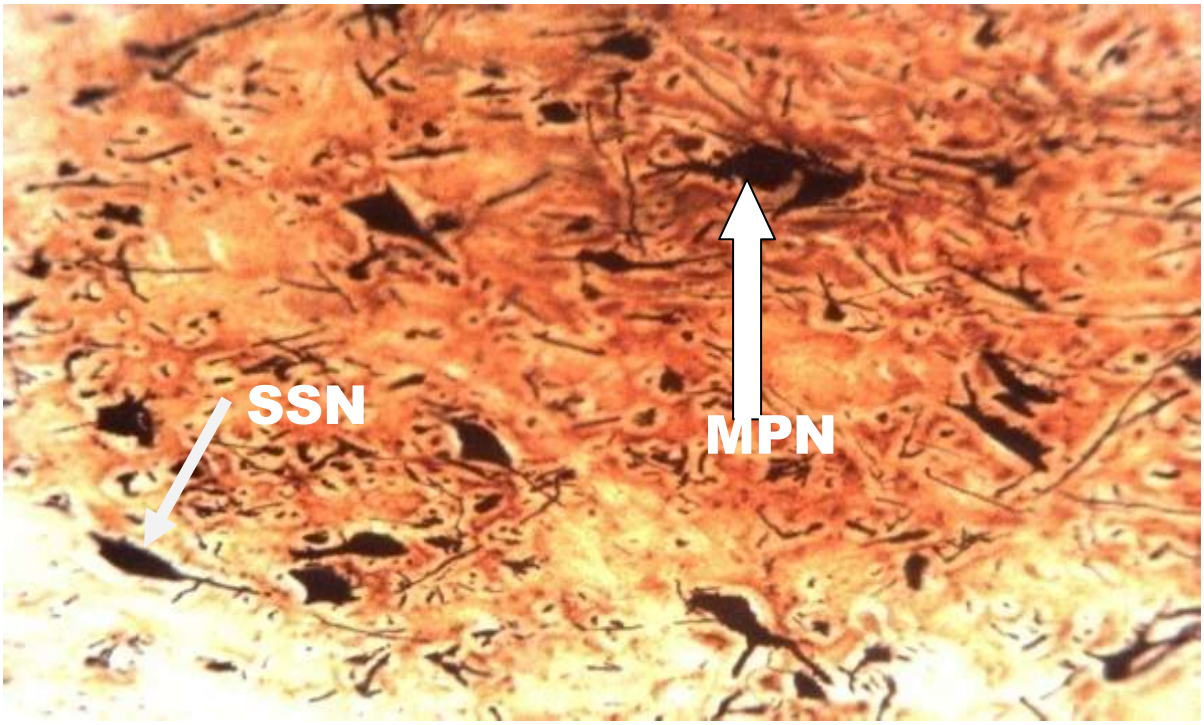
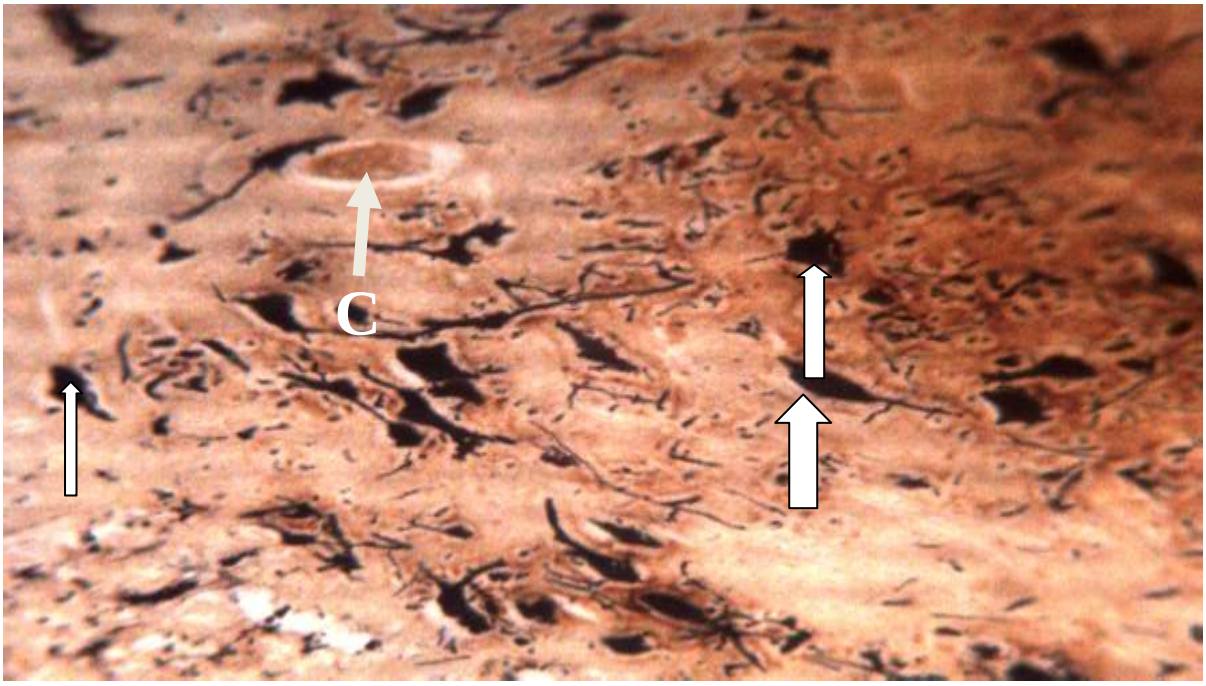
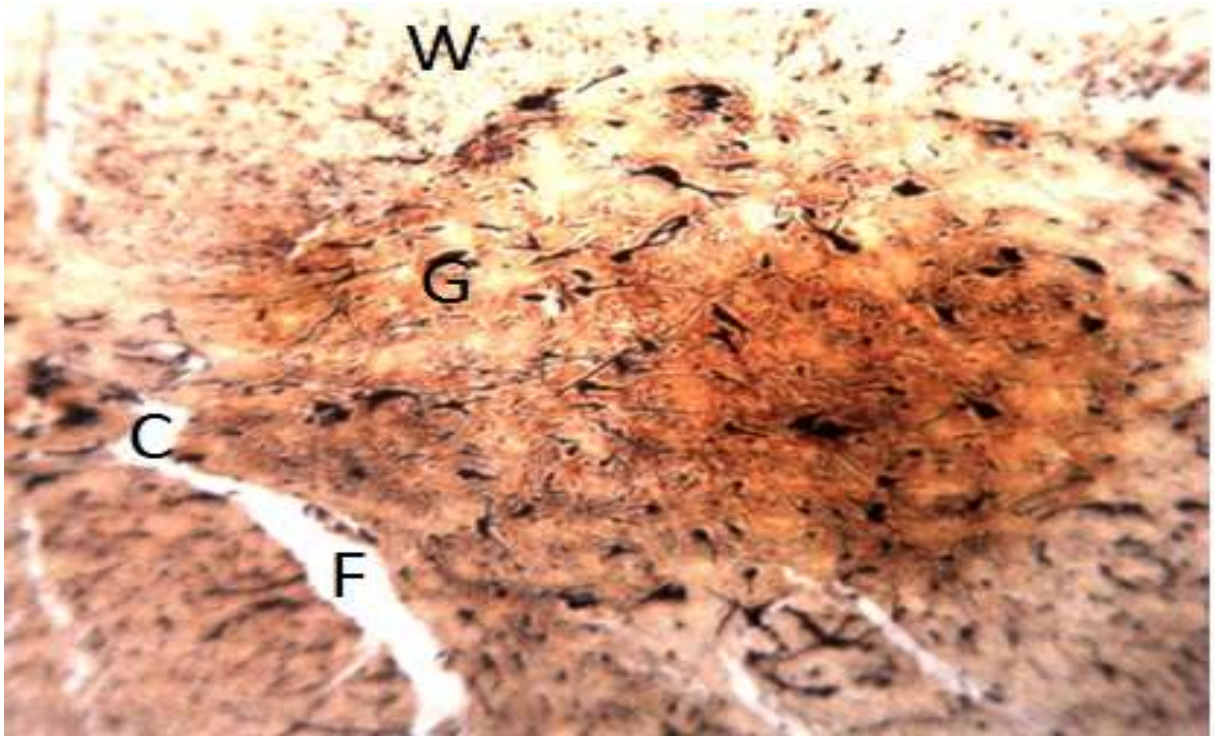


Plate XXXVI: A Transverse section of the histomorphology of the cervical spinal cord of Grasscutter at the level of $C_4$with anterior horn with the presence of large multipolar neurons (MPN). Large networks of neurons of various shapes, Spindle neuron (SSN),can also be seen in the anterior horn. Golgi stain (Mag X250).



PlateXXXVII: A transverse section ofthe cervical spinal cord of Grasscutter at the level of $C_4$with gray commisure. Arrows with the presence of different shaped neurons Spindle in the gray matter of the spinal cord segment the central canal (C) of the spinal cord of Grasscutter can be seen. Golgi stain (Mag X250).



PlateXXXVIII: A Section of the cervical spinal cord of Grasscutter at the level of $C_7$with Gray matter (G), white matter (W), Gray matter (G) has lateral horn, Anterior horn, and posterior horn. Anterior median fissure (F) and central canal (C). Golgi stain (Mag X50).

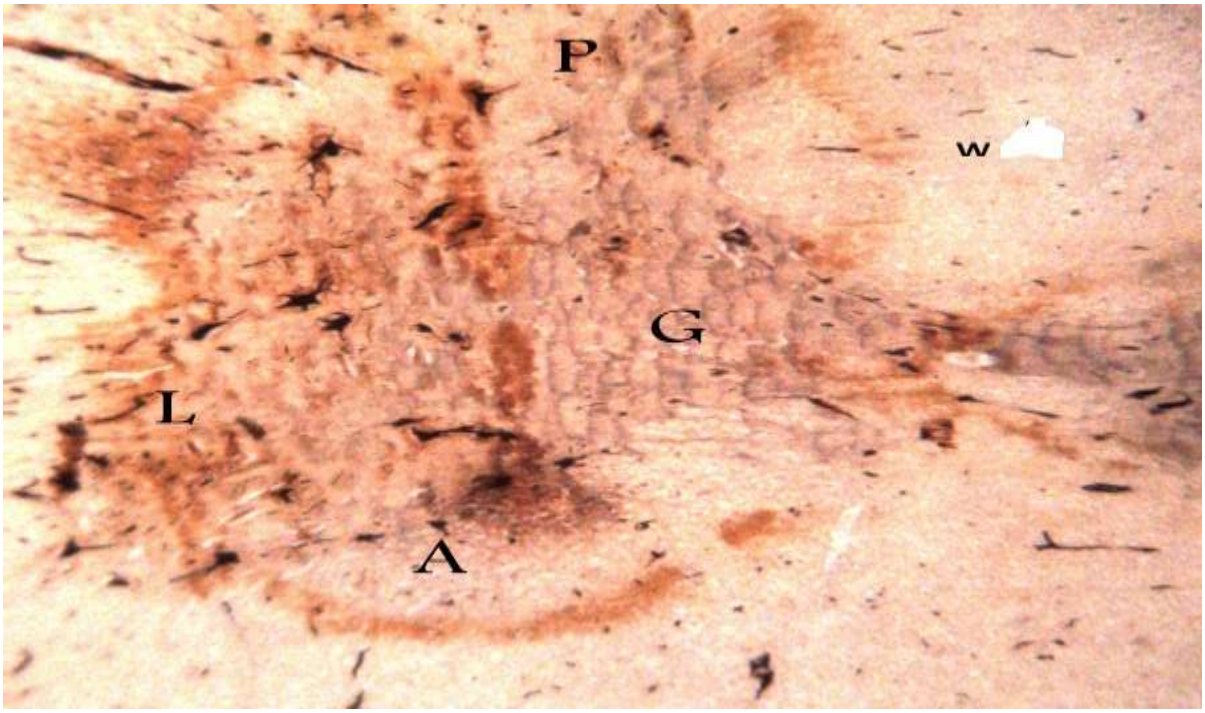


Plate XXXIX: A Transverse section of the thoracic spinal cord of Grasscutter at the level of $T_2$with gray matter (G) and white matter (W). Parts of the gray matter (G) include lateral horn (L), Anterior horn (A), and posterior horn (P) and central canal (C). Golgi stain (Mag X50).

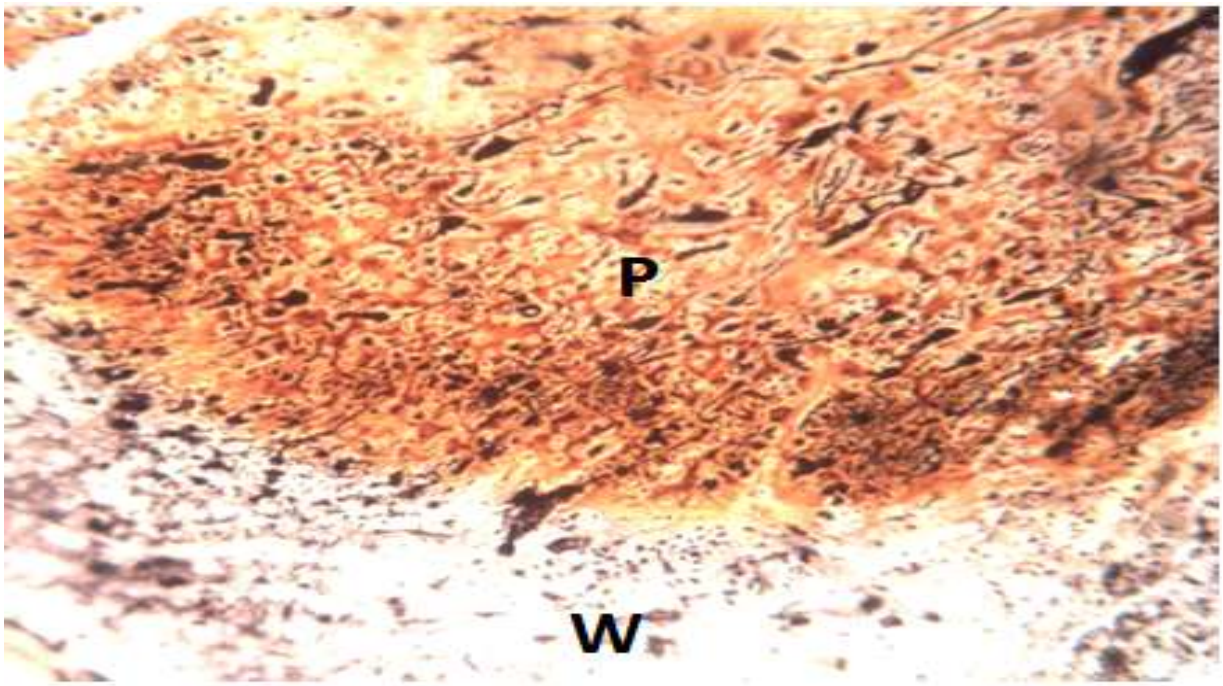
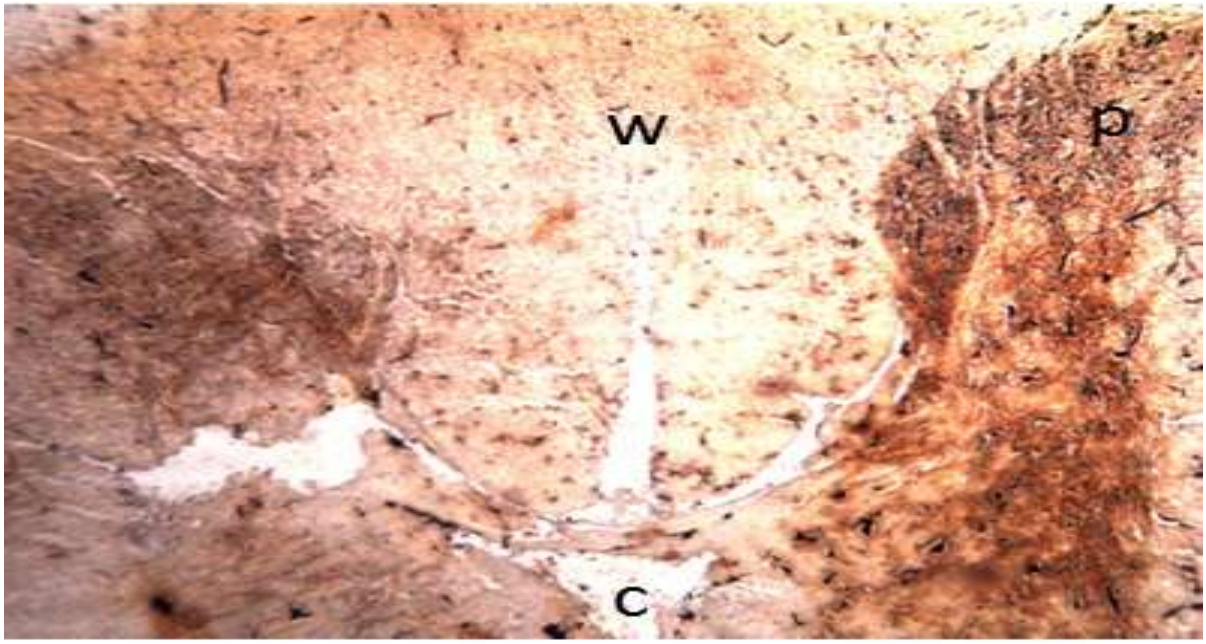


Plate XL: A Transverse section of the thoracic spinal cord of Grasscutter at the level of $T_2$with posterior horn (P) of the Gray matter (G), white matter (W). Golgi stain (Mag X100).

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PlateXLI: A Transverse section of the thoracic spinal cord of Grasscutter at the level of $T_4$with Gray matter (G) white matter (W) posterior horn (P), anterior median fissure (F) and central canal (C). Golgi stain (Mag X50).

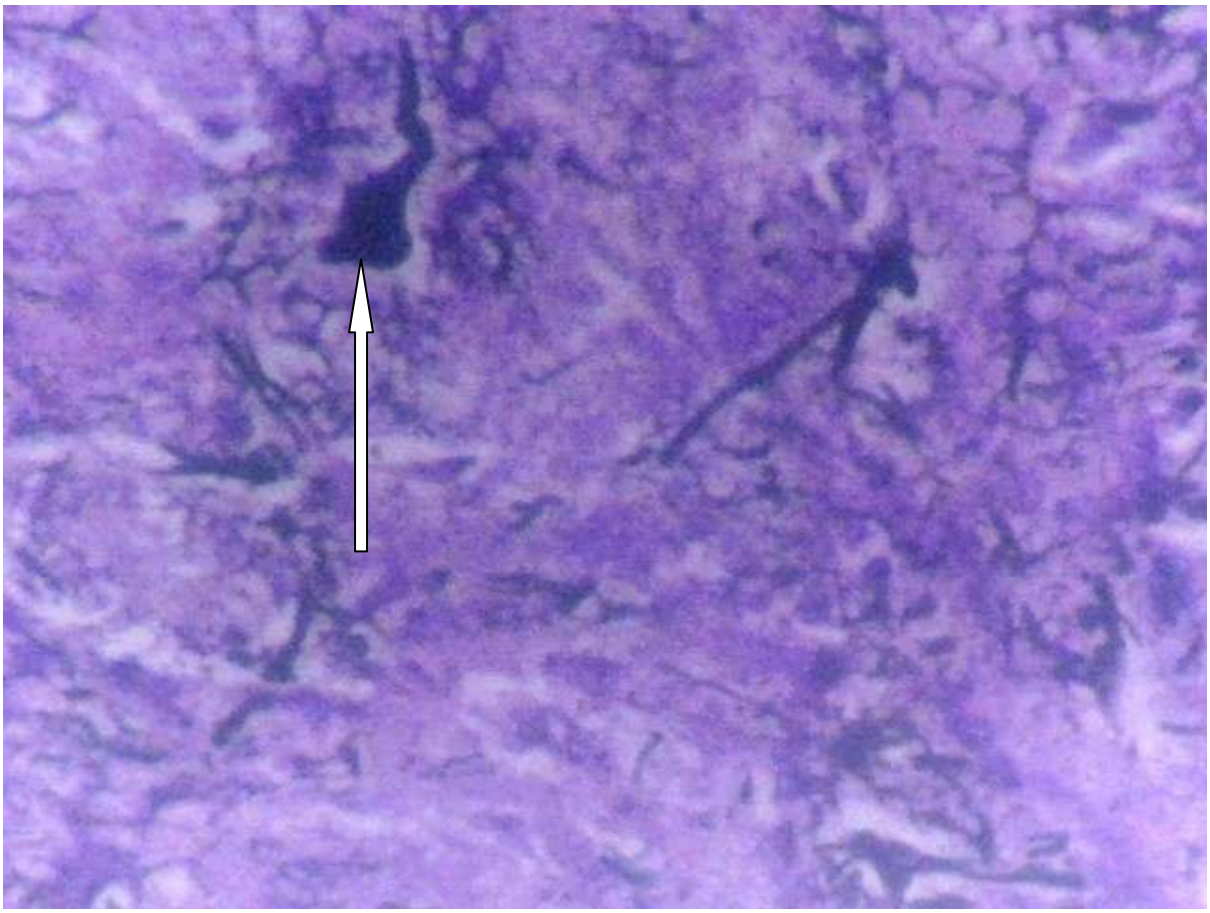
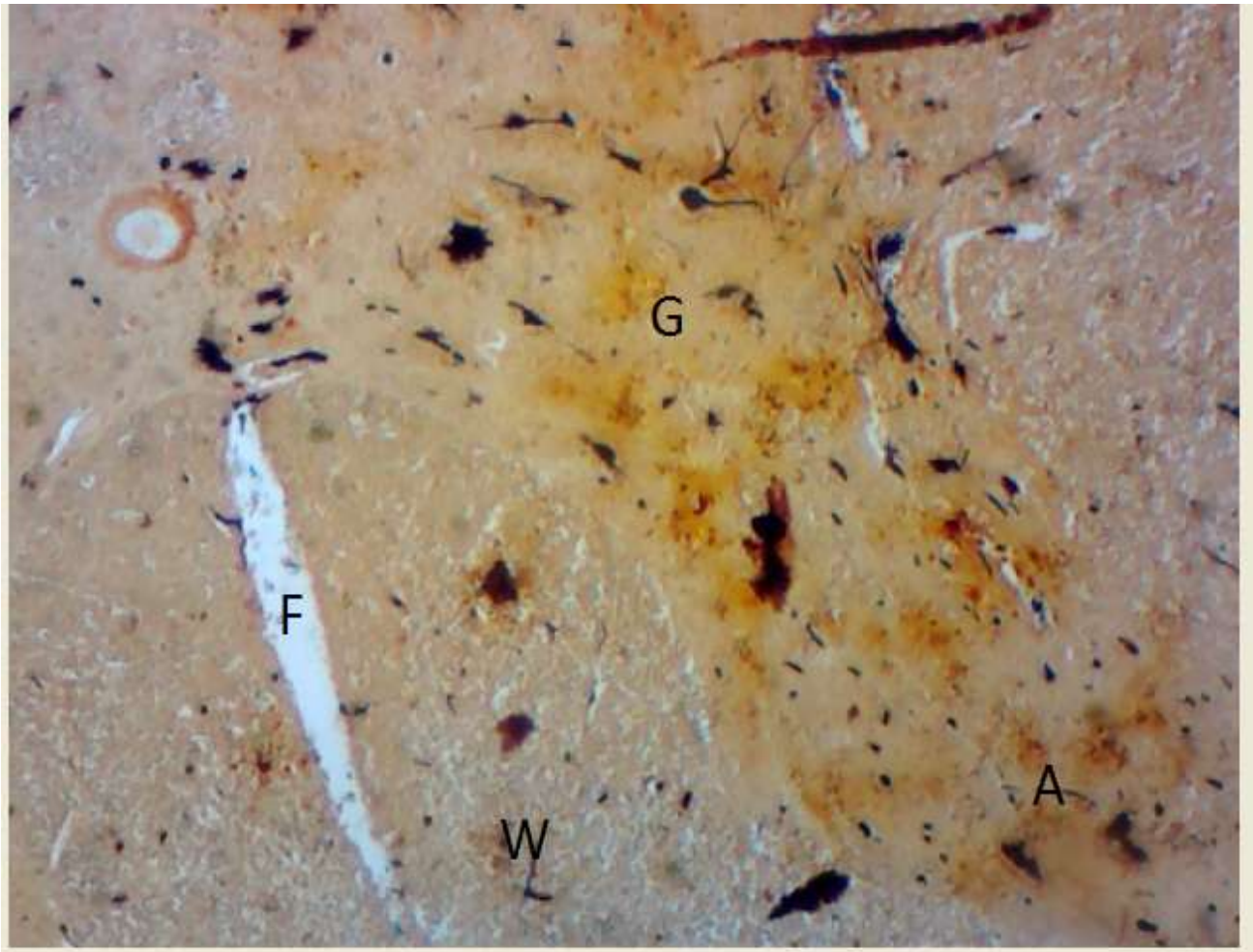


Plate XLII: A transverse section of the cervical spinal cord of Grasscutter at the level of $T_4$with posterior horn of the spinal cord. The arrow is showing neuron in the gray matter of the spinal cord segment of Grasscutter. Golgi stain (Mag X400).

