COMPARATIVE NEUROANATOMICAL STUDIES ON STRUCTURES OF FEAR RESPONSE IN LOCAL BREEDS OF SHEEP, GOAT, AND DOG

By

Itoro Oku GEORGE

BSc., Human Anatomy (A.B.U) 2010

MSc/Med/4935/11-12

DEPARTMENT OF HUMAN ANATOMY

FACULTY OF MEDICINE

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

MARCH, 2015

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AN MSC RESEARCH THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF A MASTER DEGREE IN HUMAN ANATOMY.

DEPARTMENT OF HUMAN ANATOMY, FACULTY OF MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

MARCH, 2015

DECLARATION

I, George Itoro Oku, hereby declare that this thesis titled "Comparative studies on some structures of the fear circuitry in domestic animals: sheep, goat, and dog" is an original study carried out by me under the supervision of Dr. (Mrs) J. N. Alawa and Dr. W. O. Hamman and to the best of my knowledge, the study has not been submitted in any form for the award of degree or diploma to any other university or institution. Information derived from other works had been fully acknowledged in this Dissertation.

GEORGE, Itoro Oku	----------------------------	-----------------
Name of student	(signature)	(Date)

CERTIFICATION

This thesis titled "Comparative neuroanatomical studies on some structures of the fear response in local breeds of sheep, goat, and dog" meets the requirement governing the award of degree of Master of Science (Human Anatomy) of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Chairman, Supervisory Committee ______________________ ______________

(Dr. Mrs J. N. Alawa) Date

Member, Supervisory Committee ______________________ ______________

(Dr. W. O. Hamman) Date

Head of Department (Human Anatomy) ___________________ ______________

(Prof. S. S. Adebisi) Date

Dean of Postgraduate School ______________________ ______________

(Prof. H. Zoaka) Date

DEDICATION

This research work is dedicated to Mr. and Mrs, Oku George Udofia

ACKNOWLEDGEMENT

I am grateful to Jehovah God for seeing me through this work. He is the only One who gives wisdom and knowledge. I would also like to express my sincere gratitude to my supervisors, Dr Mrs. J. N. Alawa and Dr. W. O. Hamman for the tremendous support and guidance they gave me throughout the course of the research work. They were, without doubt, the best supervisors I could have hoped for and without their time, expertise, engaging support, encouragement and, most importantly, their faith in me and what I was doing, this work would not be what it is.

Thanks must also go to Mr.Bamidele Adetiba and Mr. Enemari James, who both assisted in the histological processing of brain tissue sections which was used in this research. Appreciation also goes to Mr. James timbuak and Mr. Abel Agbon who helped me in the data analysis. I highly appreciate the fruitful collaboration of the laboratory attendants in the Histology Laboratory, Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, who were patient and worked closely with me. Thanks to all my lecturers and colleagues at the department for creating a professional and social environment that is most inspiring. I also want to express my appreciation to family and friends, especially my parents who have supported me unconditionally throughout all these years.

ABSTRACT

This study was designed to compare some fear response structures in three (3) domesticated animals: Sheep (*Ovis sp)*, Goat (*Capra sp),* and Dog (*Canis sp.*) and relate it to the different responses of these animals to threats and dangers. Seven (7) dogs, seven (7) goats, and seven (7) sheep were used in this study. The animals were decapitated, their skulls were exposed and brain tissues fixed immediately in 10% formal saline solution. After fixation, each brain was carefully removed from the skull and studied. The number of gyri and sulci on the surfaces of the cerebrum were counted. Morphometric analysis involved measurement of brain weight, volume, dimensions (cerebral and cerebellar length and width). Histomorphology and histomorphometry of layer V cells of the prefrontal cortex, CA3 region of the hippocampus, and basolateral complex and central amygdala were carried out. This also included counting the number of neurons in these brain regions to determine which region of the fear circuitry had the most number of neurons in each animal. Morphological studies revealed similar brain structure of these mammalian brains with dog having an enlongated frontal lobe, fewer gyrifications, and more caudolateral expansion of the cerebrum when compared with goat and sheep ($p<0.05$). Morphometry revealed significant differences in brain weight, brain volume, cerebral length and width, and cerebellar length and width between dog, goat, and sheep ($p<0.05$). Results showed that goat had the largest soma size (57.60±9.65µm) and dendritic aborization (153.79±35.27µm) of layer V cells of the prefrontal cortex while dog had the most number of neurons in this region. The number of CA3 cells of the hippocampus was the most in dog. However, sheep had the largest soma diameter (41.61±11.46µm) in its CA3 cells of the hippocampus. Dog basolateral cells of the amygdala were the largest in both soma size and dendritic aborization (27.93±11.10µm, 88.06±38.15µm, respectively). Sheep had the least number of neurons in its

central amygdala, while dog had more densely packed central nucleus neurons. Although sheep had the largest brain weight and volume, it had the least densely packed cells in its Prefrontal cortex which is responsible for higher cognitive function. This could explain why dog is more intelligent than sheep and goat in their response to threatening situations. Dog Hippocampus had the most densely packed neurons. This accounts for dog's better memory of a threatening situation and its quicker response than sheep and goat. The Central nucleus of Dog amygdala had the most densely packed neurons. This could explain why dog expresses fear emotions quickly either by fleeing or fighting back in comparison with sheep. Therefore, the local breeds of sheep, goat, and dog demonstrated significant differences in their brain morphology and morphometry, grossly and histologically, and this could be related to their different behaviours when it comes to responding to threats and danger in which sheep reacts very slow while dog responds faster in comparison.