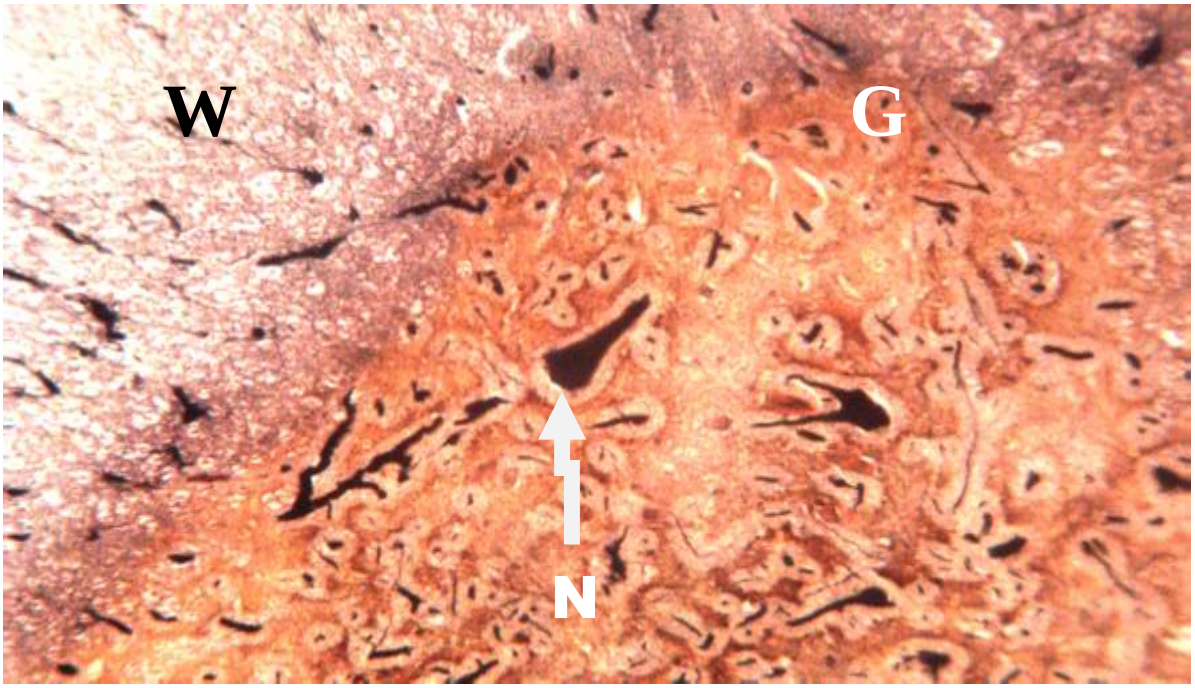
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Plate XLIII:A transverse section of the cervical spinal cord of Grasscutter at the level of $T_4$with posterior horn of the spinal cord. The arrow is showing unipolar neuron in the gray matter of the spinal cord segment of Grasscutter. Golgi stain (Mag X400).



PlateXLIV:A Transverse section of the thoracic spinal cord of Grasscutter at the level of T_4Anterior horn. (A), white matter (W) anterior median fissure (F). Golgi stains (Mag X100).



PlateXLV: A Transverse section of the thoracic spinal cord of Grasscutter at the level of T_6 with anterior horn. Gray matter (G), white matter (W), large neuron (N) and poor aborizations of different shaped neurons in the gray. Golgi stain (Mag X250).

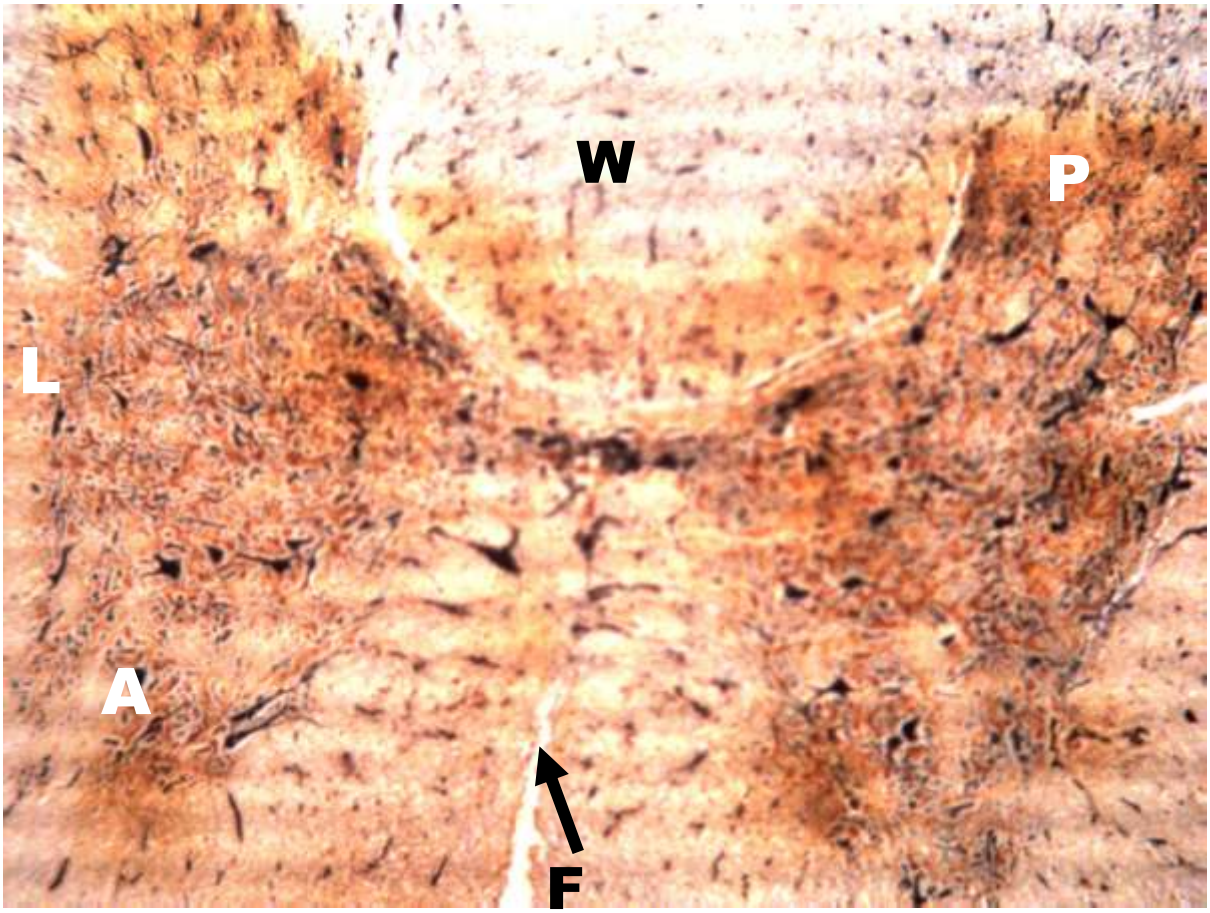


Plate XLVI: A Transverse section of the thoracic spinal cord of Grasscutter at the level of $T_6$with white matter (W), anterior median fissure (F), lateral horn (L), anterior horn (A), and posterior horn (P) are all parts of the gray matter of the thoracic spinal cord segment of Grasscutter. Golgi stain (Mag X50).



Plate XLVII: A transverse section of the cervical spinal cord of Grasscutter at the level of $T_7$with posterior horn of the spinal cord. The arrows are showing neurons in the gray matter of the spinal cord segment of Grasscutter with poor connections. Golgi stain (Mag X400).

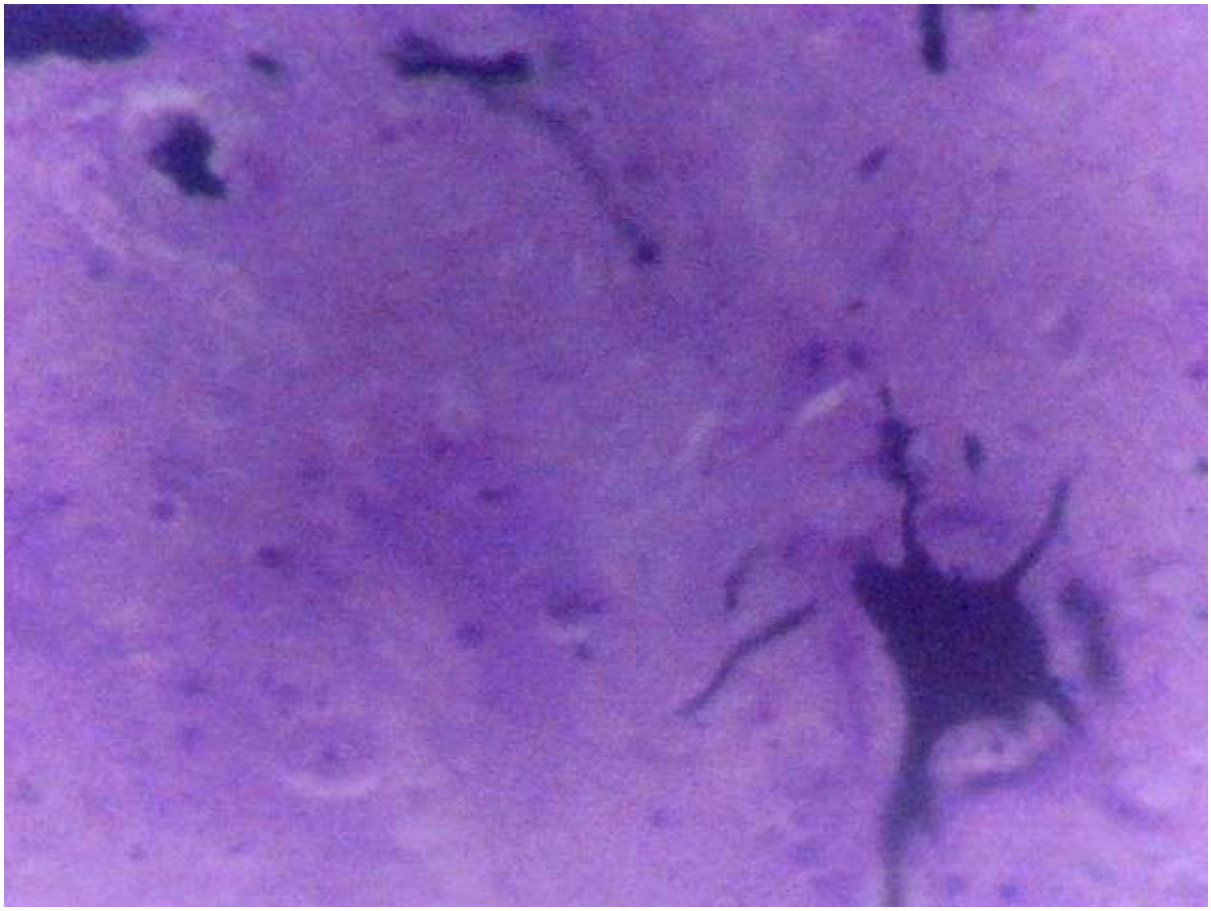
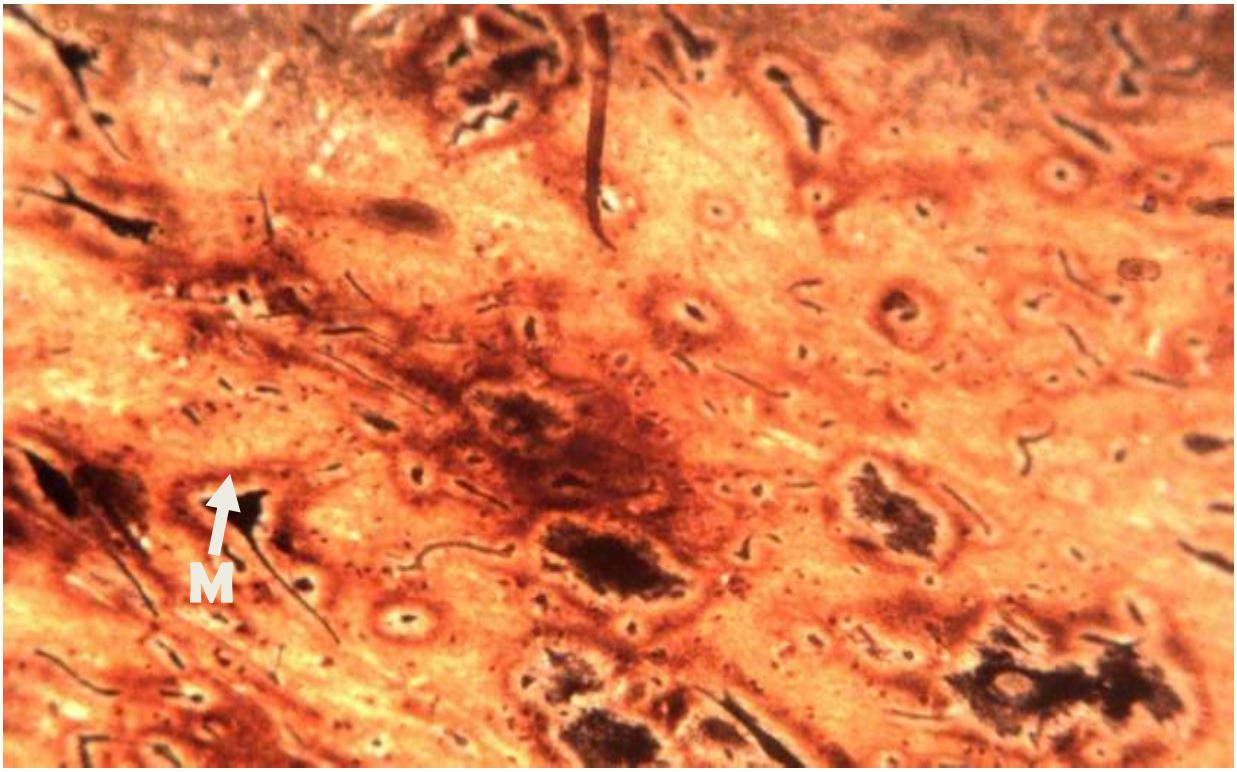
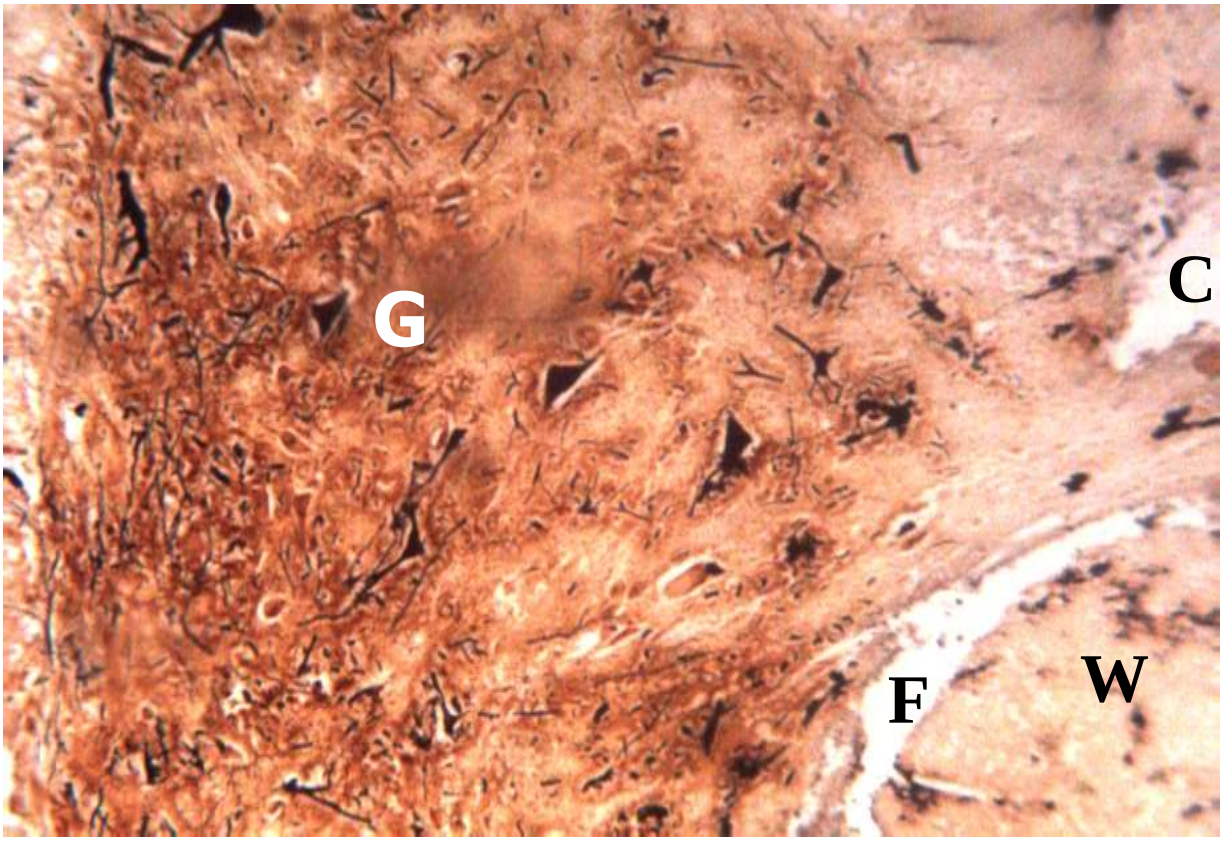


Plate XLVIII: A transverse section of the cervical spinal cord of Grasscutter at the level of $T_7$with anterior horn of the spinal cord. The arrows are showing multipolar motor neuron in the gray matter of the spinal cord segment of Grasscutter. the thoracic segmentcharacterised with poor connections. Golgi stain (Mag X400).



PlateXLIX: A Transverse section of the lumbar spinal cord of Grasscutter at the level of $L_4$with anterior horn of gray matter which contains multipolar neurons (M) of various sizes. Golgi stain (Mag X250).



PlateL: ATransverse section of the lumbar spinal cord of Grasscutter at the level of $L_4$with gray commissure of the gray matter (G) containing neurons of various sizes and shapes,central canal (C), anterior median fissure (F) and white matter (W). Golgi stain X250).

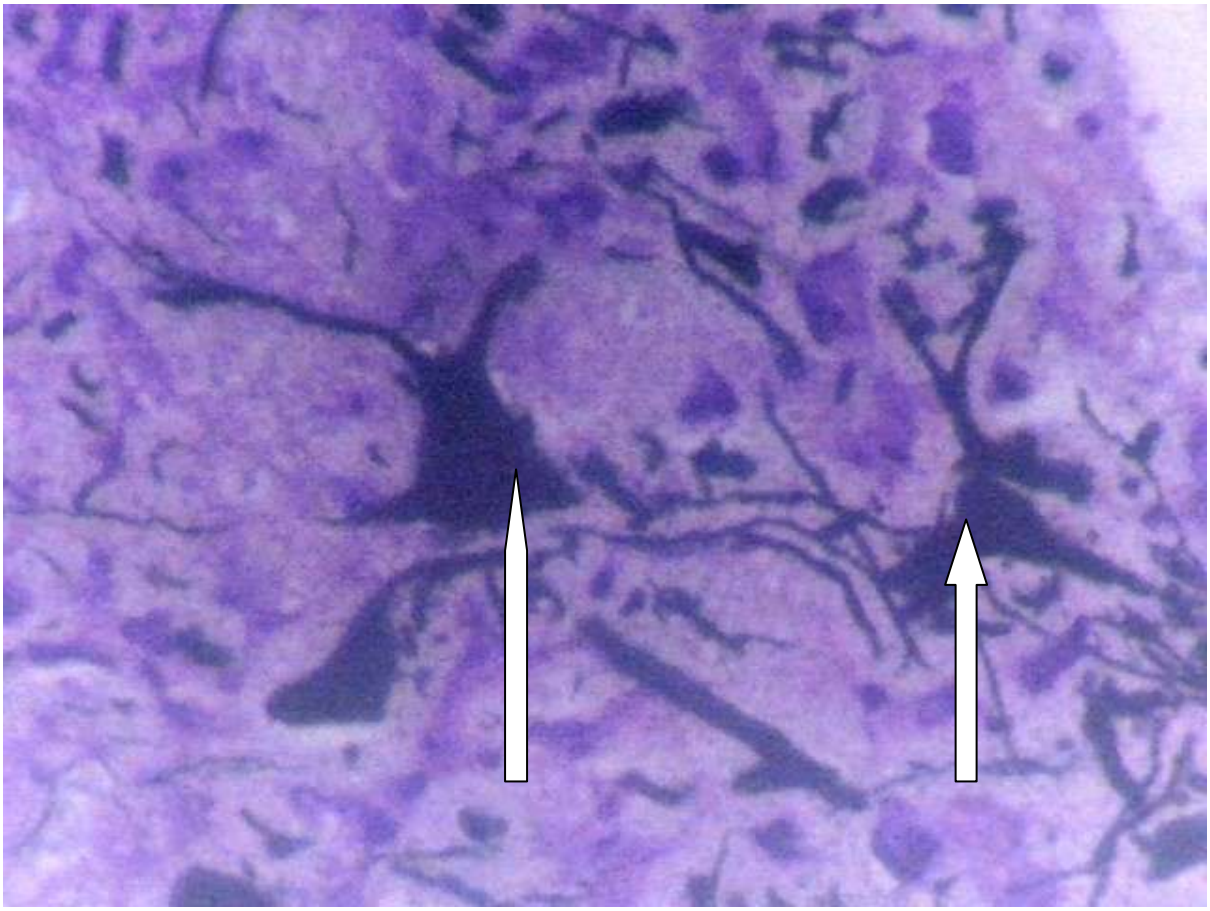


Plate LI: A Transverse section of the lumbar spinal cord of Grasscutter at the level of $L_1$with anterior horn containing neurons of various sizes and shapes with short axons (arrows), and high connections in the gray matter of the segment. Golgi stain X400.

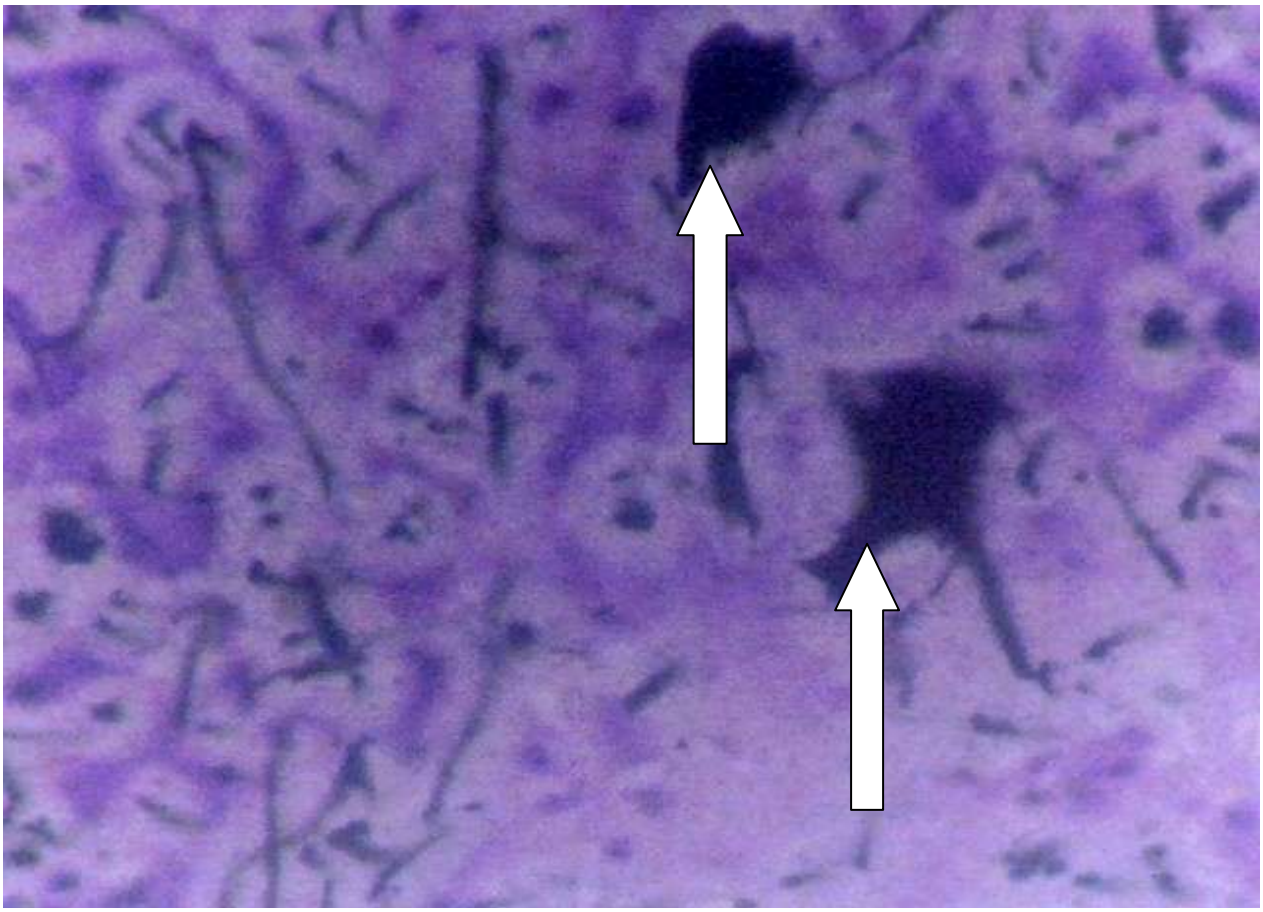
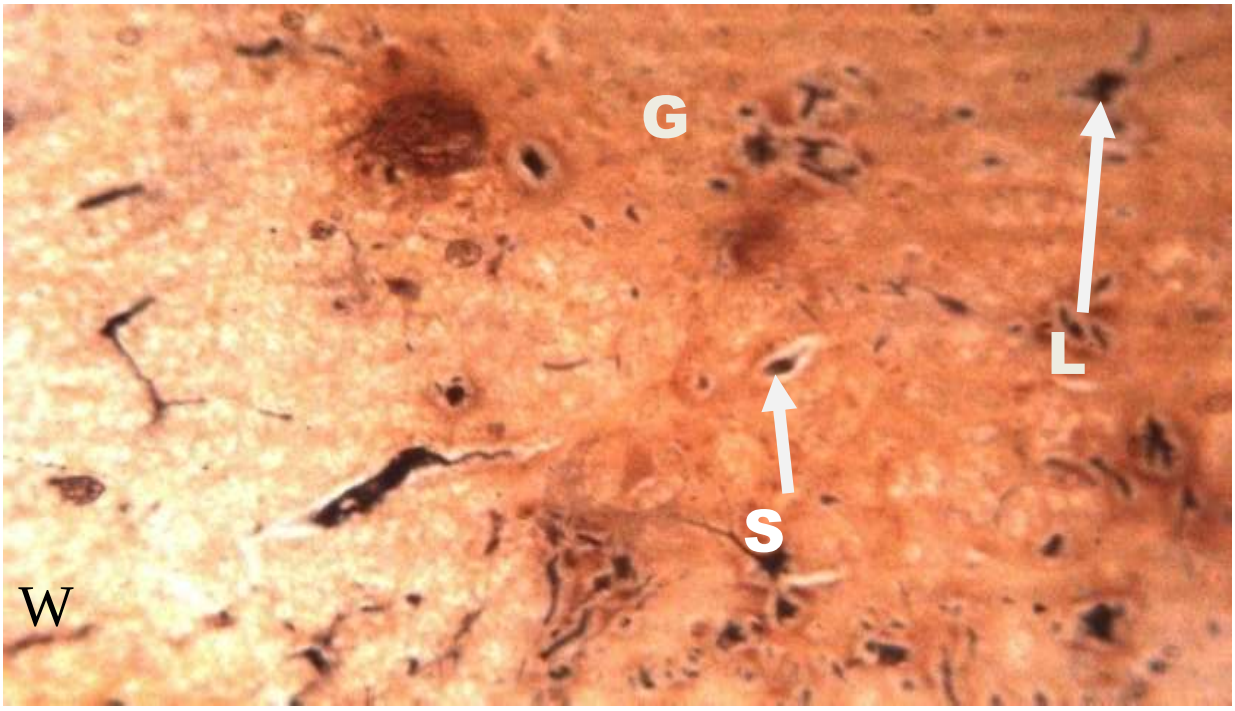


Plate LII: A Transverse section of the lumbar spinal cord of Grasscutter at the level of $L_1$with posterior horn.Aroows are with neurons in the gray natter of the spinal cord of Grasscutter. Golgi stain X400).



PlateLIII: A Transverse section of the lumbar spinal cord of the Grasscutter at the level of $L_4$with posterior horn. Many neurons present have small soma (S) which are more in number than the large neurons (L).white natter (W), gray matter (G) of the spinal cord segment has poor connections of the dendrites and axons. Golgi stain (Mag X250).

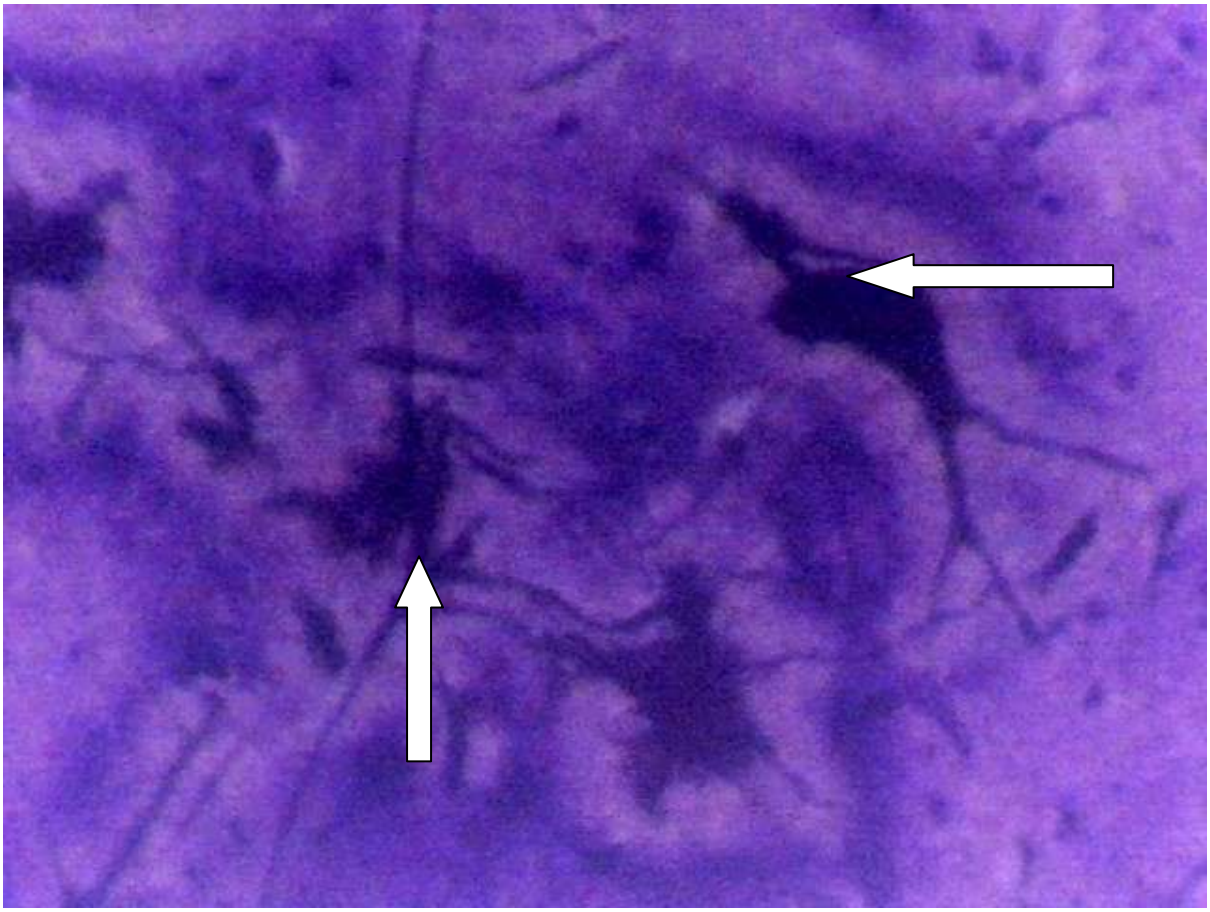


Plate LIV: A Transverse section ofthe lumbar spinal cord of the Grasscutter at the level of $L_6$with anterior horn. Arrows showing neurons present the spinal cord segment with high connection of the dendrites and axons. Golgi stain (Mag X400).



Plate LV:A Transverse section of the lumbar spinal cord of the Grasscutter at the level of $L_6$with posterior horn. Arrow showingneuron in the gray matterof the spinal cord segment Golgi stain (Mag X250).

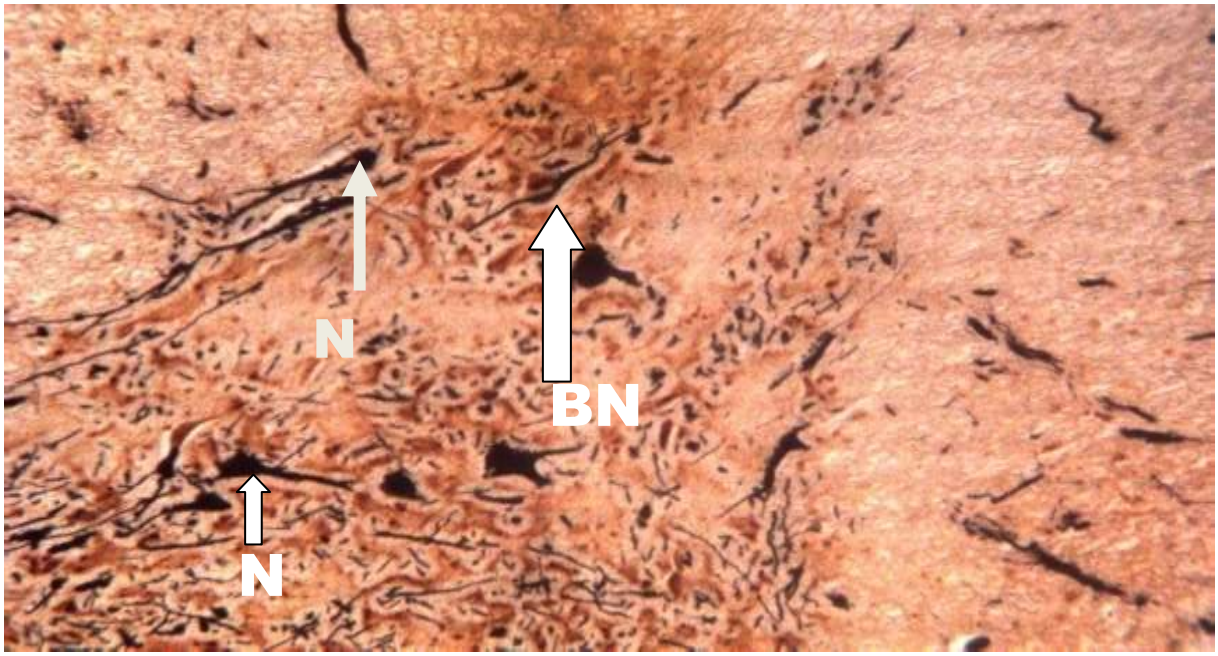
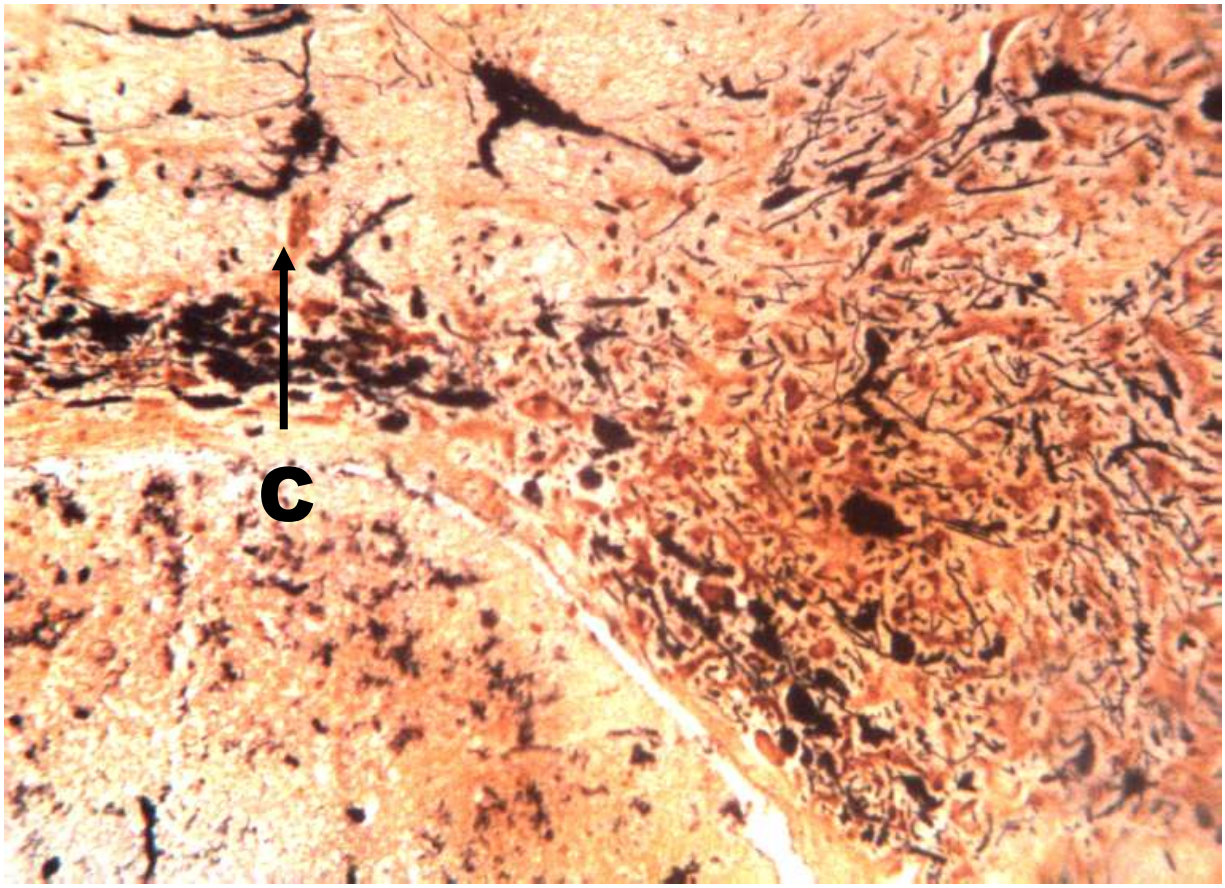


Plate LVI: A transverse section of the lumbar spinal cord of Grasscutter at the level of $L_1$with posterior horn. The presence of neurons (N), bipolar neurons (BN) can be seen in the posterior horn of the lumbar spinal cord segment. Golgi stain (Mag X250).



PlateLVII: A transverse section ofthe lumbar spinal cord of the Grasscutter at the level of $L_1$with gray commisure. The presence of numerous neurons around the central canal (C) of the spinal cord. Golgi stains (Mag X250).

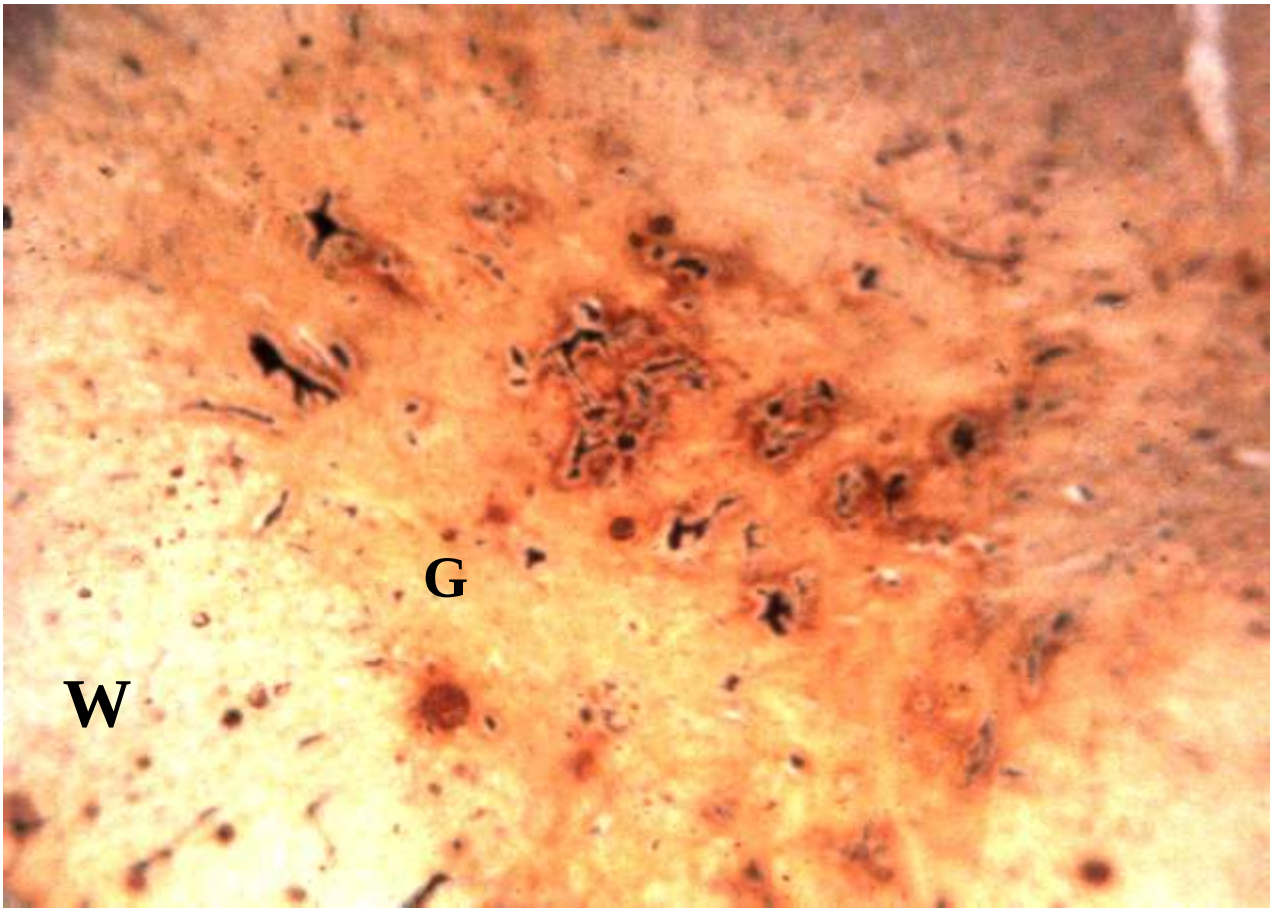


Plate LVIII: A transverse section of the sacral spinal cord of Grasscutter at the level of $S_1$with gray matter (G) and white natter (W). Gray matter (G) contains small size neurons (s) of the spinal cord of the sacral segment.Segment showed poor aborization of dendrites and axons of neurons. Golgi stain (Mag X250).

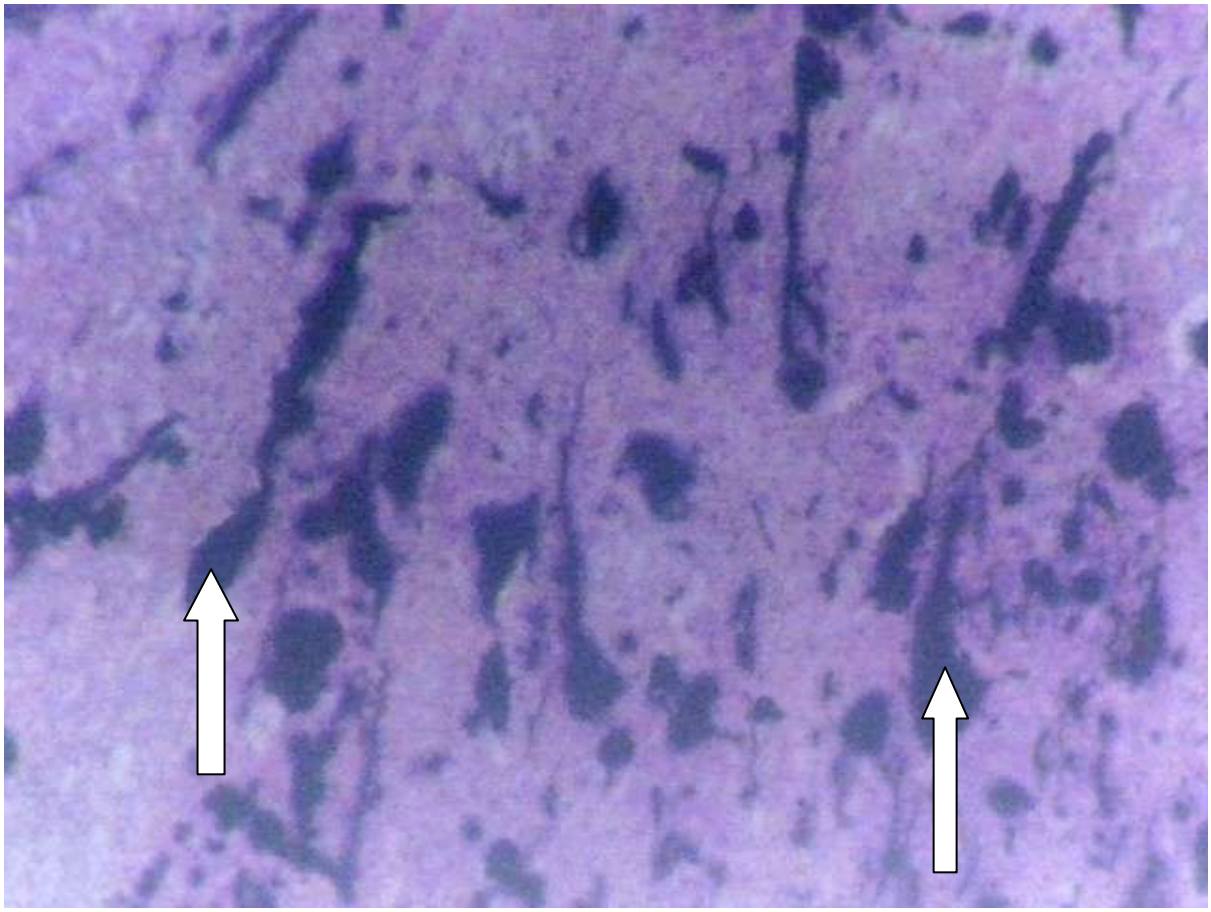


Plate LIX: A transverse section of the sacral spinal cord of Grasscutter at the level of S_1withgray matter of the sacral segment. Arrows showing neurons of the gray matter. Golgi stain (Mag X400).

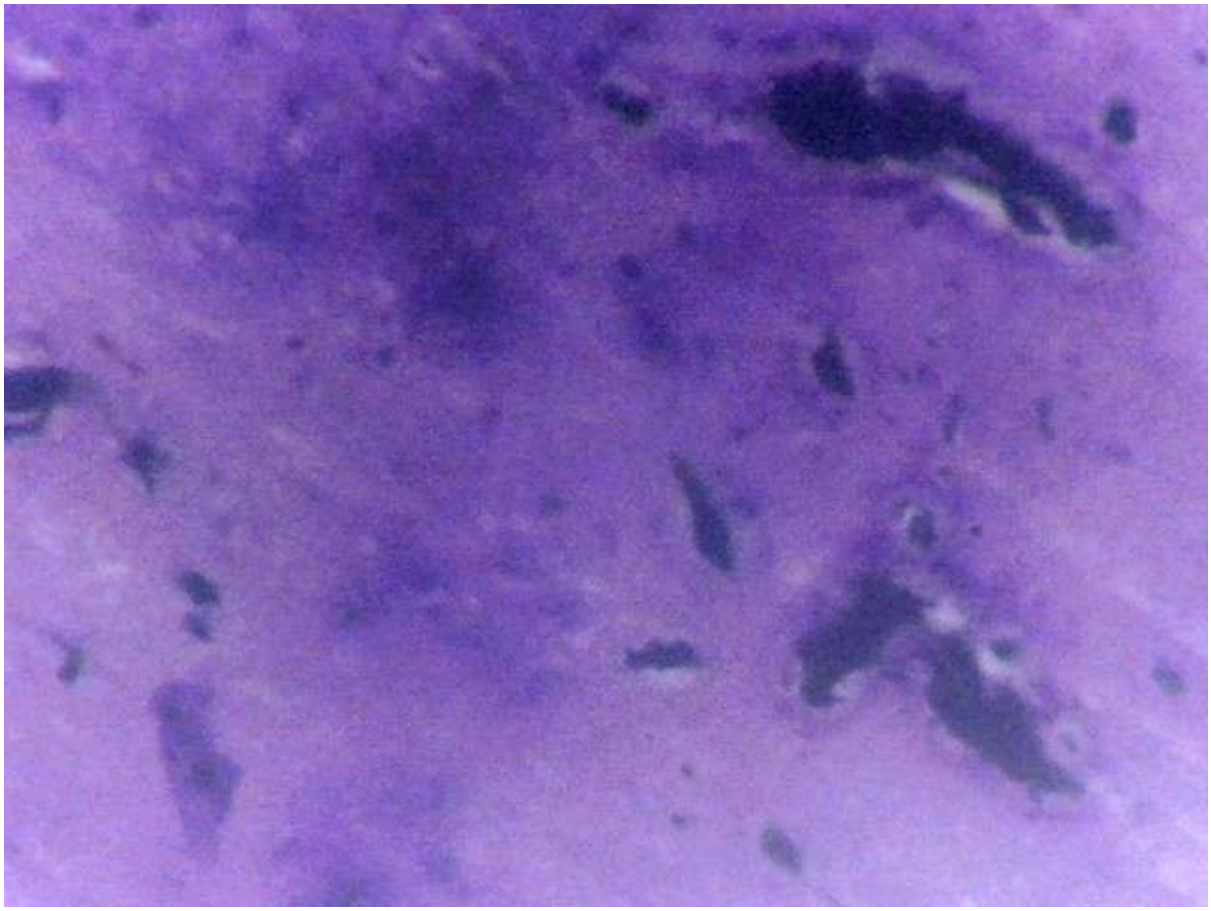


Plate LX:A transversesection ofthe sacral spinal cord of Grasscutter at the level of $S_1$with anterior horn neurons of the spinal cord of the sacral segment. Segment showed poor aborization of dendrites and axons of neurons. Golgi stain (Mag X400).

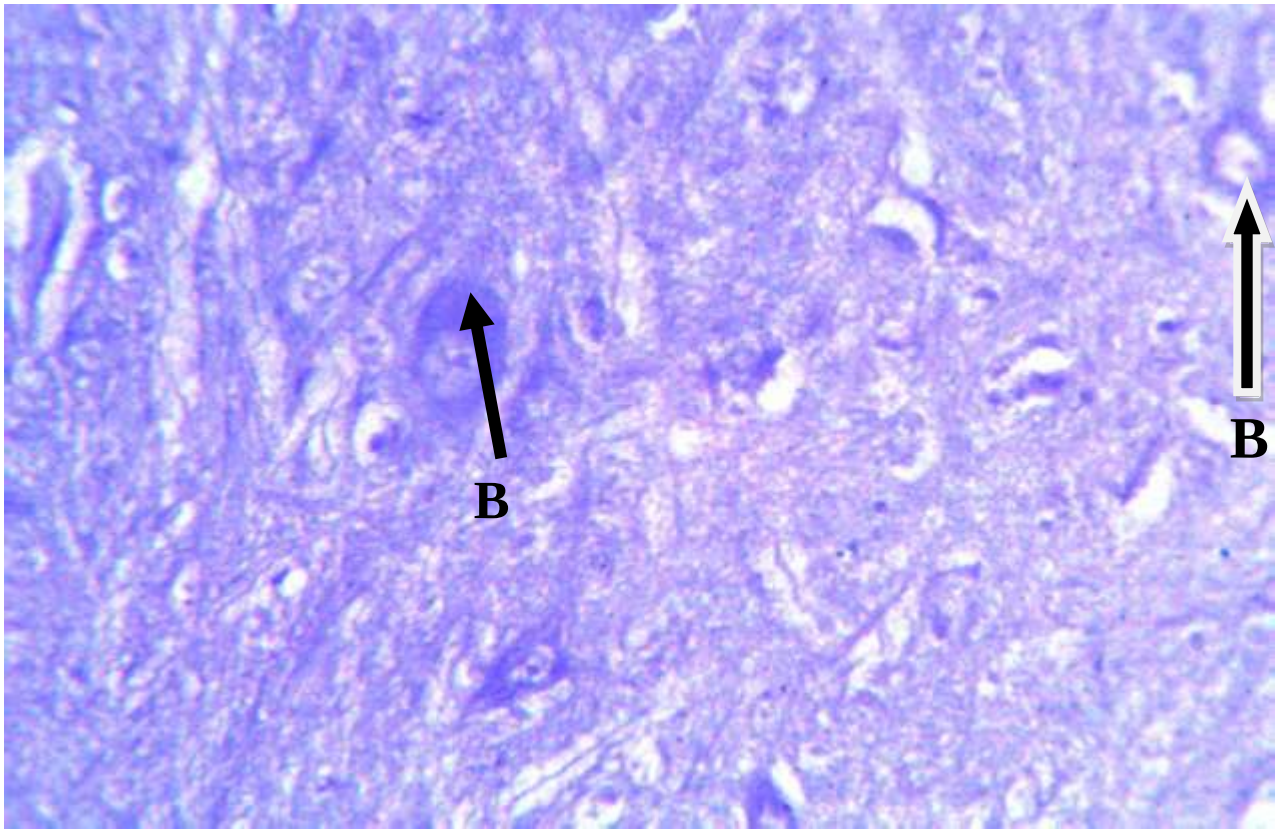
4.4.2 Crestyl fast violet

The result showed transverse sections of Crestyl fast violet(CFV) stained spinal segments ofGrasscutter with two distinct zones and a central lumen (central canal). The inner zone of gray matter stained dark purple and the outer zone of white matter stained lighter a pattern seen throughout the length of the spinal cord, at different levels namely cervical, thoracic, lumbar, and sacral spinal cord segments as shown in Plate LXI, Plate LXII, Plate LXIII, Plate LXIV, Plate LXV, Plate LXVI, Plate LXVII, Plate LXVIII, Plate LXIX, Plate LXX, Plate LXXI, Plate LXXII and Plate LXXIII.