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CHAPTER ONE

1.0 INTRODUCTION

Sheep, goats, dogs, and humans have been closely associated for a long time. Man's "bestfriend", the dog was the first specie to be domesticated, while sheep and goat occupy second and third places interchangeably in the domestication race (Schoenian, 2007). The local names of these species in three Nigerian languages are shown in table 1 below:

Table 1.0: Local names of the sheep, goat, and dog in three Nigerian languages

LANGUAGE / ANIMAL	HAUSA	IGBO	YORUBA
SHEEP	*Rago*	*Aturu*	*Aguntan*
GOAT	*Akuya*	*Ewu*	*Ewure*
DOG	*Kare*	*Nkita*	*Aja*

Sheep, goats, and dogs have evolved a unique and fascinatqying array of behavioral characteristics which have contributed to their survival and proliferation in a unique environmental niche. Studies in the ontogeny of fear behaviors in animals have revealed that fear responses differ across and within species. The limbic system structures as well as the prefrontal cortex are components of brain circuits that are involved in the regulation of the perception of memory, processing of fears, and emotional behaviours. Knowledge of the anatomy of fear response structures in the animals is essential in understanding their behavioral responses.

1.1 STATEMENT OF RESEARCH PROBLEM

Scanty literature exist on the comparative neuroanatomy of fear response structures in sheep, goat, and dog with respect to their different response to threats and dangers.

1.2 SIGNIFICANCE OF THE STUDY

Domestic animals are very useful to man. Therefore, additional insight into the anatomy of the structures of fear response will help us understand the behaviour of these animals and improve their handling. This study will also add to the body of scientific knowledge.

1.3 RESEARCH HYPOTHESIS

Variation in the structures of fear response in domestic animals is related to their behavioral differences.

1.4 AIM OF THE STUDY

To elucidate the differences in the neuroanatomy of structures of fear response in sheep, goat, and dog and relate this to their different responses when they face fearful situations.

1.5 OBJECTIVES OF THE STUDY

- To identify morphological and morphometric differences and similarities on the brains of sheep, goat, and dog.
- To compare the histomorphology and histomorphometry of the prefrontal cortex, hippocampus, and amygdala of sheep, goat, and dog using H and E (Heamotoxylin and Eosin), Toludine blue, and Golgi silver stain.
- To determine the density of neurons in the prefrontal cortex, hippocampus, and amygdala of sheep, goat, and dog.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 SHEEP, GOAT, DOG AND THEIR BEHAVIOUR

Domestic animals exist in a relatively controlled environment and their response to that environment (behaviour) as a fairly predictable composite of innate (inherited) and acquired (learned) components. Behaviour is an important part of what makes an animal. Most animals are defined by their behaviour. Ethology is the scientific and objective study of animal behavior (Nicholaus, 2013). Behaviorally, sheep are gregarious, precocial, defenseless creatures. It is rare to see a sheep by itself because of their gregarious nature. It is a strongly social animal requiring the presence of at least four (4) or five (5) sheep which, when grazing, maintain a visual link with each other. Precocial means that they have a high degree of independence at birth. This means that they can stand on their feet shortly after birth. Sheep are defenseless for the most part against predators like coyotes and wild dogs. Sheep are also very selective in their grazing habits. They have a split in their upper lip, with this they are able to pick the preferred leaves off of the plant (Claeys and Rodgers, 2003).

The goat is an animal a little like the sheep. It is also defined by its behaviour as a very sociable, lively, inquisitive and independent animal. It is quite intelligent and can learn how to open latches on farm gates. A particular behaviour of goats that is intriguing is that while they are independent, they often prefer to surround themselves with goats of their same breed in a mixed herd. Also, the kid will prefer to remain nearby their mother even if separated for years and reintroduced (Sammelwitz *et al.,* 1992). Sheep are best known for their strong flocking instinct and following instinct while goats are independent. When it comes to responding to

threats and danger, sheep is slower than goat. Hence, sheep are branded as stupid and goats more intelligent.

Dog behavior is affected by genetic factors as well as environmental factors (Coren, 2004). Domestic dogs exhibit a number of behaviors and predispositions that were inherited from wolves (Coren, 2004). However, many of the salient characteristics in dog behavior have been largely shaped by selective breeding by humans. Thus some of these characteristics, such as dog's highly developed social cognition, are found only in primitive forms in grey wolves (Hare *et al.,* 2002). The domestic dog has a predisposition to exhibit a social intelligence that is uncommon in the animal world (Coren, 2004). Dogs are capable of learning in a number of ways, such as through simple reinforcement (e.g., classical or operant conditioning) and by observation (Coren, 2004). When it comes to responding to threats and danger, dog is fastest when compared with the sheep and goat. Hence, dog is said to be more intelligent than sheep and goat.