The result showed Nissl substances in the soma of the neurons of the spinal cord of Grasscutter as shown in Plate LXI were the presence of Nissl substances were seen in the multipolar neurons of the anterior horn of cervical spinal cord segment. The result also showed the difeerent parts of the gray matter of the spinal cord segments (Plate LXII) Neurons of the anterior horns of the spinal segments were seen to be large multipolars with Nissl substances within their soma as shown in Plate LXV, Plate LXVI, Plate LXX and Plate LXXI.

The posterior horns cells of the spinal cord segments of Grasscutter were observed to also contain Nissl substances within their soma as shown in Plate LXVII, Plate LXVIII, Plate LXIX.

The white matter of the spinal cord segments was observed to be free of Nissl substances (Plate LXIII). The central canal was seen in the central core of the gray matter as a lumen which run throughout the length of the spinal cord of the Grasscutter as shown in Plate LXIII.



PlateLXI: A transverse section of the cervical spinal cord of the Grasscutter at the level of C_4 with gray matter (Anterior horn). The presence of Nissl bodies (B) in the multipolar neurons can be seen. Crestyl fast violet stain (Mag X250).

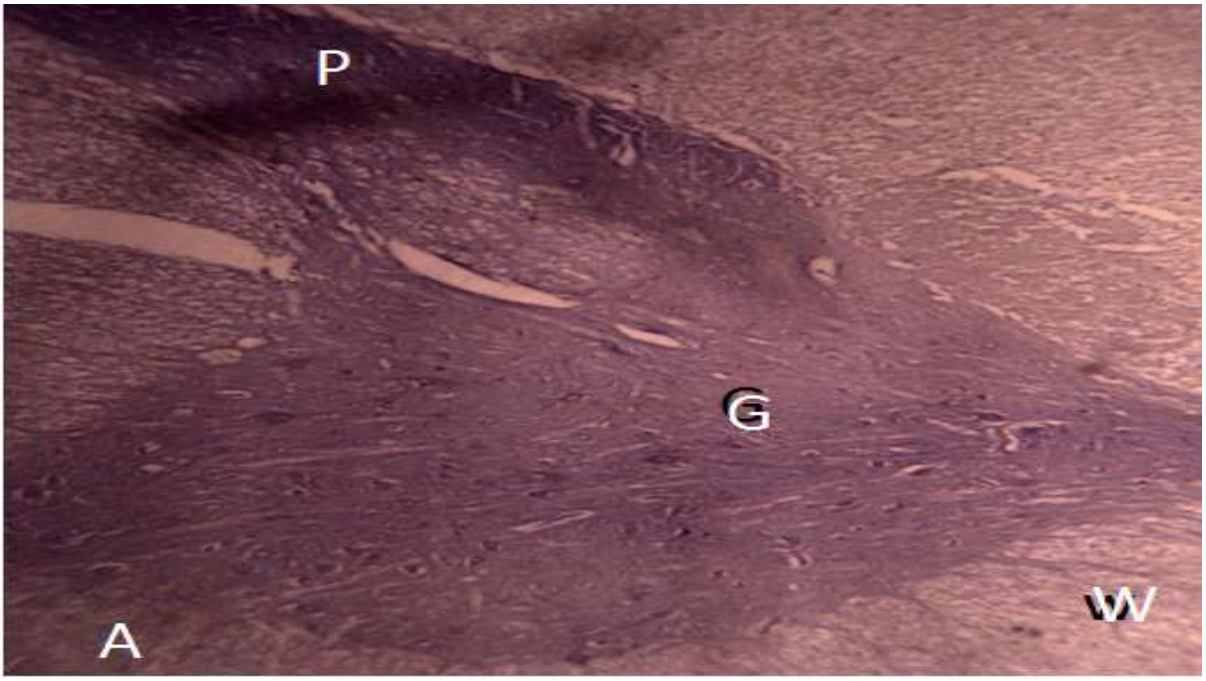


Plate LXII: A transverse section of the cervical spinal cord of the Grasscutter at the level of C_4 with Gray matter (G) white matter (W) Anterior horn (A), and posterior horn (P). Crestyl fast violet stain (Mag X100).

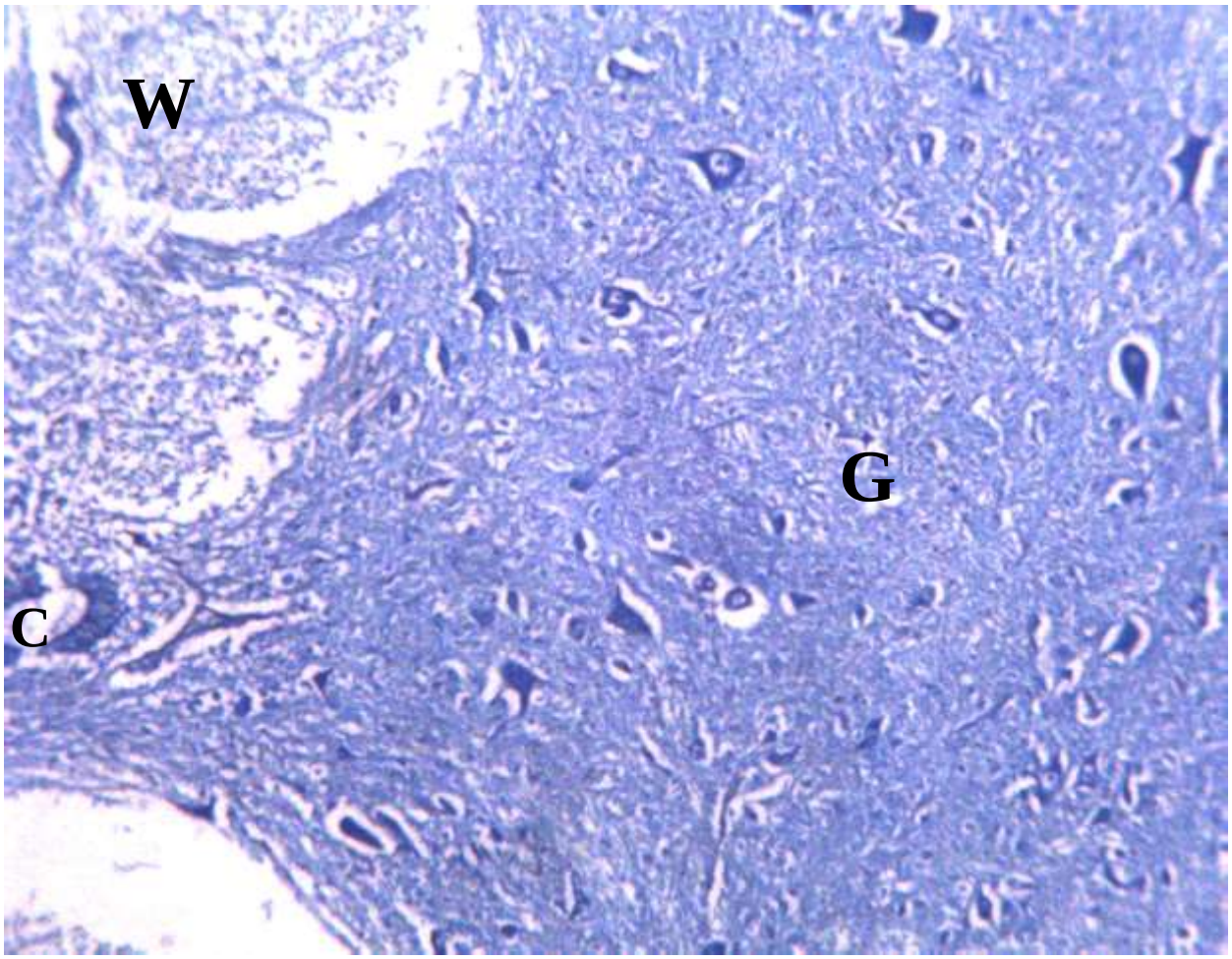
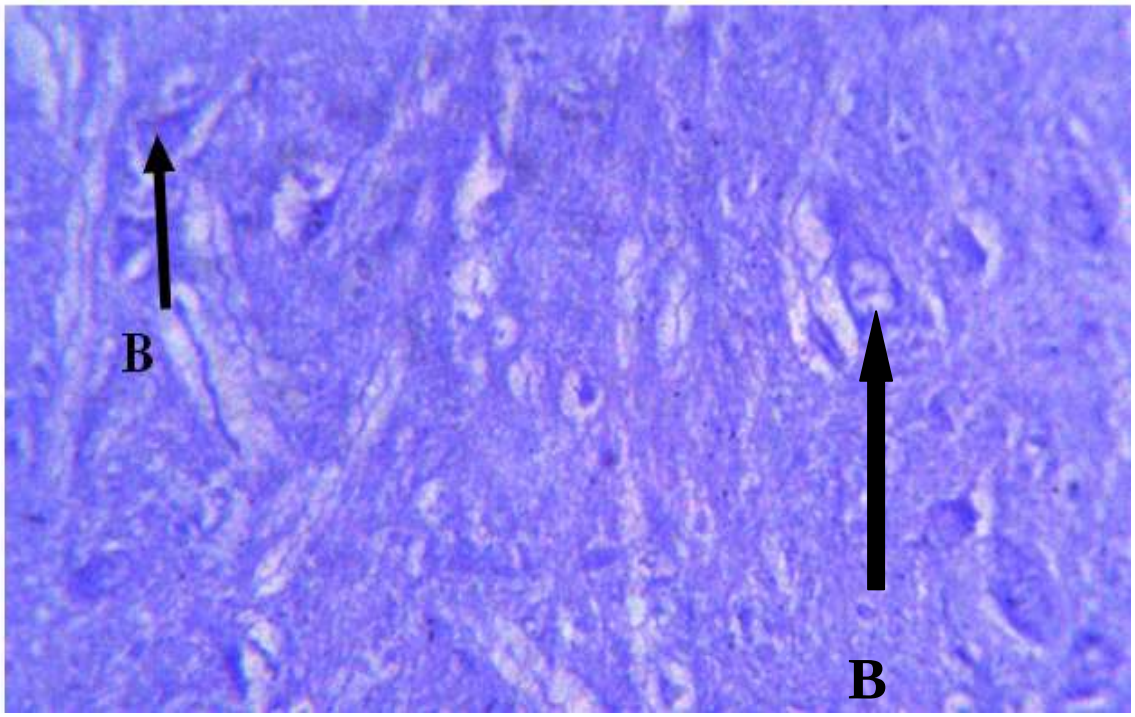


Plate LXIII: A transverse section of the cervical spinal cord of the Grasscutter at the level of $C_4$with Gray matter (G) and central canal (C). Crestyl fast violet stain (Mag X100).



PlateLXIV: A transverse section of the cervical spinal cord of the Grasscutter at the level of C4with posterior horn with presence of Nissl bodies (B) and nucleus contain the neucleous in the neuron of the posterior horn Crestyl fast violet stain (Mag X250).

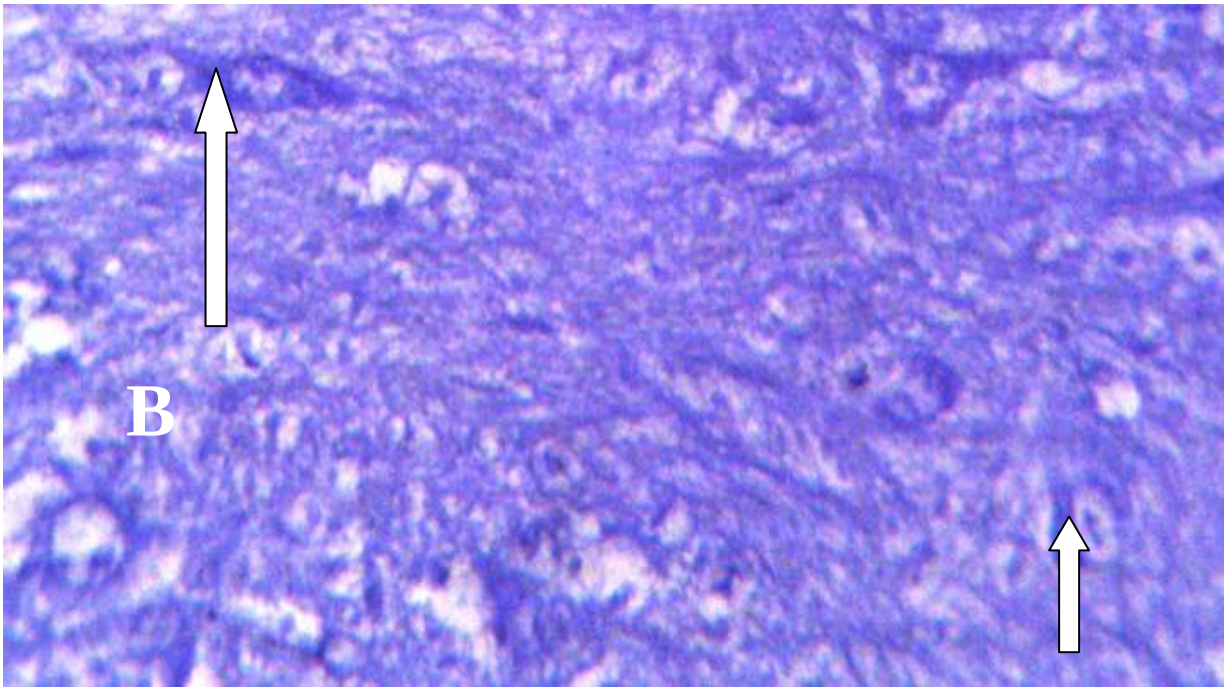


Plate LXV: A transverse section of the cervical spinal cord of Grasscutter at the level of $C_4$with Anterior horn the arrows showed the presence of Nissl bodies (B) in the neurons of the Anaterior horn. Crestyl fast violet stain (Mag X250).

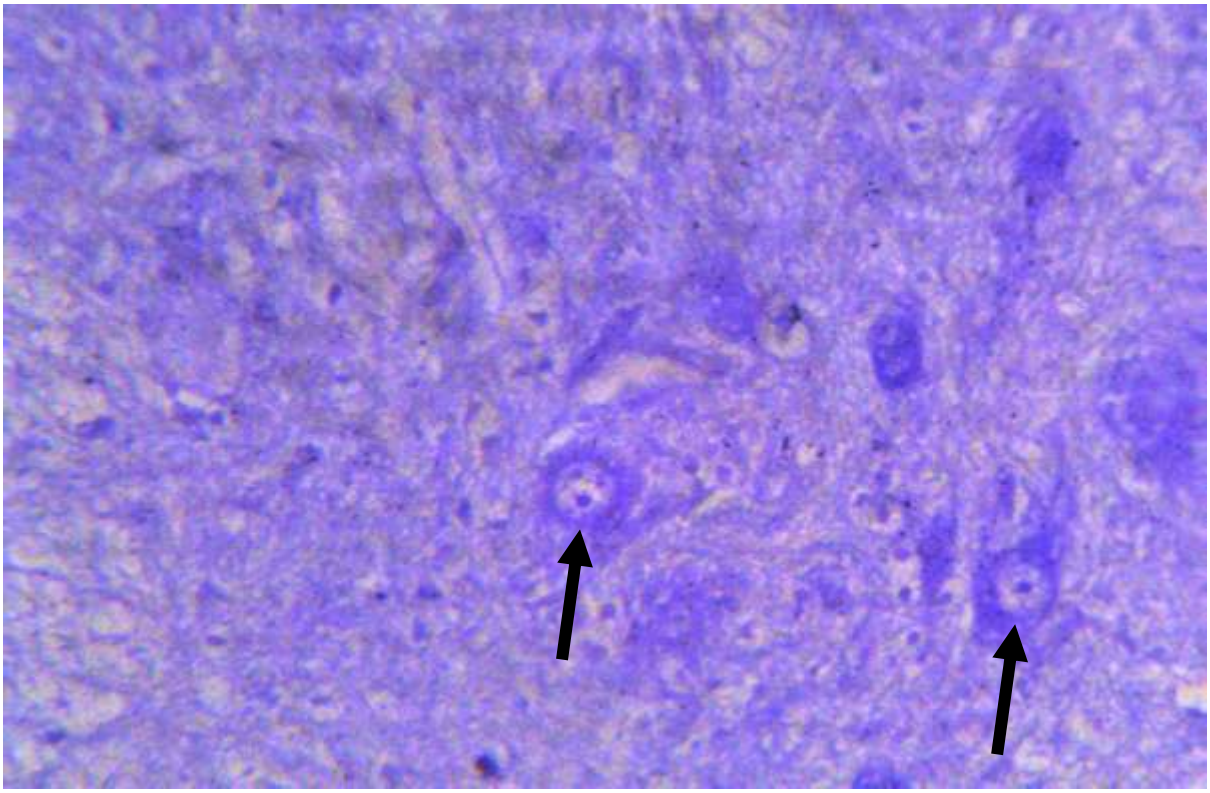


Plate LXVI: A Transverse section of the thoracic spinal cord of Grasscutter at the level of T_4withanterior horn Nissl bodies (B) can be seen in the multipolar motor neurons of the gray matter. Crestyl fast violet stain (Mag X250).

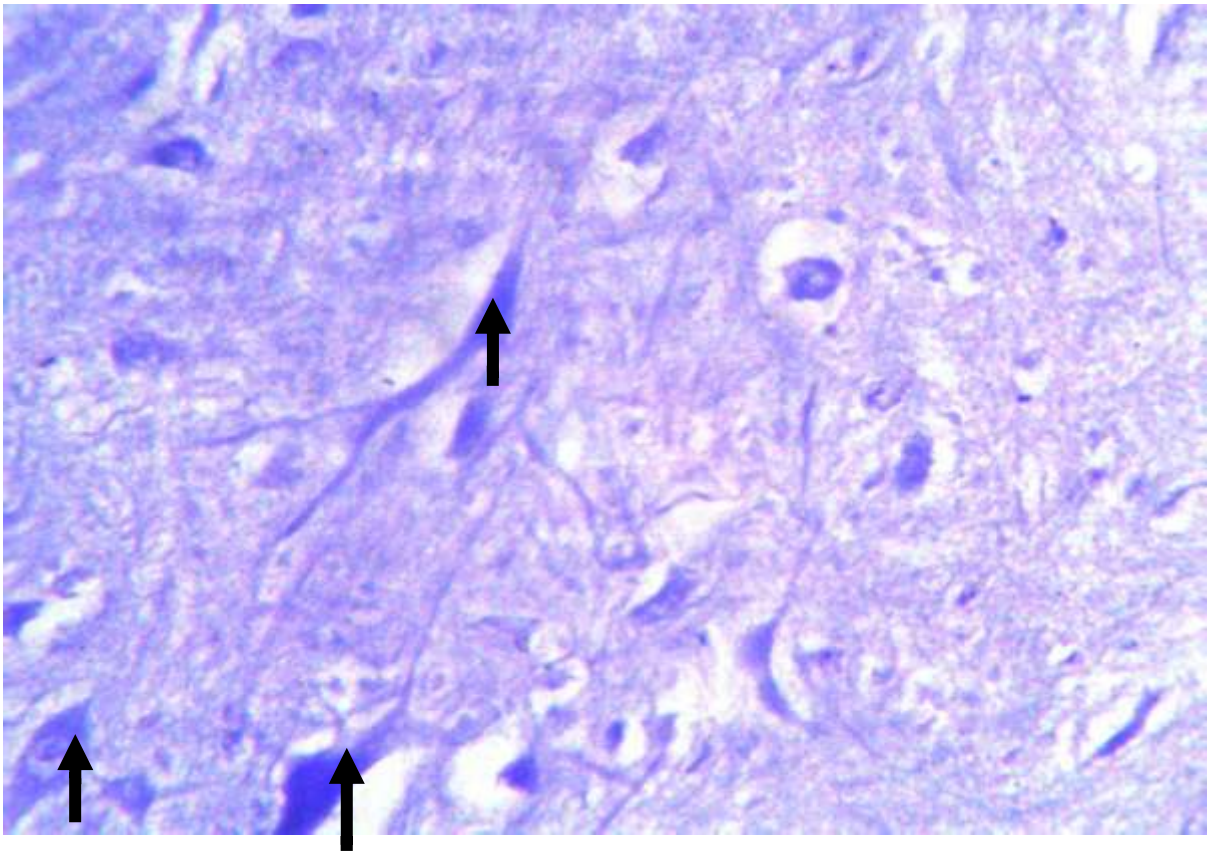


Plate LXVII: A transverse section of thoracic spinal cord of Grasscutter at the level of $T_4$with the posterior horn the arrowing Nissl bodies (arrowed structure) in the neurons of the gray matter of this region. Crestyl fast violet stain (Mag X250).

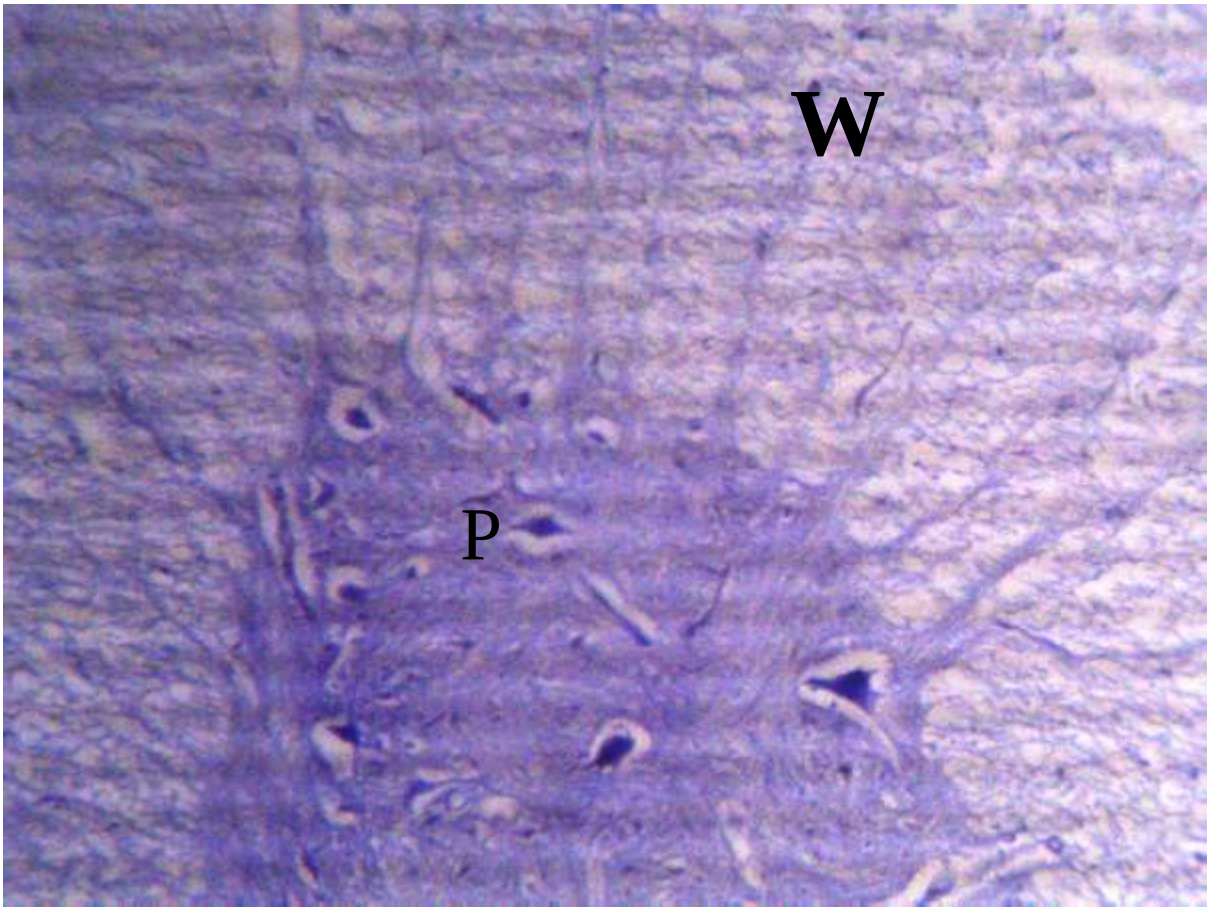


Plate LXVIII: Transverse section of thoracic spinal cord of Grasscutter at the level of $T_4$with posterior horn (P) and white matter (W). Crestyl fast violet stain (Mag X100).

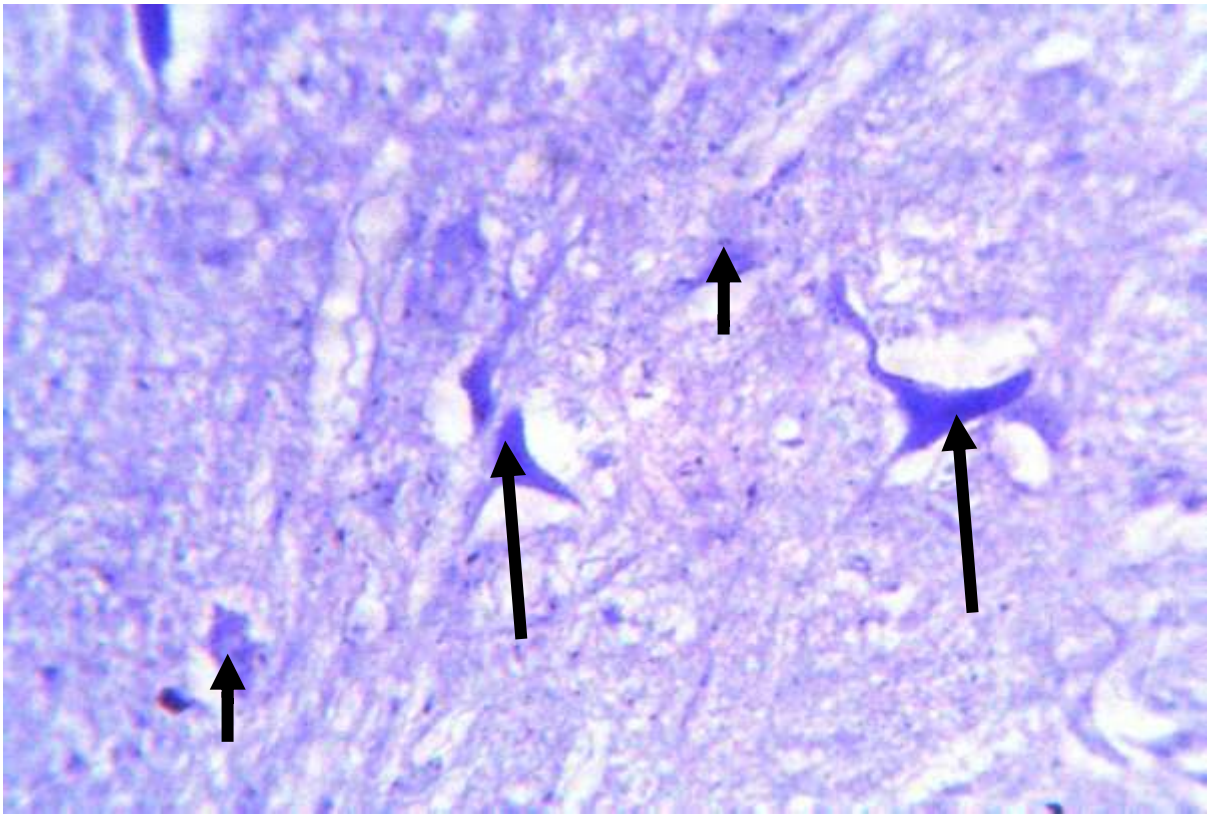


Plate LXIX: A transverse section of the lumbar spinal cord of the Grasscutter at the level of $L_2$with posterior horn with Nissl bodies (B) in the neurons of this region. Crestyl fast violet stain (Mag X250).

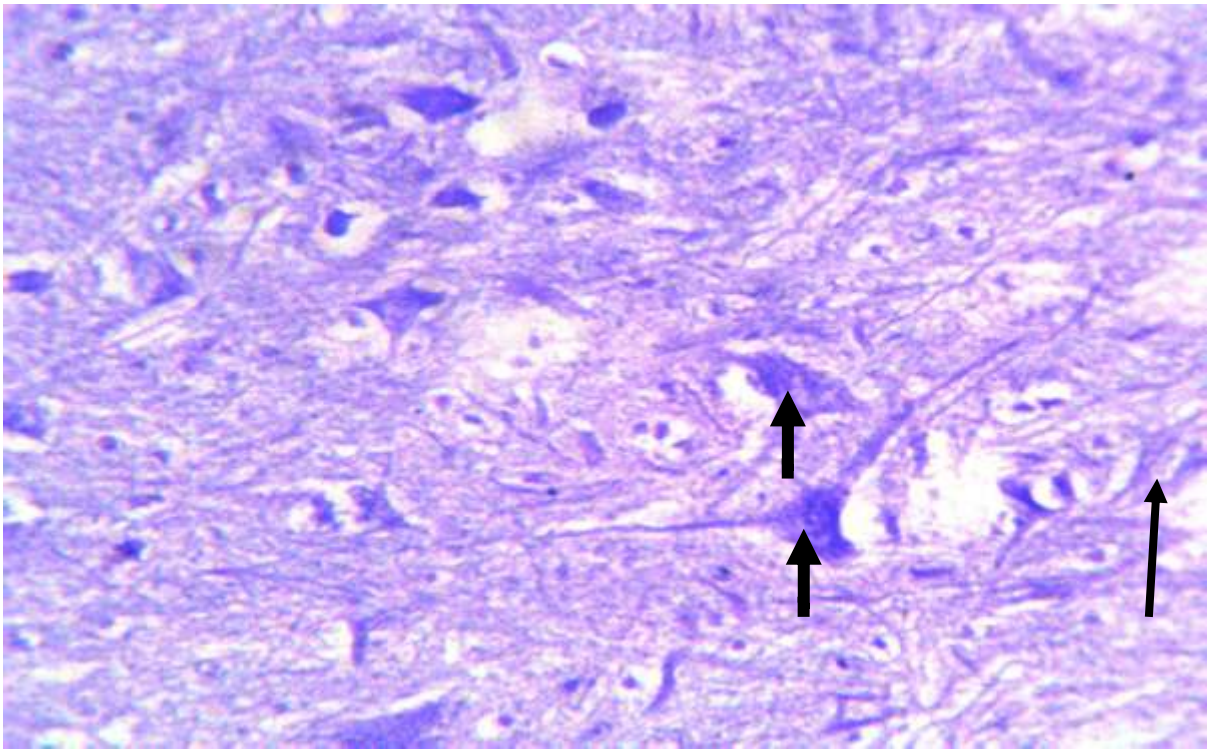
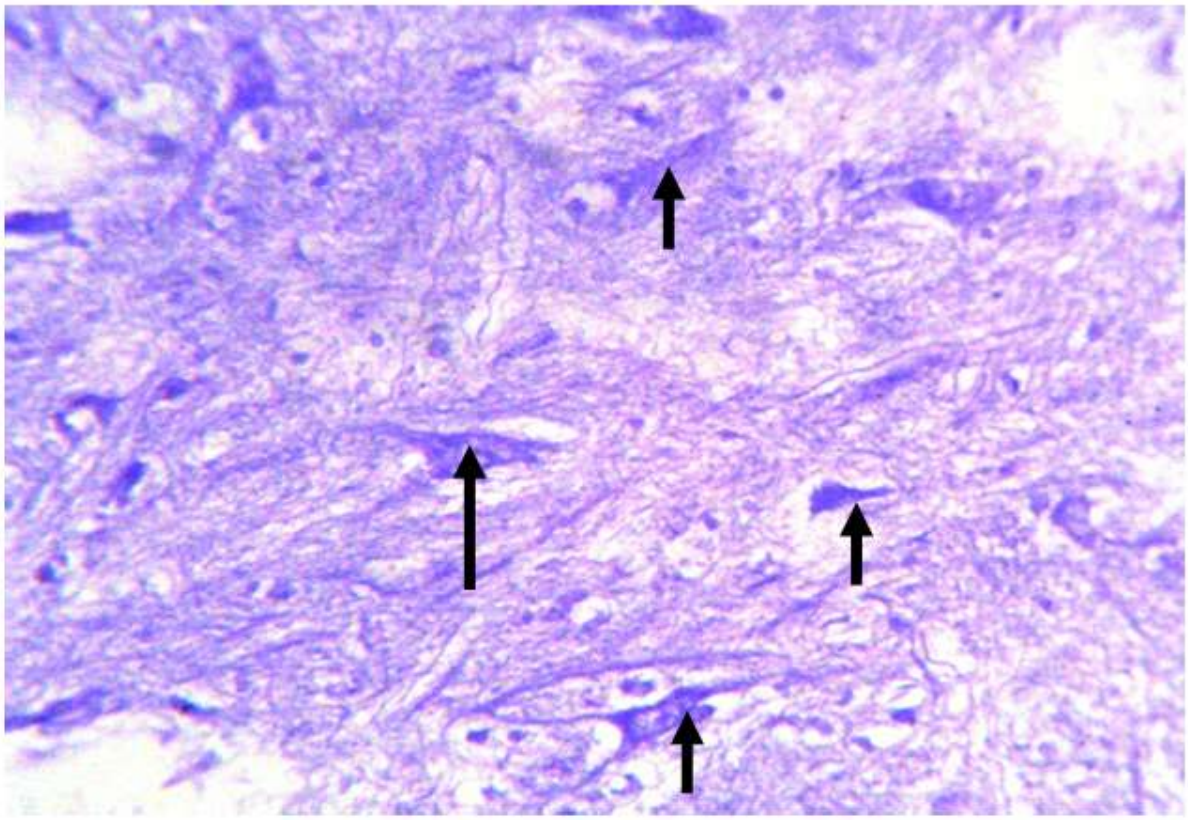


Plate LXX: A Transverse section of the lumbar spinal cord of the Grasscutter at the level of L_2withcells of the anterior horn containing Nissl odies (arrowed structures) in the cytoplasm of the large neurons of the region. Crestyl fast violet stain (Mag X250).



PlateLXXI:ATransverse section of the lumbar spinal cord of Grasscutter at the level of $L_6$with anterior horn. The Nissl substance in the cytoplasm of the neurons in the region shown by the arrow Crestyl fast violet stain (Mag X250).

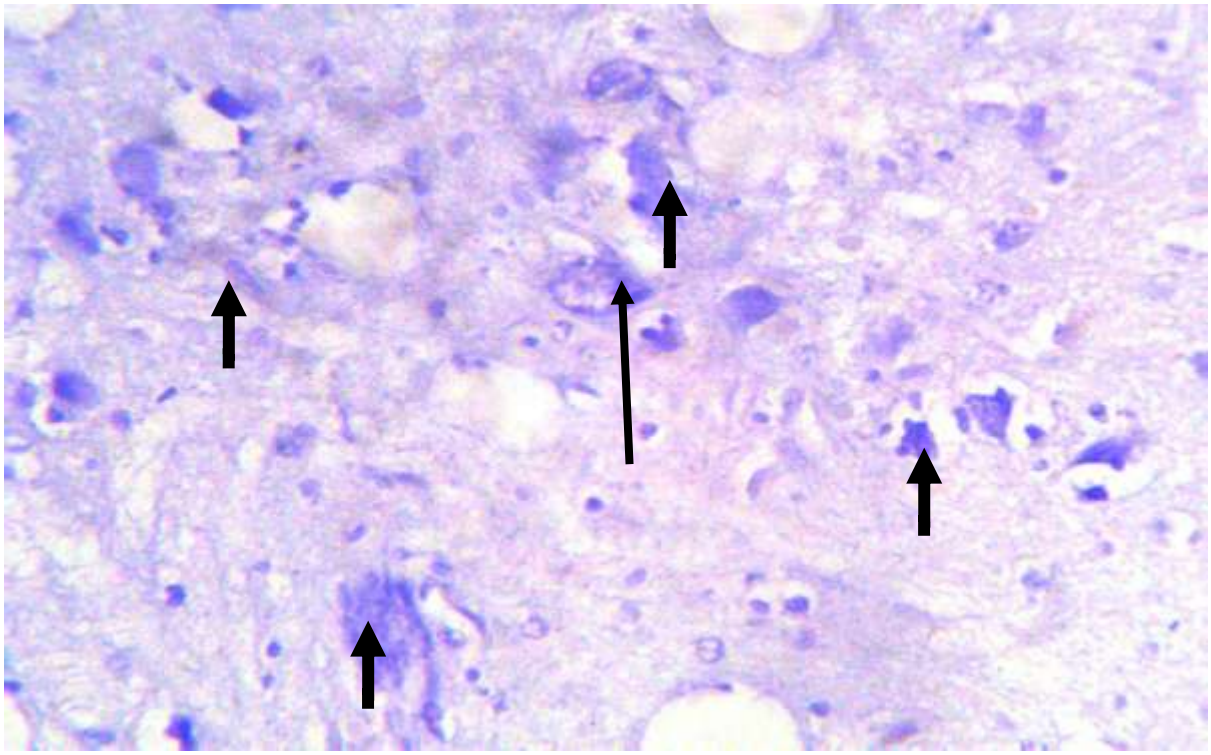


Plate LXXII: A Transverse section of the sacral spinal cord of the Grasscutter at the level of $S_1$with Nissl bodies the neurons of the gray matter of this spinal cord segment shown by arrows. Crestyl fast violet stain (Mag X250).

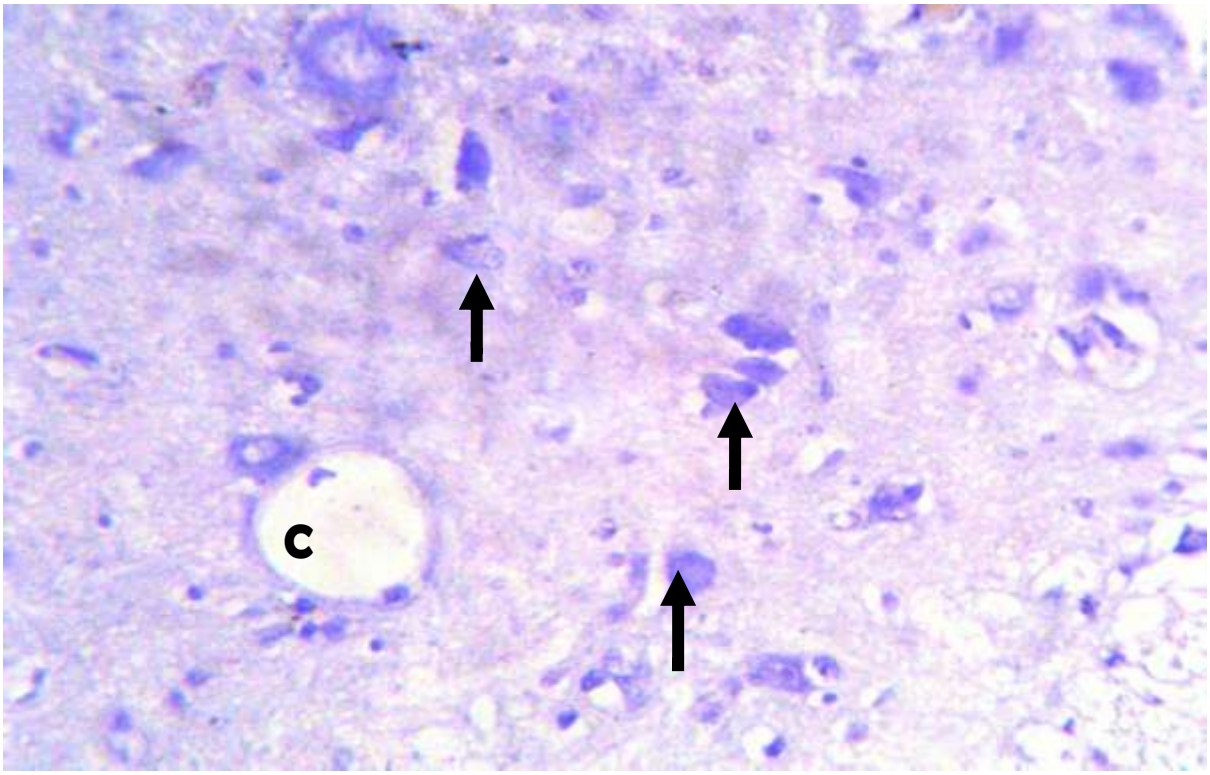


Plate LXXIII: A transverse section of the sacral spinal cord of Grasscutter at the level of S_2withNissl bodies (arrows) in the neurons of the gray matter of the segment and central canal (C). Crestyl fast violet stain (Mag X250).

4.5 Immunohistochemical studies

The result showed the transverse section of the spinal cord segments of theGrasscutter with distribution of dopamine immune reactivity observed from all spinal cord segments of the Grasscutter. In the presencestudy dopamine-positive neurons were observed along the length of the spinal cord of the Grasscutterwhen the spinal cord segments were stained with Alpha-1 antityrosine hydroxylase.

In the cervical spinal cord segments, dopaminergic neurons (DPN)were seen in the gray matter of the spinal cord segments of Grasscutter as shown in Plate LXXIV,Plate LXXV, Plate LXXVI and Plate LXXVII.

In the thoracic spinal cord segments, dopaminergic neurons (DPN) were seen in the gray matter of the spinal cord segments of Grasscutter to be lightly stained as shown in Plate LXXVIII, Plate LXXV, Plate LXXIX and Plate LXXX.

In the lumbosacral spinal cord segments, dopaminergic neurons (DPN) were seen in the gray matter of the spinal cord segments of Grasscutter to be deeply stained as shown in Plate LXXX, Plate LXXXI, Plate LXXXII, Plate LXXXIII, Plate LXXXIV, Plate LXXXV, Plate LXXXVI, Plate LXXXVII and Plate LXXXVIII.

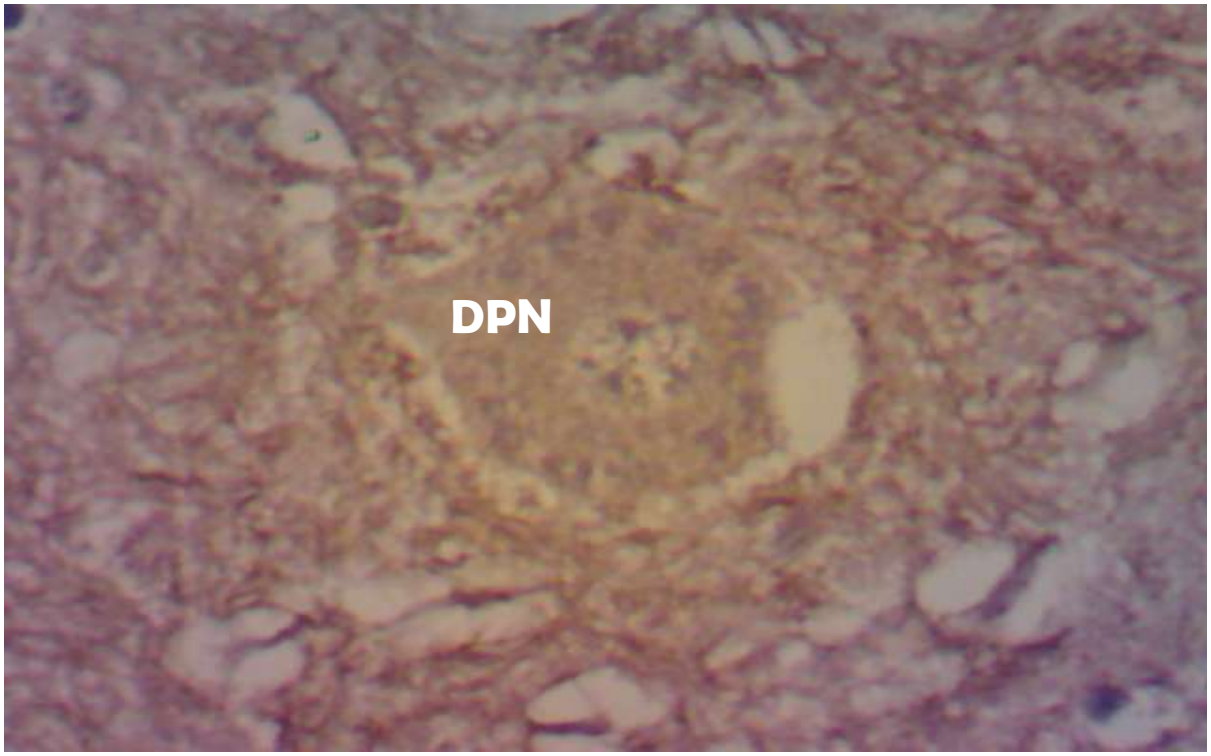
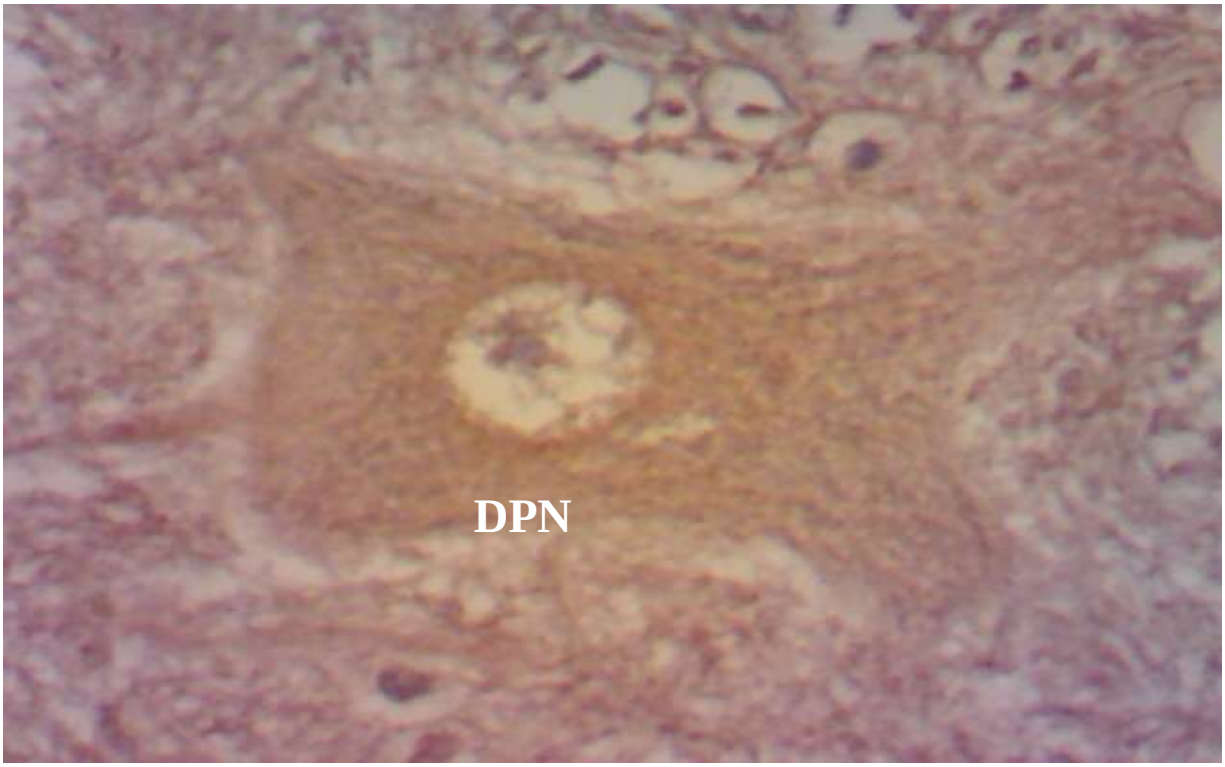


Plate LXXIV: A Transverse section of the cervical spinal cord of the Grasscutter at the level of $C_2$with anterior horn gray matter positive reaction of the presence of dopaminergeric neurons (DPN) in the region. Alpha-1 antitrysine hydroxylase method (Mag X400).



PlateLXXV: A Transverse section of the cervical spinal cord of the Grasscutter at the level of C_2arrows with posterior horn positive reaction of the presence of dopaminergeric neurons (DPN) in the region. Alpha-1 antitrysine hydroxylase method (Mag X400).



PlateLXXVI: A Transverse section of the cervical spinal cord of the Grasscutter at the level of $C_2$with anterior horn with positive reaction of the presence of dopaminergeric neuron (DPN) in the spinal cord. Alpha-1 antitrysine hydroxylase method (Mag X400).



Plate LXXVII: A Transverse section of the cervical spinal cord of the Grasscutter at the level of C_4arrow with posterior horn dopamine positive neurons (DPN). Reaction can be seen in the neurons to be weak. Alpha-1 antitrysine hydroxylase method (Mag X400).

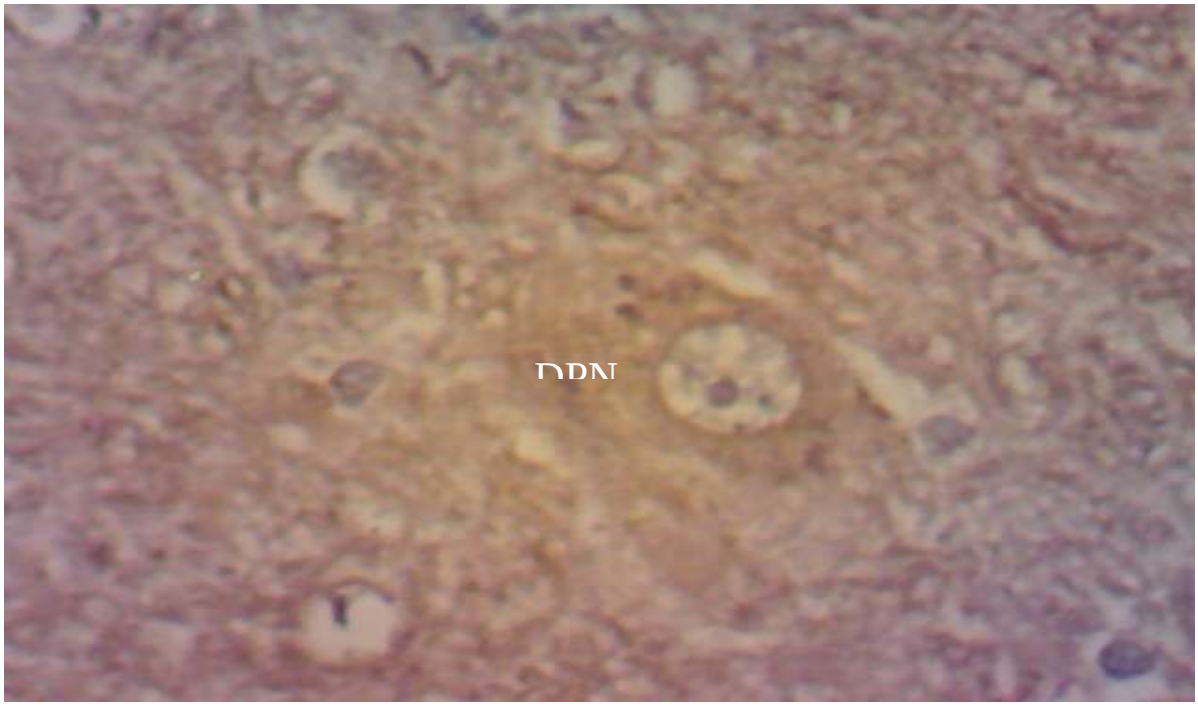
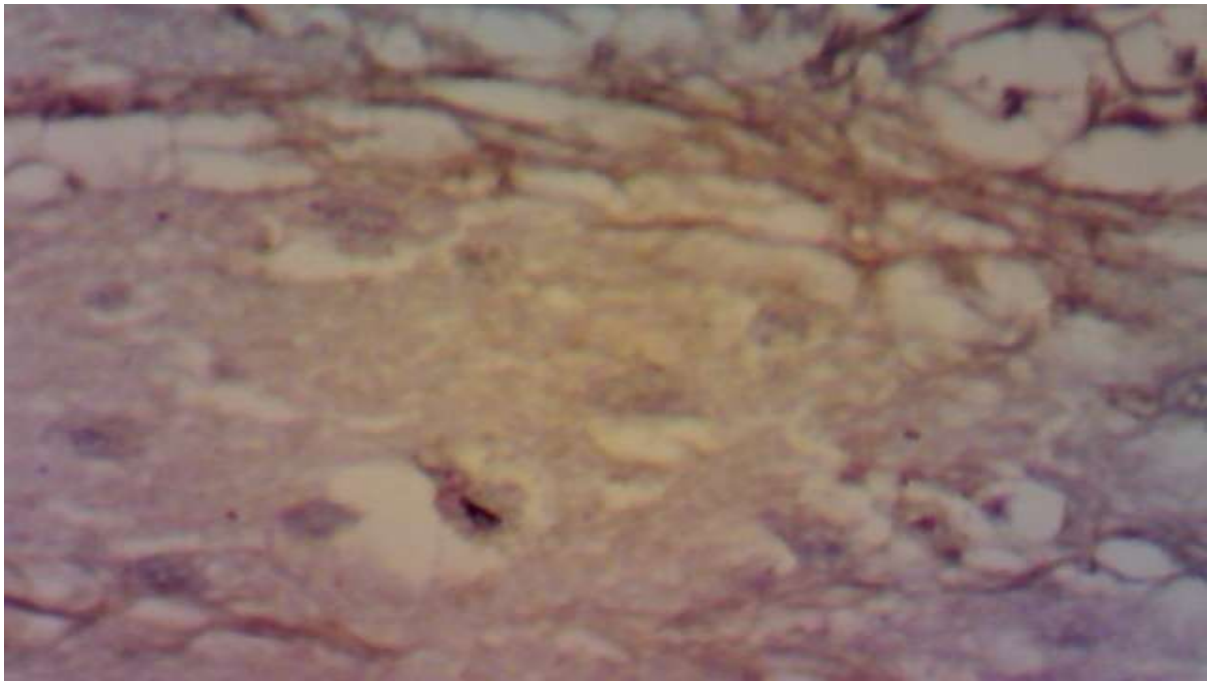


Plate LXXVIII: A Transverse section of the thoracic spinal cord of the Grasscutter at the level of $T_2$with anterior horn lighly stained dopaminergeric neuron (DPN) in the region shown to be very weak. Alpha-1 antitrysine hydroxylase method (Mag X400).



PlateLXXIX: A Transverse section of the thoracic spinal cord of the Grasscutter at the level of $T_1$with posterior horn dopaminergeric neuron in the region the very weak reaction shown. Alpha-1 antitrysine hydroxylase method (Mag X400).



PlateLXXX: A Transverse section of the thoracic spinal cord of the Grasscutter at the level of T_2 Posterior horn with weak reaction of the presence of dopaminergeric neurons in the region Alpha-1 antitrysine hydroxylase method (Mag X400).

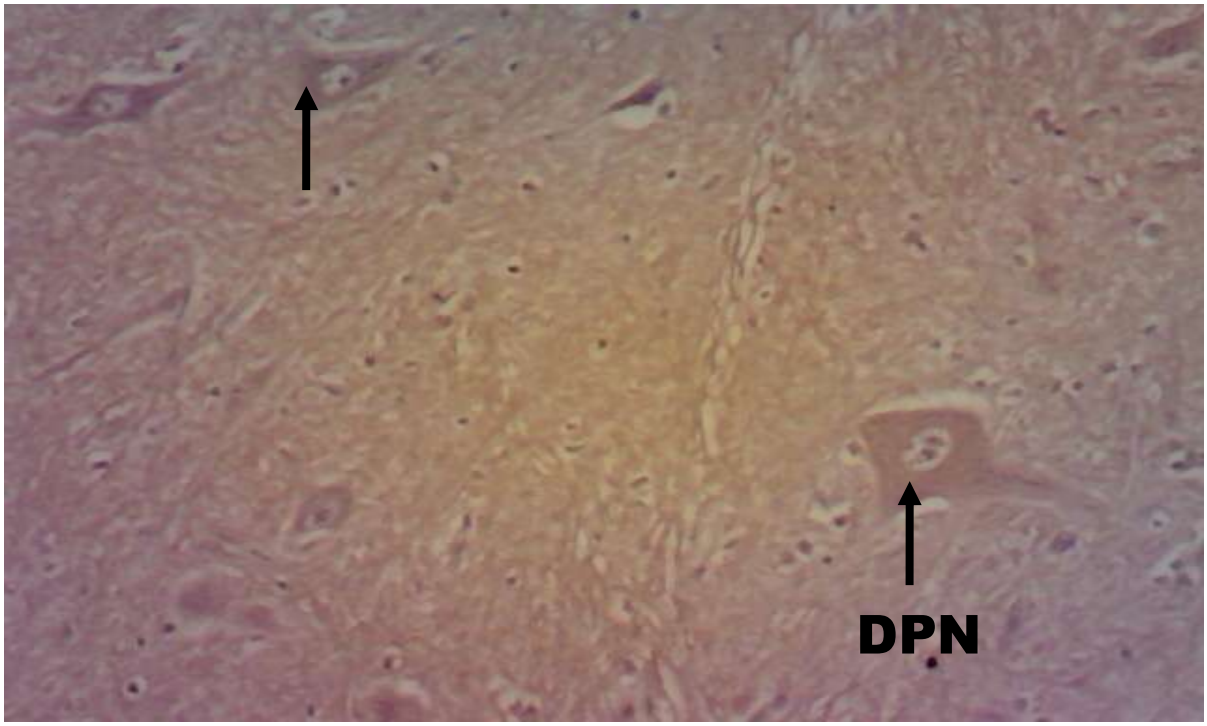
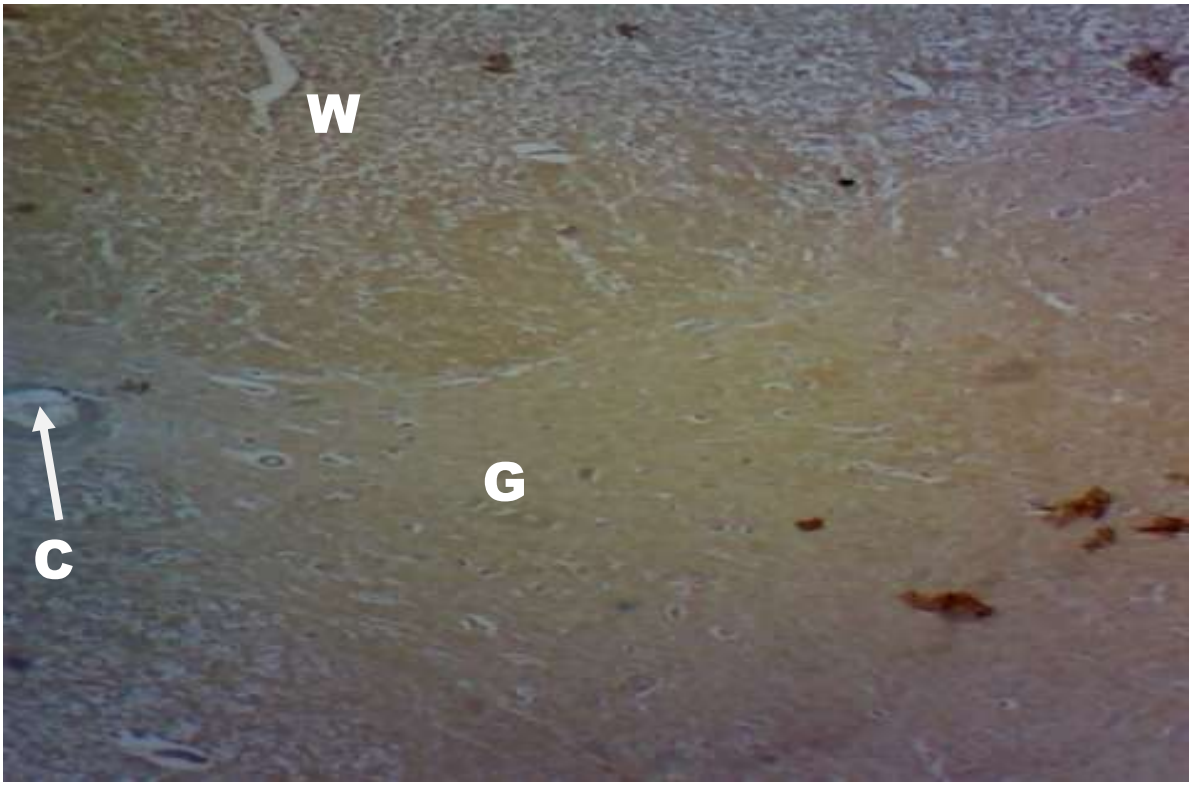
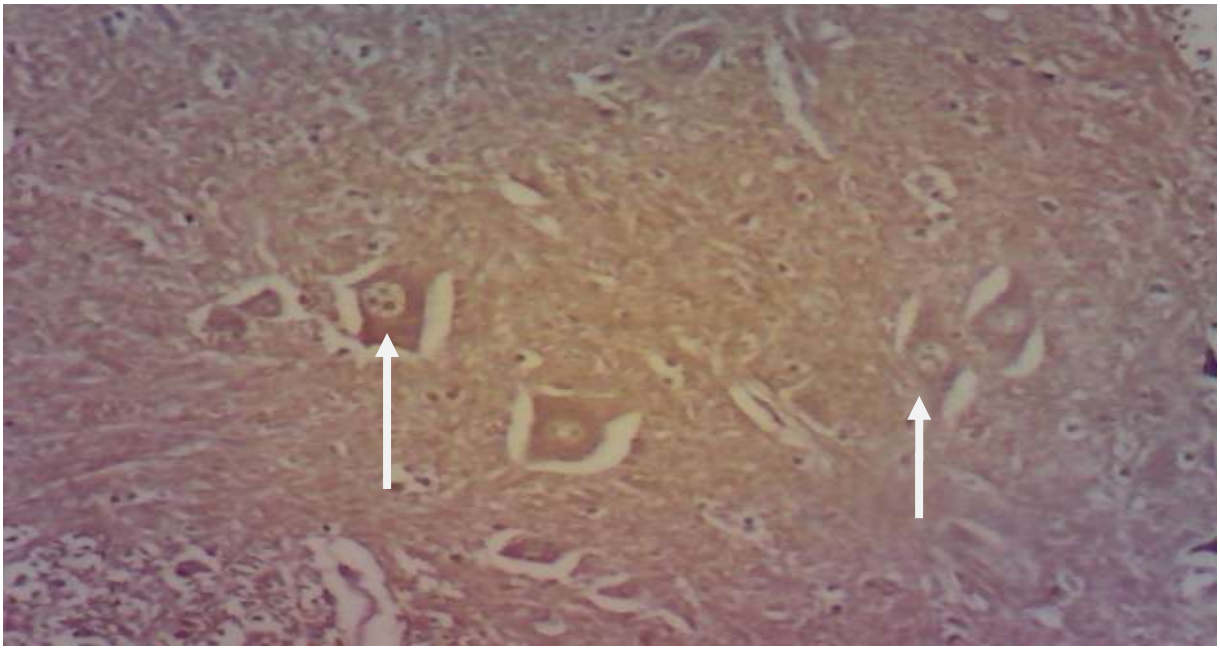


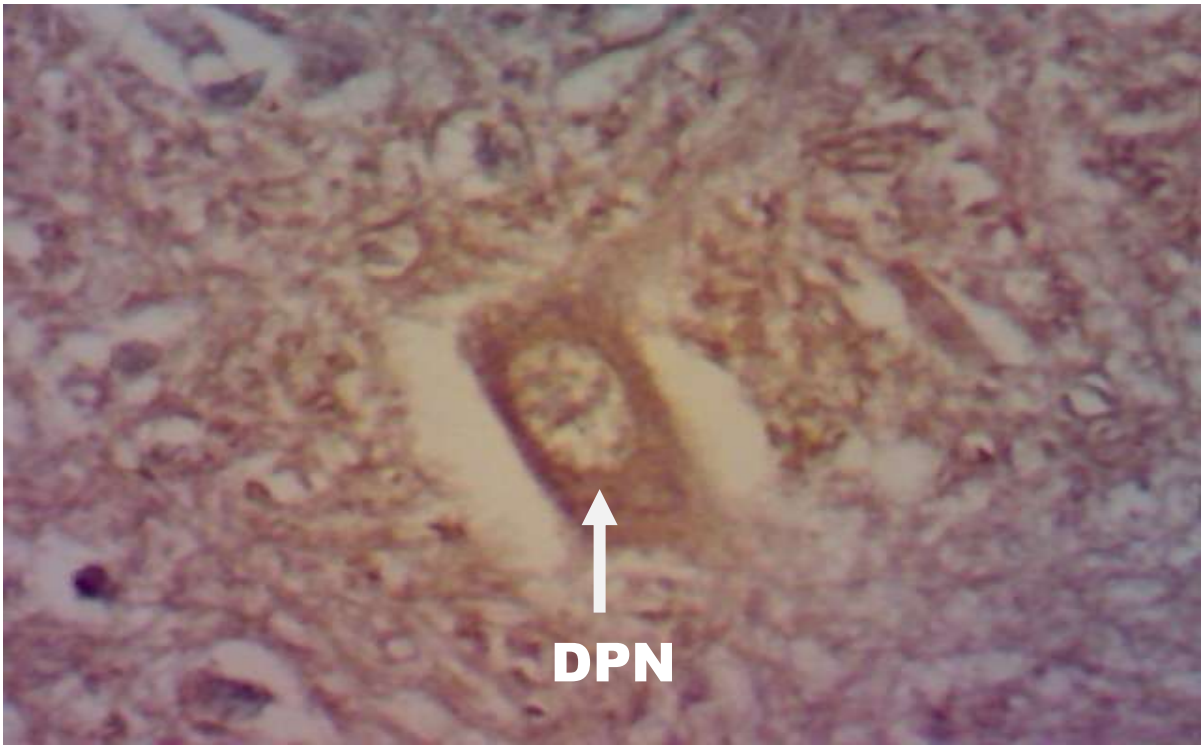
Plate LXXXI: A Transverse section of the lumbar spinal cord of the Grasscutter at the level of $L_2$with posterior horn deeply stained dopaminergeric neurons (DPN) in the gray matter of the spinal cord in the spinal cord segments of Grasscutter. Alpha-1 antitrysine hydroxylase method (Mag X400).



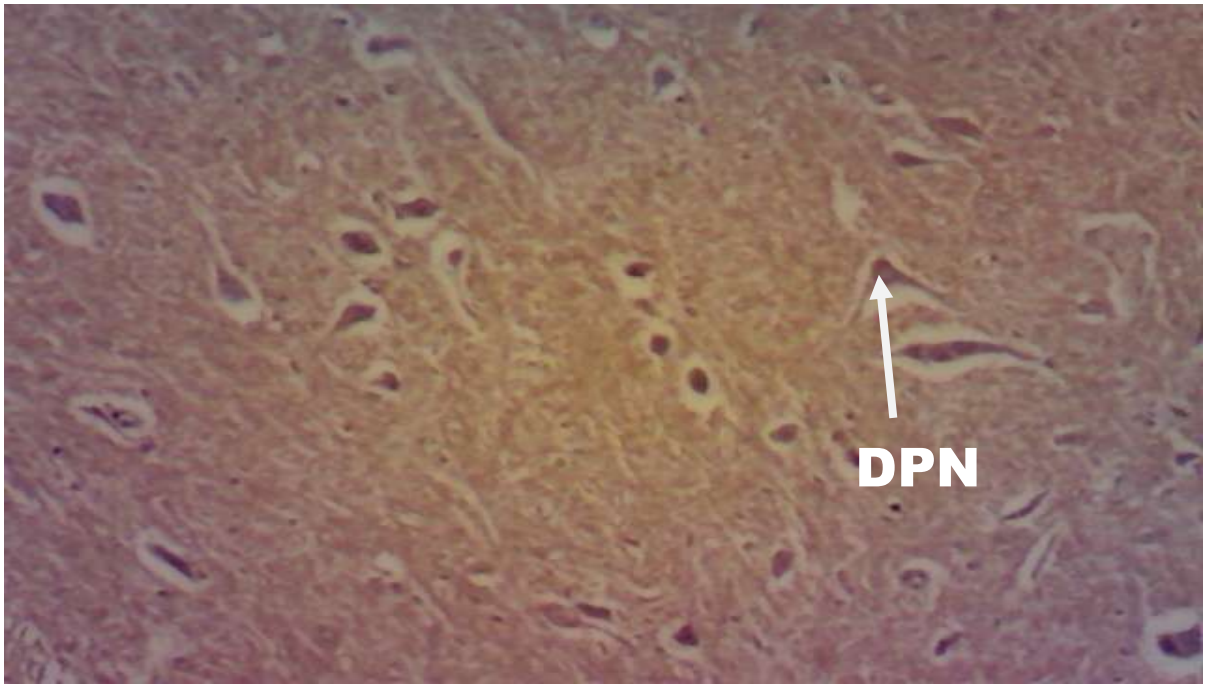
PlateLXXXII: A Transverse section of the lumbar spinal cord of theGrasscutter at the level of $L_2$with Gray matter (G), white matter (W), central canal C with the presence of dopamine in the neurons in the region. Alpha-1 antitrysine hydroxylase method (Mag X100).



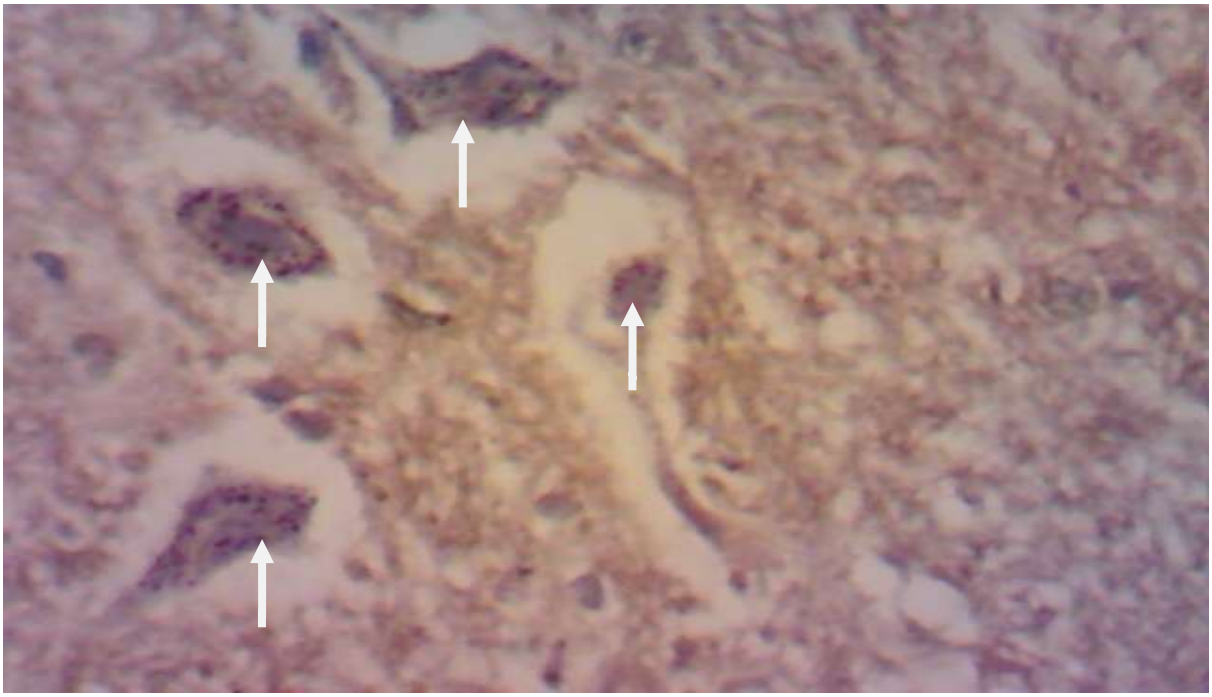
PlateLXXXIII: A Transverse section of the lumbar spinal cord of the Grasscutter at the level of L_4 with posterior horn containing deeply stained DPN (arrowed structure) in the spinal cord segments of Grasscutter. . Alpha-1 antitrysine hydroxylase method (Mag X400).



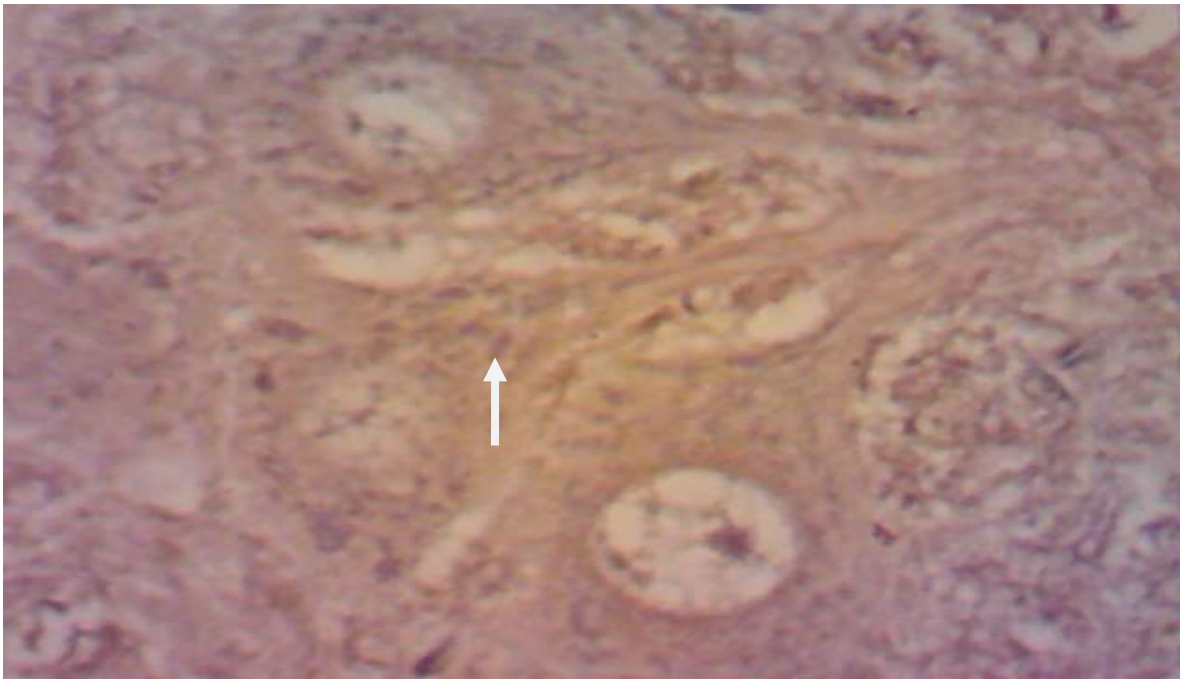
PlateLXXXIV:A Transverse section of the lumbar spinal cord of theGrasscutter at the level of L_4 with anterior horn deeply stained dopaminergeric multipolar neurons (DPN) in the gray matter of the region. Alpha-1 antitrysine hydroxylase method (Mag X400).



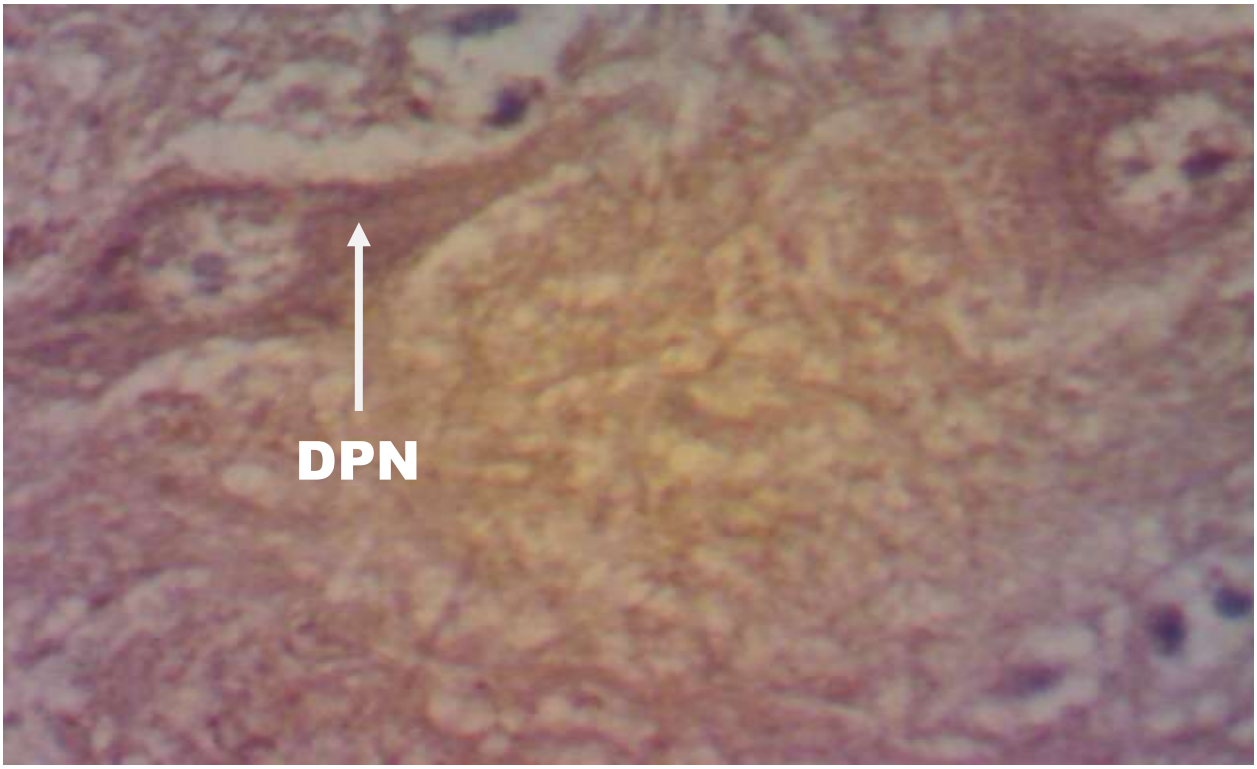
PlateLXXXV: A Transverse section of the lumbar spinal cord of the Grasscutter at the level of $L_6$with posterior horn the deeply stained of many dopaminergeric neurons (DPN) in the segment of the spinal cord of Grasscutter. Alpha-1 antitrysine hydroxylase method (Mag X250).



PlateLXXXVI: A Transverse section of the lumbar spinal cord of the Grasscutter at the level of L_6arrows withthe deeply stained dopaminergeric neurons (DPN) in the spinal cord segments of Grasscutter. Alpha-1 antitrysine hydroxylase method (Mag X400).



PlateLXXXVII:A Transverse section of the Sacral spinal cord of Grasscutter at the level of S_2anterior horn arrow with dopaminergeric neurons in the spinal cord segment of the region. Alpha-1 antitrysine hydroxylase method (Mag X400).



PlateLXXXVIII: A Transverse section of the Sacral spinal cord of the Grasscutter at the level of S_2arrow with posterior horn of the presence of dopaminergeric neurons (DPN) in the sacral spinal cord segment of Graccutter. Alpha-1 antitrysine hydroxylase method (Mag X400).

4.6 Histometry

The morphological characteristics of the cells of the cervical, thoracic,lumbar and sacral areas from the spinal cord of the Grasscutters showed mean values and SEM, minimum and maximum values for cell area (A), cell perimeter (cell size) (P), of neurons in cervical, thoracic and lumbar areas and sacral areas from spinal cord of Grasscutters. The greatest value for mean and SEM in this study was for cell perimeter lumbar ventral horn (P) and cell area (A) were calculated to be 9.30± 0.67µM and 3.28± 0.59 μM^2respectively while the least values cell perimeters and cell areas were calculated to be 3.86± 0.24µm and 0.83± 0.09μm^2 respectively were observed in the cervical spinal cord segment. Details of the measurements are shown in listed in Table 4.4

The result in Table 4.5 when analysed using *t*- test showed that the cell area in cervical ventral horn was (CVH)significantly higher than cell area in the sacral ventral horn (SVH) ($P<0.05$). In table 4.6 the *t*- test showed that cell area in the dorsal horn of cervical spinal cord (CDH) was significantly higher than the cell area in the dorsal horn of the thoracic spinal cord segment (TDH) of grasscutter while the cell area of the dorsal horn of the lumbar spinal cord segment (LDH) was significantly higher than the cell area of the dorsal horn cervical spinal cord segment (CDH) of grasscutter($P<0.05$).

In table 4.7 Data analysis was also done using student *t*-test among the various spinal cord segments. The cell perimeter (cell size), P of the cells of the ventral horn of lumbar spinal cord segment (LVH) was significantly larger than perimeter of the cells of the ventral horn of the cervical spinal cord segment (CVH), ($P<0.05$). Also cell perimeter (cell size) (P) of the cells of the ventral horn of cervical spinal cord segment (CVH) was significant larger than perimeter of the cells of the ventral horn of the sacral spinal cord segment (SVH) ($P<0.05$) and it was also observed that the perimeter of the ventral horn of lumbar spinal cord

segment (LVH)was significant larger than perimeter of the cells of the ventral horn of the thoracic (TVH) and sacral spinal cord segments (SVH) ($P<0.05$).

In table 4.8 the result for data analysis of the cell sizes or cell perimeter of the dorsal spinal cord horns using student t-test is as follows cell perimeter (P) of the cells of the dorsal horns of lumbar spinal cord segment was significantly higher when compared with the cell perimeter (P) of of the dorsal horn of the cervical (CDH) ($P<0.05$) and dorsal horn of thoracic spinal cord segments (TDH) ($P<0.05$).

Table 4.3: Nuronal perimeter, P (µM)and neuronal area, A (μM^2) of neuronal cells in cervical, thoracic, lumbar and sacral spinal cord segments of Grasscutter with minimum, maximum and mean values

Variables	Minimum	Maximum	Mean ± SEM
CVH A	1.65	3.71	2.56± 0.13
CVH P	5.05	8.08	6.36± 0.16
CDH A	0.91	1.40	1.43± 0.09
CDH P	2.91	5.07	3.86± 0.24
TVH A	1.01	1.48	1.05± 0.60
TVH P	1.31	2.01	2.11± 0.18
TDH A	0.06	0.91	0.46± 0.11
TDH P	1.15	1.01	1.10± 0.15
LVH A	2.04	4.37	3.30± 0.42
LVH P	7.36	11.48	9.30± 0.67
LDH A	1.25	5.50	3.28± 0.59
LDH P	4.77	10.09	7.56± 0.67
SVH A	1.11	1.73	2.15± 0.18
SVH P	3.07	5.86	5.47± 0.46
SDH A	1.46	1.82	1.73± 0.21
SDH P	3.26	5.41	4.00± 0.37

Descriptive statistics of the values of cell area (A) (μm^2) cell perimeter (cell size), (P) (µm) CVH is veneral horn of cervical spinal cord segment, CDH is dorsal horn of cervical spinal cord segment, TVH is veneral horn of thoracic spinal cord segment,TDH is dorsal horn of thoracic spinal cord segment, LVH is veneral horn of lumbar spinal cord segment, LDH is dorsal horn of lumbar spinal cord segment, SVH is veneral horn of sacral spinal cord segment, SDH is dorsal horn of sacral spinal cord segment

Table 4.4: Analysis of data showing comparison between the cell area (A) (µm2) of the cells of ventral horns of cervical,thoracic lumbar and sacral spinal cord segments of grasscutter using student's t-test

	Mean ± SEM	***T***	**p-value**
CVH A	2.56± 0.13	-1.189	0.170
TVH A	1.05± 0.60		
CVH A	2.56± 0.13	-1.623	0.071
LVH A	3.30± 0.42		
CVH A	2.56± 0.13	-1.710	0.913
SVH A	2.15± 0.18		
TVH A	1.05± 0.60	4.919	*****0.014
LVH A	3.30 ±0.42		
TVH A	1.05± 0.60	5.988	*****0.003
SVH A	2.15± 0.18		
LVH A	3.30 ±0.42	-2.976	0.704
SVH A	2.15± 0.18		

Analysis of data was done using student's *t*-test of cell area (A) (μm^2) between the cell area (A) of the following CVH (veneral horn of cervical spinal cord segment) and TVH,(veneral horn of thoracic spinal cord segment), CVH (veneral horn of cervical spinal cord segment) and LVH, CVH (veneral horn of cervical spinal cord segment) and SVH (veneral horn of sacral spinal cord segment), then between TVH (veneral horn of thoracic spinal cord segment) and LVH (veneral horn of lumbar spinal cord segment), TVH (veneral horn of thoracic spinal cord segment) and SVH (veneral horn of sacral spinal cord segment), LVH (veneral horn of lumbar spinal cord segment) and SVH (veneral horn of sacral spinal cord segment). Data were expressed as mean ± standard error of mean (±SEM) of values collected, where * is significantly different ($P<0.05$) between the the various spinal cord segments of Grasscutter.

Table 4.5: Analysis of data showing comparison between the cell area (A) (μm^2) of the cells of ventral horns of cervical,thoracic,lumbar and sacral spinal cord segments of grasscutter using student's t-test

	Mean ± SEM	***T***	**p-value**
CDH A	1.43± 0.09	-5.291	*0.002
TDH A	0.46± 0.11		
CDH A	1.43± 0.09	4.165	0.033
LDH A	3.28± 0.59		
CDH A	1.43± 0.09	-2.564	0.293
SDH A	1.73± 0.21		
TDH A	0.46± 0.11	-6.309	*0.001
LDH A	3.28± 0.59		
TDH A	0.46± 0.11	1.093	0.419
SDH A	1.03 ±0.21		
LDH A	3.28± 0.59	1.832	0.141
SDH A	1.73± 0.21		

Analysis of data was done using student's *t*-test of the values of cell area (A) (μm^2) from the various spinal cord segments between cell areas of the following regions CDH (dorsal horn of cervical spinal cord segment) and TDH,(dorsal horn of thoracic spinal cord segment), CDH (dorsal horn of cervical spinal cord segment) and LDH (Dorsal horn of lumbar spinal cord segment),, CDH (dorsal horn of cervical spinal cord segment) and SDH (dorsal horn of sacral spinal cord segment), then between TDH (dorsal horn of thoracic spinal cord segment) and LDH (dorsal horn of lumbar spinal cord segment), TDH (dorsal horn of thoracic spinal cord segment) and SDH (dorsal horn of sacral spinal cord segment), LDH (dorsal horn of lumbar spinal cord segment) and SDH (dorsal horn of sacral spinal cord segment). Data were expressed as mean ± standard error of mean (±SEM) of values collected, where * is significantly different ($P<0.05$) between the the various spinal cord segments of Grasscutter.

Table 4.6: Analysis of data showing comparison between the cell perimeter (cell size) (P) (μm) of the cells of ventral horns of cervical,thoracic,lumbar and sacral spinal cord segments of grasscutter using student's t-test.

	Mean ± SEM	***t***	**p-value**
CVH P	6.36± 0.16	-3.801	*0.002
TVH P	2.11± 0.18		
CVH P	6.36± 0.16	-4.250	*0.013
LVH P	9.30± 0.67		
CVH P	6.36 ±0.16	3.636	*0.015
SVH P	5.47± 0.46		
TVH P	2.11± 0.18	-4.005	*0.016
LVH P	9.30 ±0.67		
TVH P	2.11± 0.18	1.709	*0.003
SVH P	5.47± 0.46		
LVH P	9.30 ±0.67	6.236	*0.001
SVH P	5.47± 0.46		

Analysis of data was done using student's *t*-test between the perimeter (cell size) (P) (μm) of CVH (veneral horn of cervical spinal cord segment) and TVH,(veneral horn of thoracic spinal cord segment), CVH (veneral horn of cervical spinal cord segment) and LVH, CVH (veneral horn of cervical spinal cord segment) and SVH (veneral horn of sacral spinal cord segment), then between TVH (veneral horn of thoracic spinal cord segment) and LVH (veneral horn of lumbar spinal cord segment), TVH (veneral horn of thoracic spinal cord segment) and SVH (veneral horn of sacral spinal cord segment), LVH (veneral horn of lumbar spinal cord segment) and SVH (veneral horn of sacral spinal cord segment). Data were expressed as mean ± standard error of mean (±SEM) of values collected, where * is significantly different ($P<0.05$) between the the various spinal cord segments of Grasscutter.

Table 4.7: Analysis of data showing comparison between the cell perimeter (cell size) (P) (μm) of the cells of dorsal horns of cervical,thoracic,lumbar and sacral spinal cord segments of grasscutter using student's *t*-test

	Mean ± SEM	***T***	**p-value**
CDH P	3.86± 0.24	-4.046	*0.002
TDH P	1.10± 0.15		
CDH P	3.86± 0.24	-5.111	*0.001
LDH P	7.56± 0.67		
CDH P	3.86± 0.24	-1.237	*0.004
SDH P	4.00± 0.37		
TDH P	1.10± 0.15	-3.245	*0.018
LDH P	7.56± 0.67		
TDH P	1.10± 0.15	1.776	*0.002
SDH P	4.00± 0.37		
LDH P	7.56± 0.67	2.273	*0.001
SDH P	4.00± 0.37		

Analysis of data was done using student's *t*-test between the perimeter (cell size) (P) (μm); CDH (dorsal horn of cervical spinal cord segment) and TDH,(Dorsal horn of thoracic spinal cord segment), CDH (dorsal horn of cervical spinal cord segment) and LDH (Dorsal horn of lumbar spinal cord segment),, CDH (dorsal horn of cervical spinal cord segment) and SDH (dorsal horn of sacral spinal cord segment), then between TDH (dorsal horn of thoracic spinal cord segment) and LDH (dorsal horn of lumbar spinal cord segment), TDH (dorsal horn of thoracic spinal cord segment) and SDH (dorsal horn of sacral spinal cord segment), LDH (dorsal horn of lumbar spinal cord segment) and SDH (dorsal horn of sacral spinal cord segment). Data were expressed as mean ± standard error of mean (±SEM) of values collected, where * is significantly different ($P<0.05$) between the the various spinal cord segments of Grasscutter.

CHAPTER FIVE

5.0 DISCUSSION

In the present study it was observed that the mean body weight of the male Grasscutters (*Thryonomysswinderianus)* was significantly higher than that of the female Grasscuttersand this was in agreement with the findings in other studies on the same animals. Opara, (2010) report similar mean weight when he studied the haematology and major parasitesof Grasscutters. Other workers include Opara, (2010) reported similar weight when he studied the occurrence and prevalence of gastrointestinal helminthes in the captive-reared Grasscutter (*Thryonomys swinderianus*, *Temminck*). Also, in the studies onits reproductive characteristics, Olukole *et al*.,2010 reported similar findings.

The spinal cord of the Grasscutter was observed to be elongated, approximately cylindrical part of the central nervous system, occupying the vertebral canal which was in agreement with the findings in other mammals as in the horse and cattle (Habel,1975, Claridge *et al.,* 2010, S*adu*llah *et al.*,2013), Ovines(Rao, 1990), dogs (Miller *et al*.,1964), Buffalos (Rao, 1976, Abu-zaid,1982), cats (Thomas and Combs,1962),rabbits (Santos,*et al.*,1999,Farag *et al.,* 2012,Al-Saffar and Al-Haaik,2017), males and females Indian gray mongoose (Rasouli*et al*.,2015) donkeys (Hifny *et al.*,1982, Ocal and Haziroglu 2015), African giant rats (Olude *et al*.,2015) and humans (Kameyama *et al.,* 1996;Ariane *et al.,* 2017).Also work done in the following animals are in agreement with findings in this present study. In goats (Sharma*et al*.,1973, Kahvecioğlu *et al*., 1995Abd El-ghany,1995), impala (Rao *et al*.,1995), sheep (Done *et al.*,1985) and Wistar rat (Molander *et al.,* 1989 and Vera and Meyer-siegler, 2003).

The basic structural features of the Grasscutter spinal cord are typical of therodents'spinal cord and the spinal cord was seen to be enclosed in the dura, arachnoid and pia maters which

are in agreement with studies on the spinal cords of other rodents (Hebel and Stromberg, 1976; Bjugn *et al.,* 1989).

The spinal cord accounts for about 0.06% of body weight of the Grasscutter. It is smaller than that of the spinal cord of rabbit which weighs 5-7g (about 0.5% body weight) (Farag *et al.,* 2012) but are about the same with that of the horse which weighed 250-300g and about 0.06% body weight.

Themeanspinal cordlength of Grasscutter was observed to be 20.30 ± 1.50cm in the males and 19.40 ±1.04 cm in the females. Spinal cord length has been described in several animals amongst which are: 34.7 cm in Wistar rats about 70.4% body length (Hebel and Stromberg, 1976; Aguh *et al.,* 2013); 34.7 cm in rabbits about 99.1% of body length (Farag *et al.,* 2012); 53.8 cm in goats (61.6% of body length) (Kahvecioglu *et al.,* 1995); 167.2cm in horses about 68.6% of body length (S*adu*llah *et al.,* 2013); 106.8 cm in donkeys (53.4% of body length) (Ocal and Haziroglu, 2015); 61.5 cm in brockets about 64.7% of body length (Lima *et al.,* 2010). Bjugn *et al.,*(1989) reported the spinal cord length of mice as 4.4cm about 55.7% of body length.

The positive linear relationship between the tail length and the spinal cord weight of the males and the females established that the variability observed in the spinal cord weight may be explained by the tail length in both sexes, indicating that the filum terminale contributed to the weight of the spinal cord. The mean tail length value was11-10 ±0.56 cm in the males and 10..20 ±0.98 cm in the females and these findings were different from other animals as 107cm in the African giant rat (Olude *et al,*2015), 47cm in the fox squirrels, 87cm in the red squirrel, 50cm in the California ground squirrel and in mice (Brian and William,2000).

The enlargements at the cervical and lumbosacral segments provide innervations to the fore and hind limbs respectively, contributing to the brachial and lumbosacral plexuses (Bjugn *et*

al., 1989; Rahmanifar *et al.,* 2008). Worthy of note is that the extents of the enlargements, which begin earlier in the spinal segments, are more extensive in the rodents and may characteristically add to limb efficiency than other mammals. Thus; regional anaesthesia for surgical manoeuvres in the Grasscutter can be readily achieved based on the knowledge of the extent and anatomical locations of these enlargements (Jonathan and Gerbrand, 2005). The cervical enlargement was between $C_2 – C_7$ in Grasscutters and this finding was different from the findings from other mammals. In the deer, this enlargement includes segment $C_4 – T_1$ (Lima *et al.,* 2010), while in the rabbit, it is between $C_5–T_1$ (Farag *et al.,* 2012). It is formed between $C_7 – C_8$ inthe pig (Dellmann and McClure, 1975), $C_6 – T_1$ in the dog (Miller *et al.,* 1964), between $C_6 – T_2$ in the buffalo (Abu-zaid, 1982) and camel (Mansour, 1980) and $C_5–T_2$ in the Indian sheep (Rao,1990) and donkey (Mansour,1980). Moreover, this dilatation corresponds to C_5-T_2 in the sheep (Rao, 1990) and $C_6 – C_8$ in the cat (Thomas, 1962).

It can be agreed that the extent of spinal cord enlargement in the cervical region of the Grasscutter may be the reason for the extreme mobility of its fore limbs. The lumbosacral enlargement in the Grasscutter was shown to extent from L_1 to the $S_{2..}$ This finding was not the same with what is recorded in other mammals. In the rabbit, lumbosacral enlargement was formed between $L_4 – S_3$ (Farag *et al.,* 2012). In the donkey, it is found between $L_2 – S_1$ (Mansour, 1980, Haziroglu and Ocal,1988), between $L_6 – S_1$ in the camel (Mansour, 1980), between the last three lumbar and first two sacral in Buffalo (Abu-zaid, 1982), between $L_4 – S_1$ in the sheep as shown by Rao (1990), between $L_6 – L_7$ in the pig, between $L_5 – L_7$ in the cat (Thomas, 1962), and between $L_4 – S_1$ in the dog (Dellmann and McClure, 1975). In the deer, this enlargement of the spinal cord was found between L_3-L_6 (Lima *et al.,* 2010).

The basic histological features of the Grasscutter spinal segments observed in the present study was typical with inner gray matter core surrounded by an outer white matter which are similar to those reported in other rodents (Hebel and Stromberg, 1976; Portiansky *et al*., 2004, Watson *et al.,* 2008; Olude *et al.,* 2015,Gilmore *et al.*, 2017, Al-Saffar *et al.,* 2017) and in other mammals like Wistar rats (Portiansky *et al.,* 2004), donkeys (Ocal and Haziroglu,2015)and humans (Choi *et al*.,1996, Ellingson *et al*.,2015) in the Buffalos (Rao, 1976), in the deer (Lima *et al.,* 2010) in dog (Fletcher *et al.,* 1979) in horse (Bolat*et al*.,2012) and in the rabbit (Santos *et al.*, 1999, Greenaway*et al*., 2001,. Kirmani and Ullah, 2011,Farag *et al.,* 2012). The gray matter included bodies of different size and types of neurons which were observed in a butter-fly shaped structure of two dorsal horns and two other ventral horns in addition to the two intermediate lateral horns with a central canal located in the central point of the gray matter. The white matter surrounds the gray matter. Freire *et al*.,(2009) showed that neurons tend to concentrate in these three main regions of gray matter mentioned above and current description of this matter on the spinal cord of Grasscutter was similar to those described for other mammalian species such as rat (Saito, *et al*.,1994,Reuss *et al.*,2001) and in humans and rabbit (Kolesar *et al*.,2015). The gray matter and nerve cell were distributed in the spinal cord of Grasscutter and the spinal cord segments showed thinner dorsal horns compared to the ventral horns and they are extended up to reach the dorsal border of the spinal cord whereas the ventral horns were wider and shorter and they did not extend to reach the ventral border of the spinal cord.

Although the basic structural patterns were seen throughout the spinal cord segments of the Grasscutters the shape and structure of the spinal cord vary at different levels namely cervical, thoracic, lumbar, and sacral segments. The thoracic region of the spinal cord of

Grasscutter differs from the cervical region illustrated in the resultsshown in the present study.

The thoracic spinal cord segments exhibit slender posterior gray horns and smaller anterior gray horns with fewer motor neurons. The lateral gray horns, on the other hand, were shown to be well developed in the thoracic region of the spinal cord which contains the motor neurons of the sympathetic division of the autonomic nervous system. These Findings are similar to those reported for other rodents (Hebel and Stromberg, 1976; Watson *et al.,* 2008; Elkarmoty, 2012, Olude *et al.,* 2015). The remaining structures in the thoracic region of the spinal cord closely correspond to the structures shown in the cervical cord region. These are the posterior median sulcus, anterior median fissure, fasciculus gracilis and fasciculus cuneatus,the posterior white column, lateral white column, central canal, and the gray commissure.

The gray matter contains abundant presence of neuronal cell bodies while the outer zone of white matter is relatively lighter due to scanty presence of neuronal cell bodies. The gray matter was characterized and consist of ventral, dorsal and lateral horns and these findingsare in agreements with other mammals such as dogs (Getty *et al.,* 1975), Buffalos (Rao, 1976), deer (Lima *et al.,* 2010) and in rabbit (Farag *et al.,* 2012). Ventral horns of the spinal gray matter are known to contain numerous motor neurons (Butler and Hodos, 2005) and receive extra axonal contributions from the brachial and lumbosacral plexi (Gruener and Biller, 2008).

The ventral horns of the gray matter in the Grasscutter were noted to be wider than dorsalhorns of the spinal cord of Grasscutter. The ventral horn of gray matter coordinates the motor neuron; this explains its relative larger in size compared to the dorsal horn (Gruener

and Biller, 2008). The ventral horn appears wider at the cervical region and the lumbosacral region than other segments. This corresponds to the cervical and lumbosacral enlargements (Gruener and Biller, 2008, Gilmore *et al.,* 2017).

Differently to the local rabbit (Farhan*et al.,* 2011) the ventral horn (motor neurons) of Grasscutter cervical segments showed an extensive area occupied mainly by large and medium sized motor neurons. However, the Grasscutter uses its tail to defend itself and has been reported to stand on its hindlimbs. The forelimbs of Grasscutterhave shown high locomotor dexterity (Olude *et al.,* 2015). This probably accounts for the ventral horns of cervical and lumbosacral regions being more developed than other segments as shown in the present study.

The dorsal horn of the spinal cord is one of the gray columnsof the spinal cord observed in the present study and the finding is in agreement with those in the other mammals. The dorsal hornreceives several types of sensory information from the body, including fine touch, proprioception, and vibration. This information is sent from receptors of the skin, bones, and joints through sensory neurons (Getty, 1975).

Shape variations were observed in the central canal of theGrascutter spinal cord and in the Grasscutter, the cervical spinal cord segments are horizontally ellipsoidal as compared to a vertical slit in the laboratory Wistar rat (Hebel and Stromberg, 1976) and African giant rat (AGR), (Olude *et al.,* 2015), while shape of the thoracic spinal cord segments aretrapezoid but with vertical slit in the laboratory rat and African giant rat (Hebel and Stromberg, 1976; Olude *et al.,* 2015); the shape of the central canals of the sacral spinal cord segments were oval while those of laboratory rat and African giant rat were vertically slit (Hebel and Stromberg, 1976; Olude *et al.,* 2015).

The inner gray matter of the spinal cord of Grasscutter in the present study stained dark purple due to the abundant presence of nissl substances in the neuronal cell bodies while the outer zone of white matter stained relatively lighter due to scanty neuronal cell bodies in the region. Nissl-stained dark neurons were observed in the spinal cord of the Grasscutter and these findings are in agreements with the findings in other rodents(Dellmann and McClure. 1975).

The cells in the anterior gray horns of the cervical, thoracic,lumbar and sacral segments of the spinal cord of Grasscutter containmultipolar motor neurons. Their cytoplasms are characterized by a prominent vesicular nucleus, a distinct nucleolus and coarse clumps of basophilic material, the Nissl substances. The Nissl substances extend into the dendrites but not into the axons of neurons of the spinal cord of Grasscutter. This findingis in agreement with the findings in the Wistar rat (Brichta and Grant, 2015) and in horse (Bolat *et al*., 2012, Bahar *et al*.,2013) and sheep (Done*et al*.,*1*985) and donkey (Hifny *et al*.,1984).

Nissl substances had been reported to play key roles in cellular metabolism (Dyce *et al*., 2010)and Nissl substances range inappearance from rhomboid blocks in large neurons to particulate materials in the cell bodies of smaller neurons. These bodies are nodal points in the endoplasmic reticulum, which permeate the cell body and dendrites and absent at the axon and axon hillock (Farag *et al*., 2012). It had been reported that Nissl body comprises of broadsheet of rough or granular endoplasmic reticulum, free ribosomes and ribosomes in clusters or rosette. These ribosomes had been found in large amounts in the cytoplasm of neurons as cytoplasmic riboxyribonucleic acid under the control of nuclear deoxyribonucleic acid (DNA), cytoplasmic RNA is concerned with protein synthesis. The protein synthesized is transported down through the axons by axoplasmic flow or transport(Fitch *et al*., 2008). Each nerve cell requires large amounts of protein to maintain their integrity and perform their

functional activities. The presence of Nisslbodies in the neurons of cervical, thoracic,lumbar and sacral regions of the spinal cord of Grasscutter could be responsible for the synthesis of protein molecules that are essential for the transmission of nerve impulses between neurons.

Nissl bodies also serve to maintain nerve fibers. The synthesized proteins travel along the dendrites and replace proteins that are destroyed in cellular activity. Subsequently, the surplus proteins that produce Nissl bodies are transmitted to the Golgi apparatus. There they are stored temporarily and to some are added carbohydrates (Fix, 2008). The anterior horn cells of the spinal cord segments of thegrasscuuter contain nissl substances**.** The progressive selective staining of the Nissl substance produces an extremely clear and characteristic cellular picture in the spinal cord of Grasscutterwhich is of fundamental importance in neurocytology, since it furnishes the most delicate and accurate criterion for the well being of the neurons **as** well as providing information for any deviation from the normalhealthy state of the neurons.

Series of studies have shown that the material from which the Nissl substances areelaborated areformed primarily round the nucleolus inside the nucleus and migrates towards the periphery of the nucleus, and then diffuses gradually through the nuclear membrane to form Nissl bodies in the cytoplasm (Ghazi and Gholami,1993).The original descriptions of Nissl bodies specified them to be basophilic masses within the cytoplasm of neurons. Later cytochemical investigations demonstrated that the basis for their basophilia resides in their content of ribonucleic acids (Ghazi and Gholami, 1993).

The elegant study by Guillery, (2005) of the fine structure of mammalian neurons has conditioned the expectation that Nissl bodies everywhere will be found to consist of more or

less ordered and compact arrays of granulated endoplasmic reticulum. The structure of Nissl bodies consists of a granular and concentrated section of the endoplasmic reticulum. The Nissl substance that creates the bodies is made up of RNA, proteins, lipids and acid polysaccharides that aid in the creation of proteins. It is believed that the majority of proteins created by Nissl bodies are used inter-cellularly(Humberto *et al.*,2015).

In the present study, Nissl bodies were found only in the soma and dendrites of a neuron and do not appear in the axon and the proteins formed by the neurons of the spinal cord of Grasscutter are likely carried to the axon by axontransmitters. Nissl bodies are essential to the well-being of a neuron; a decrease in the number of Nissl bodies in a neuron indicates neural degeneration (Lima *et al*., 2010).Nissl body structures change abruptly in the case of inflammatory disease, oxygen deprivation or trauma to the nervous system(Lima *et al*., 2010).

Bovine and human spinal motor neurons usually have a stellate appearance, with their primary dendrites emerging from several points around the circumference of a rounded cell body. Studies have shown that neurons from the spinal cords of grass frogs have dendrites emerging from opposite ends of a triangular or fusiform cell body. The cell bodies of most motor neurons in the posterior horns of the spinal cord are much smaller than those of motor neurons of the anterior horns of the spinal cord segments of the Frog. These observations in the spinal cord of grass frog suggest that larger neurons may contains more nissl bodies than smaller ones (Reece *et al*.,2011; Llinás, 2014).

In the animal kingdoms, a positive correlation exists between the size of a cell and its Nissl bodiescontent. Spinal motor neurons of the anterior horn cells are among the largest mononucleate cells in animals, they can be suspected to have more Nissl bodies in their soma than posterior horn cells of the spinal cord of Grasscuuter which are smaller in size. Spinal

motor neurons organize their ribosomes into Nissl bodies so most of the RNA in motor neuron cell bodies are ribosomal RNA (López-Muñoz *et al.,* 2006).

Furthermore, Nissl bodies help to maintain motor neuronal size and shape. An interesting question arises: are the positions of ribosomes embedded within intersections of the cytoskeletal struts determined by genetic instructions that co-localize specific ribosomes and cytoskeletal junctions, thereby specifying size, shape and protein synthesis in each neuron. Therefore, the presence of Nissl bodies in the spinal cord of Grasscutter contributes to the shapes and sizes of neurons in the spinal cord of Grasscutter.

Also, Nissl bodies serve, mainly, to synthesize and to release proteins that are essential for neuronal growth and regeneration of axons in the peripheral nervous system. It is important to know that in some physiological conditions and in certain pathologies, Nissl bodies can change and even dissolve and disappear. As in the conclusion of the chromatolysis (Monica *et al.,* 2018).In addition, when there is damage to the neuron or problems in its functioning, the bodies of Nissl are mobilized and gather in the periphery of the cytoplasm to try to mitigate the damages (Monica *et al.,* 2018).On the other hand, Nissl bodies can store proteins to prevent their release into the cytoplasm of the cell. Thus, it does not interfere with the functioning of the neuron, releasing only when needed(Ochi *et al*., 1979).

The sections of spinal cord of Grasscutter stained with Golgi-Cox method revealed both the cell bodies of neurons, and their axons and dendrites. Generally, a cell body gives rise to a single axon and fewprimary dendrites that branch and taper along their lengths. The various shapesand sizes of dendritic trees are one of the main attributes of neurons seen in the spinal cord of Grasscuter. Several sizes and shapes of unclassified neurons were seen in the spinal cord of the Grasscutter.

Some neurons were observed with long axons and others were observed with shoer neurons with short axons. Neurons with long axons and other unipolar neurons are found mainly in the posterior horns of the spinal cord of Grasscutter especially in the cervical spinal segments. Some of the neurons of the posterior horns were characterized by a spindle or fusiform cell body giving rise to two or three dendrites which show recurrent pattern of branching. The dendritic extent appears to be restricted to a narrow band of gray matter within the posterior horn. These findings could be compared to those of Matsushita (1969) and Mannen and Suguira (1976) who showed and described in their Golgi studies on the spinal cord of kitten whichwas similar to the neurons in the posterior horn, having a fusiform or spindle shaped cell body. Beal and Cooper (1978) also described similar type of neurons in the posterior horn of spinal dorsal horn of monkey, while Todd and Lewis (1986) revealed the presence of neurons in Wistar rat spinal posterior horn.

In the anterior horns of the spinal cord segments of Grasscutter neuron with short axon and several multipolar neurons were identified in the gray matter of the spinal cord. These neurons whichare mainly found in the anterior horns of the spinal cord of Grasscutter are characterized by rounded or polygonal soma giving rise to three or four primary dendrites. Secondary branches arise from a short distance away from the soma. Their dendritic extent appears to be extensive. Similar neurons were observed by Beal and Cooper (1978) in lamina IV of spinal gray matter of macaque monkey spinal cord (Bhardwaj *et al*., 2001). These findings were in agreement with the report of Al-Hassan *et al*., (2008) which showed that axonsof neurons of posterior horns send out few branches and retaining their identity while that of anterior horns neurons send out many branches leading to complex ramifications.

In the enlarged segment of the spinal cord of Grasscutter dendenrite ramifications are elaborate, suggesting the complex communications which involves various input into the spinal cord circuits as seen in the innervations of the limbs. Very few dendenritic aborization

were seen in the thoracic region which showed fewer complex innervations to the body wall. This may explain the great dexterity of the fore limbs and the ability of the Grasscutter to balance on its hind limbs specially when eating or fighting and these findings have given insight into the morphology of the neurons of the spinal cord of Grasscutter.

The result of the present study showed that dopaminergic neurons are present in the spinal cord of Grascutters.Findings arein agreement with the findings in cat,rat and monkey (Ridet *et al*., 1992, Holstege *et al*.,1996). It have been established that dopamine is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation (Van*et al.*,1996; Barbeau and Rossignol, 1991; Puhl *et al.*,2012; Ramer *et al.*, 2014, Nutt *et al*.,2015).

Fleetwood-Walker *et al*., (2014) had shown that multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function, and gastrointestinal motility. The diencephalic dopaminegic neurons are divided into four groups named A11, A12, A13, and A14 in rodents. Only the A11 dopaminergic neurons appear to project directly to the spinal cord without intermediate neurons. A11 diencephalospinal dopaminergic neurons originate in prosomere 1 of the diencephalon and their projecting axons innervate the entire rostrocaudal extent of the spinal cord in the mouse (Barraud *et al.*,2010). Dopaminergic neurons of the A12, A13 and A14 group do not project to the spinal cord (Hasegawa *et al*., 2017). The diencephalic A11 dopaminergic neurons possess long axons extending over several segments and possibly traversing the entire length of the spinal cord. Axons of these neurons terminate predominantly in the dorsal horn, with less dense projections to the intermediolateral cell column and ventral horn (VanderHorst and Ulfhake, 2006; Fisher *et al.*,2017).In the present

study the expressions of dopamine were in the gray matter of the spinal cord of the Grasscuuter. This finding was in agreement with the work of Zhao*et al*., (2017) which showed that the expression of all dopamines are largely in the gray matter of mice spinal cord.

Dopaminergic neurons observed in the spinal cord of Grasscutter in the present study were more in the cervical and lumbosacral segments than in the thoracic spinal cord segments of the spinal cord of the Grasscutters.Dopaminergic cellsare clustered in the cervical and lumbosacral regions, with only few cells labelled at thoracic segments. The distribution pattern of dopaminergic neuron partially agreed with the previous data from mammals (Crisp and Mesce,2004, Darren*et al*., 2017) but differs from distribution pattern in the Wistar rats. The presence of dopaminergic neurons fibers and termination in the posterior horn of spinal cord of Grasscutter might suggestthe involvement of spinal dopaminergicneurons in the processing of nociceptive information.

Studies haverevealed that autonomic neurons in thelaboratory rat lower spinal cord expressed dopamine receptors (Stafford and Coote, 2006), suggesting that dopamine released within the spinal cord helps regulate autonomic functions. Though dopamingic neurons are known to reside in the spinal cord of non-mammalian species, *e.g*. birds and fish (Roberts and Meredith, 1987), they are thought to be restricted to the brain in mammals (Bjorklund and Dunnett, 2007). Thus, dopamine in the spinal cord is assumed to come from diencephalon spinal pathways that originate mainly from the A11 cell group (Skagerberg *et al*., 1982; Taniguchi *et al*., 2011; Sharples *et al*., 2013). Reports also showed that spinal interneurons are critical for the micturition reflex in neonatal rats and spinal cord-injured rats(Mouchet *et al*., 1986),

In the present study a similar distribution of dopamonergic neurons were seen in the spinal cord of Grasscutter, confirming the previous observations in other animals (Mouchet *et al*., 1986).The majority of these neurons are aggregated in the lumbosacral segments, particularly within the dorsal horns. Importantly,the location of these neurons in the lumbosacral region of the spinal cord of Grasscutter suggests their involvement in pelvic visceral activity, such as micturition (Mouchet *et al*., 1986).

To specifically identify if these neurons play a role in urinary function in Wistar rats, it was reported that a complete spinal cord injury model was done to remove descending control and retain only spinal micturition neural circuitry. In their findings they reported that the lumbosacal spinal cord contains dopamine neuron that controls micurations. Interruption of supraspinal micturition pathways causes acute flexic bladder paralysis. Yet, over a few weeks, there was a partial recovery of urinary function *via* involuntary bladder and urethral reflexes (Fowler *et al*., 2008). Observationshave been made remarkable on the plasticity of lumbosacral dopaminergic neurons after spinal cord injuries that contributed to a low level of sustained, local spinal dopamine expression. Spinal dopamine receptors regulating bladder reflex are active, indicating that this spinally-derived dopaminemodulates micturition function.

As a matter of fact, dopamine neurons are widely distributed throughout the brain in mammals. Dopamine cells convert L-tyrosine to L-DOPA; L-DOPA is then released and taken up by the neurons, which convert L-DOPA to dopamine. This is considered to be a compensatory reaction (Ugrumov, 2009). Interneurons in the lumbosacral cord receive afferent input from the lower urinary tract which is involved in the regulation of micturition (Araki and de Groat, 1997).

Across invertebrate and vertebrate species, dopamine has profound and diverse effects on rhythmically active motor networks. These actions are a result of a complex modulation of intrinsic cellular properties and synaptic connectivity. Remarkably, in some species dopamine demonstrates the ability to reconfigure circuits or networks to generate completely different motor behaviors (Puhl and Mesce, 2008, Sarkar *et al.*,2010, Crisp *et al.*, 2012; Puhl *et al.*, 2012). Studies have highlighted the ability of dopamine to shape a motor network during development, producing a change in motor behaviors from immature to more mature adult-like behaviors (Lambert *et al.*, 2012). Furthermore, dopamine also possesses the capacity to promote developmental and adult motor neurogenesis (Reimer *et al.*,2013).

In the present study, the presences of dopamine in the lumbosacral region of the spinal cord of Grasscutter also suggest the role dopamine plays in locomotive activities of the Grasscutter. Early exploration of the role of dopamine in locomotor behaviors in the 1960's determined that L-DOPA could modulate reflex circuits and promote locomotor activity, although at the time it was thought to be of noradrenergic origin (Jankowska *et al.*,1967; Grillner and Zangger, 1979). For many years, L-DOPA was used to evoke locomotor activity in spinalized cats. It was later demonstrated that L-DOPA could promote air stepping in the neonatal rodent (Sickles *et al*., 1992; McCrea *et al*., 1997), an effect that is blocked by both noradrenergic (Taylor *et al.*,1994) and dopaminergic antagonists (Sickles *et al*., 1992).

Studies now suggest that dopamine contributes to the control of locomotion in a different way than the predominant spinal serotonergic system that contributes to both evoking and modulating locomotor behavior (Kiehn *et al.*,1999; Jordan *et al*., 2008). The presence of dopamine in the cervical regions may suggest a reason why Grasscutter have a greater use of the forelimbs than other rodents.

Large amount of the influence of dopamine on the spinal locomotor networks has been studied from *in vitro*investigations of locomotor activity of the neonatal rodent spinal cord studied in isolation (Barrière *et al.*, 2004; Gordon and Whelan, 2006; Humphreys and Whelan, 2012; Christie and Whelan, 2005). Unlike serotonin, which is a potent activator of the locomotor central pattern generator network across a variety of mammalian species (Harris-Warrick and Cohen, 1985; Schmidt and Jordan, 2000, Gabriel*et al.*, 2009), dopamine alone can elicit fictive locomotor activity in the rat (Kiehn and Kjaerulff, 1996, Barrière *et al.*, 2004, Sharples *et al.*, 2013). Application of dopamine on its own is sufficient to evoke rhythmic motor activity. It is likely that dopamine is acting to promote locomotor activity through excitatory influences of dopamine receptors in rodents, whereas the slowing effect on fictive locomotor frequency is through dopamine receptor mechanisms. Indeed, there is consistent evidence across adult and neonatal preparations that the dopamine promotes locomotor activity (Barrière *et al.*, 2004 and Dvorak and Kwon 2017).

Furthermore, the presence of dopamine in the spinal cord of Grasscutter especially in the cervical spinal segments may contribute to the ability of Grasscuuters to efficiently use their fore limbs when fighting and eating.Dopamine in the lumbosacral region contributes to Grasscutters standing on their hindlimbs since dopamine has influence of individual spinal reflex circuits and components of the spinal locomotor network. Dopamine does elicit potent effects on spinal sensorimotor reflex circuits and has been an area of interest in the context of restless leg syndrome (Carp and Anderson, 1982; Tamae *et al.*, 2005,Barriere *et al.*, 2008).

The presence of dopamine in the dorsalhorns of spinal cord of Grasscutter may suggest that it mediate dopaminergic suppression of the monosynaptic reflex. (Hammar *et al.*, 2004) and has been implicated with restless leg syndrome given that D_3 receptor agonists exert potent

therapeutic effects at alleviating motor hyperactivity (Cheng and de Groat, 2004,Manconi *et al.,* 2011, Tamae *et al*., 2005).

Dopamine has also been shown to modulate synaptic connectivity and intrinsic properties of some known components of the locomotor network (Han *et al.,* 2007; Han and Whelan, 2007). The ability of dopamine to promote on-going locomotor activity may be due to combined effects exerted on motor neurons, premotor and interneurons that participate at all levels of the spinal locomotor network (Han *et al.*, 2007).

In the neonatal mouse, dopamine acts to depolarize motor neurons as well as premotor interneurons that project through the ventrolateral funiculus (Han *et al.,* 2007 Ogawa *et al* .,2006). It appears that dopamine is a necessary component to evoke stable rhythmic bursting activity in classes of genetically identified spinal interneurons. The dopaminergic system is involved in sensory, autonomic, and motor control in the spinal cord. The dense dopaminergic neuron projection to the intermediolateral column, and its hypothalamic origin, are consistent with its role in the regulation of sympathetic outflow (Gentleman *et al.,* 1981,Paulus,2006).Antinociception can be evoked by electrical stimulation of the A11 dopaminergic cell group in rats and cats, and this is mediated by dopamine at the level of the dorsal horn (Fleetwood *et al.,* 2014). Dopaminergic projections have also been shown to be involved in the generation of spinal flexor reflexes in the mouse (Peyron 1995).

In rats, strong labeling of dopamine fibers occurs at the lumbrosacral cord (van Dijken *et al.,* 1996 Seki*et al.*, 2001) but in the present study dopaminergic neurons were strongly labelled in the cervical and lumbosacral regions but weakly labelled in the thoracic region. These dopaminergic neurons are present in the gray matter throughout the spinal cord of the Grasscutter at which the majority resided in the lumbosacral spinal segments. Dysfunction of

spinal dopaminergic neurons could be involved in the pathophysiology of certain conditions such as Parkinson's disease.

In this study, cell area, cell perimeter (cell size) of neurons in cervical, thoracic, lumbar and sacral spinal cord regions of the Grasscutters. The descriptive statistics showed that the greatest mean value for cell perimeter and cell area were calculated in the lumbar ventral horn while the least values cell perimeters and cell areas were calculated in the dorsal horn of the thoracic spinal cord region of the spinal cord of Grasscutter.Findings similar to findings in other mammals(Junqueira *et al.*,2013) and findings suggest that the cells of the ventral horns of the spinal cord of the Grasscutter were generally larger than those of the posterior horns of the spinal cord segments of Grasscutter the presence of large cells in the ventral horn of the lumbar spinal cord segment also suggest the ventral horn of the lumbar region are well developed and could explain why the animal can stand on its hind limbs for a long period of time. The data for cell areas were collected from different spinal cord regions namely cervical, thoracic, lumbar and sacral regions, values were analysed using *t*- test which showed that the cell area in cervical ventral horn was significantlyhigher than cell area in the sacral ventral horn ($P<0.05$). The ventral hornscontain the cell bodies of motor neurons that send axons through theventral roots of the spinal nerves to terminate on striated muscles. Since area in cervical ventral horn was significantlyhigher than cell area in the sacral ventral horn it might suggest that more motor neurons in the ventral horn of the cervical spinal cord segment than in the ventral horn of the sacral spinal cord segment. This might also explain the dexreity of the forelimbs of the Grasscutter.

Also observed that the perimeter of the ventral horn of lumbar spinal cord segment was significantly larger than cell perimeter of the cells of the ventral horn of the thoracic and sacral spinal cord segments (SVH) ($P<0.05$). These findings in similar to findings in other

rodents the reason might be thoracic spinal cord segment supplies the thoracic muscle wall while the cells of ventral horn of the lumbar spinal cord segment supply the hindlimbs of the Grasscutter.

CHAPTER SIX

6.0 CONCLUSION AND REMMENDATIONS

6.1 Summary

The mean weight of the Grasscutter (*Thryonomysswinderianus)* of both sexes was determined to be 4700.27 ± 0.50g and 3633.40 ± 0.80g for males and females Grasscutters respectively. There was positive linear relationship between the tail length and spinal cord weight in both male and femaleGrasscutter. The enlargements at the cervical and lumbosacral segments provide innervations to the fore limbs and hind limbs respectively contributing to the brachial and lumbosacral plexuses in the Grasscutters.

The basic histological features of the spinalcord segments of Grasscutters were observed in the present study using the routine H and E method which showed inner gray matter core surrounded by an outer white matter. Although the basic structural patterns were seen throughout the spinal cord of the Grasscutters, the shape and structure of the spinal cord vary at different levels namely cervical, thoracic, lumbar, and sacral segments. The thoracic and lumbar spinal segments of the spinal cord of Grasscutter differs from the cervical and sacral spinal cord segments illustrated in the results shown in the present study because of the presence of lateral horn of the gray matter of the spinal cord of the Grasscutter.

Also, the presence of central canal was seen throughout the length of the spinal cord of Grasscutter. The shape of the central canal was not the same across the various regions of the spinal cord of the Grasscutter. Progressively the amount of gray matter increases from the cervical spinal cord region and become greatest at the sacral spinal cord region while the amount of white matter increases progressively from the sacral region and became greatest at the cervical spinal cord region of the Grasscutter.

The presence study using Crestyl fast violet revealed the inner gray matter stained dark purple due to the abundant presence in the neuronal cell bodies of Nissl substances while the outer zone of white matter stained relatively lighter due to the presence of neuronal fibers. The cells in the anterior gray horn of cervical, thoracic, lumbar and sacral segments of the spinal cord of Grasscutter contain large multipolar motor neurons. Their cytoplasms are characterized by a prominent vesicular nucleus, a distinct nucleolus and coarse clumps of basophilic material(Nissl substance) while cells in the posterior gray horns of cervical, thoracic,lumbar and sacral segments of the spinal cord of Grasscuttercontain sensory neurons. Their cytoplasms are characterized by a prominent vesicular nucleus, a distinct nucleolus and basophilic material, the Nissl substance. The Nissl substance extended into the dendrites but not into the axons of neurons of the spinal cord of Grasscutter.

The spinal cord of the Grasscutter when stained with Golgi method revealed neurons which were of various shapes and sizes. Nurons were identified in the gray matter of the spinal cord. These neurons were composed of several dendrites and one axon. Cells found mainly in the posterior horns of the spinal cord of Grasscutter (that is in the cervical, thoracic, lumbar and sacral) spinal segments were observed to be smaller than cellsof the anterior horns of spinal segments of Grasscutter. The complexity of dendrites of these neurons may play a role in the unique ability of the Grasscutter to stand on it hind limbs and efficient use of its forelimbs especially during fighting and feeding. The histometrical studies showed the neurons of the spinal cord of Grasscutter have various sizes and have different areas.

The immunohistochemical study of the spinal cord of Grasscutterstained withanti-tyrosine hydroxylaseshowed that dopaminergic neuronswere present in the spinal cord of Grasscutter. Dopaminergic neurons weredeeply stained in lumbosacral segment than in the other spinal cord segments of Grasscutter. Dopamine performs a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation.

This catecholamine also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function, and gastrointestinal motility. The presence of dopamine in the lumbosacral region suggests dopamine could play a role in micuration.

6.2 Conclusion

Using Anatomical and immunohistochemical approaches the Anatomical features of the spinal cord of Grasscutter were similar to that of other rodents

6.3 Contributions to knowledge

I. This present study has documented baseline data on the morphologic features of the spinal cord of Grasscutter to extend from the first cervical vertebrae (C_1) to the second sacral vertebrae (S_2).

II. The study has provided morphologic descriptions of the spinal cord of Grasscutters establishing that the spinal cord of Grasscutters has two enlargements which are identified as cervical and Lumbosacral enlargements. The cervical enlargement was shown to innervate the fore limbs and began at the junction between the second cervical (C_2) and the third cervical (C_3) vertebral discs and ended between the seventh cervical (C_7) and the first thoracic (T_1) vertebrae while the result showed that lumbosacral enlargement which innervates the hind limbs, began at the junction of the first lumbar (L_1) and the second lumbar (L_2) and ended at S_2 vertebrae.

III. The present study has documented baseline data on the morphometeric features of the male and female spinal cord of the Grasscutters which showed that the mean length and weight of spinal cord of the male Grasscutters were 20.30± 1.50cm and 2.60 ±

0.02 g respectively while the mean length and weight of spinal cord of the female Grasscutters to be 19.40 ± 1.04cm and 2.02 ± 0.01g respectively. Studies have provided morphometric descriptions of the spinal cord of the Grasscutters establishing that the parameters of the spinal cord of the Grasscutters namely weight and length showed that there was positive correlate between the body weights of both male and female Grasscutters in both sexes.

IV. The present study established that the result of histological studies using hematoxylin and eosin stained sections of the transverse sections of the spinal cord of Grasscutter showed outer white matter and an inner dark gray matter,the gray matter was divided into ventral (Anterior) and dorsal (posterior) horns. The ventral horn showed a moderate number of large basophilic multipolar motor neurons, while the dorsal horn showed a large number of small sensory neurons.

V. The present study has established that result of the Golgi method of silver impregnation of neurons in the spinal cord of the Grasscutter showed positive Golgi reaction in which different neuron sizes and shapes identified in the gray horn of the spinal cord

VI. The present study also established the result of histochemical studies using Crestyl fast violetstained that the neurons in the anterior gray horns and posterior gray horns of cervical, thoracic,lumbar and sacral segments of the spinal cord of the Grasscutters have neuronswhich containNissl substancein their cytoplasms.The presence of Nissl substances in the spinal cord of Grasscutter suggests active protein synthesis

VII. The immunohistochemical study of the spinal cord of Grasscutterstained withAlpha-1 anti-tyrosine hydroxylaseestablished that dopaminergic neuronsare present in the spinal cord of Grasscutter. Dopaminergic neurons are more in the cervical and lumbosacral spinal cord segments than in the thoracic spinal cord segment of the

spinal cord of Grasscutter. This finding sugest that Dopaminergic neurons play roles in pelvic visceral activity, such as micturition

VIII. The histometrical studies of cell areas and cell sizes of the spinal cord of Grasscutters showedthat largest value for mean for cell perimeter and cell area was observed in the lumbar spinal cord segment to be 9.30± 0.67µm and 3.28± 0.59 μm^2respectively while the least values cell perimeters and cell areas were calculated to be3.86± 0.24µm and0.83± 0.09μm^2 respectively.

6.4 Recommendations

The Gross anatomy of the spinal cord of Grasscutter was similar tothe features of the spinal cord in other mammals. Some unique differences were observed in the arrangement and distributions of neurons. The following recommendations for further studies are suggested

I. Neuronal tracing should be carried out to detect neuronal communications with higher centers

II. Immunohistochenistry should be done to describe the dendritic spines variations in the sizes and shapes of the dendrites of the neurons of the spinal cord of the Grasscutter.

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