Fig 2.1: Mongrel breed of dog, goat (middle: Retrieved from www.goatworld.com, 06/02/2013), and Adult Uda breed of sheep (right). Dog and sheep pictures were obtained with permission from slaughterhouses in Zaria, Kaduna state, Nigeria

TABLE 2.1 BRIEF COMPARISON OF SHEEP, GOAT, AND DOG

FEATURE	SHEEP	GOAT	DOG
Taxonomy	Order: Artiodactyla Family:Bovidae Genus: Ovis sp.	Order: Artiodactyla Family:Bovidae Genus: Capra sp.	Order:Carnivora Family: canidae Genus: Canis sp.
No. of chromosome	54	60	78
ppearance of the tail	Tail hangs down	Tail goes up	Tailset (position) varies
Eating behaviour	Herbivores	Herbivores	Carnivores
Physical differences	lost grow wool and need to be sheared annually	air coat of most goats do not reguire shearing or combing	Exhibit a diverse array of coat textures.
Horn	May be present	Naturally have horns	Hornless
General Behaviour	tronger flocking instinct	ndependent and curious	have the ability to sense and leliver a wide variety of cues via body language

2.2 CIRCUITRY OF FEAR

Saunders Veterinary Dictionary defined fear as a normal emotional response to consciously recognized external sources of danger such as those often associated with loud noises, threatening gestures, strange people, and thunderstorms. It is manifested in animals by flight, by attack or by cringing. Fear is also an extreme dislike to some conditions/objects such as fear of darkness, fear of ghosts, etc. It is one of the basic emotions. Much of what is known regarding the mechanisms of persistent maladaptive anxiety disorders (such as post-traumatic stress disorder) has emerged from animal research into Pavlovian Fear Conditioning (Ledoux, 1995). Scientific researches have mapped the fear circuits in the brains of animals. Circuitry is a network of neurons in the nervous system, especially in the brain as defined by Merriam Webster Dictionary. It is also the neuronal pathways of the brain along which electrical and chemical signals travel. Therefore, the Circuitry of fear is the neuronal pathways of the brain along which electrical and chemical signals related to the emotion of fear travel so as to bring about a response. In the intervening years, studies have shown the amygdaloid complex to be essential for fear conditioning and an important component in the emotion of fear (Ledoux, 1995).

In 2008, Neurocognition and Performance Technologies Newsletter published mapped out areas, specifically three structures in the brain, that are responsible for the emotion of fear. In this review, only amygdala, prefrontal cortex, hippocampus will be considered. These three structures form the circuitry of fear as shown below:

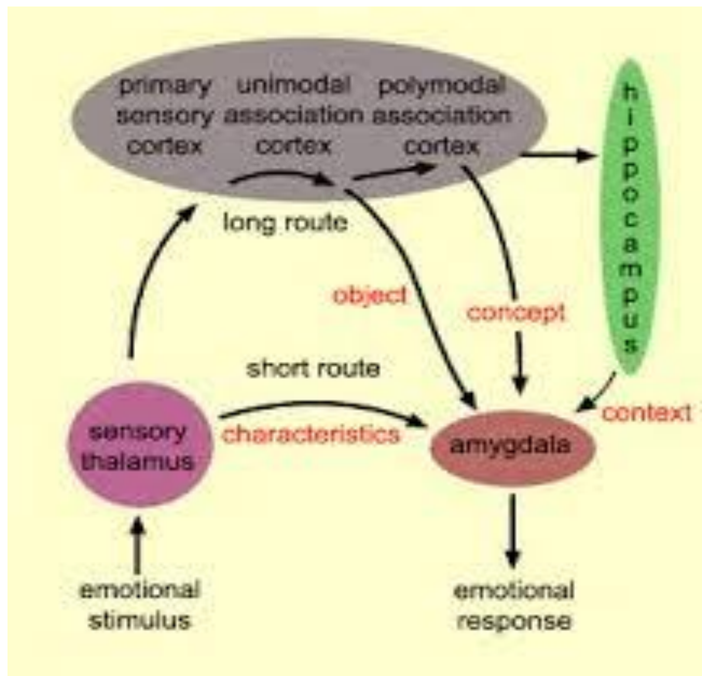

FIG. 2.2 The Circuitry of fear (Pathways of fear). Picture was adapted from Bruno Dubuc (2001) From Top to Bottom: The Amygdala and its allies-. (Canadian Institute of Neurosciences, Mental health, and Addiction. Canadian Institute of Health Research. www.thebrain.mcgill.ca

2.3 SENSORY INPUT CONVERGING AT THE AMYGDALA

Extensive research works have examined the neurocircuitry associated with fear responses, mainly in rodents, using primarily fear conditioning, inhibitory avoidance, and fear-potentiated startle models. The key components of the fear circuitry include the amygdala (and its subnuclei), nucleus accumbens (including bed nucleus of stria terminalis, BNST), hippocampus, ventromedial hypothalamus, periaqueductal gray, a number of brain stem nuclei, thalamic nuclei, insular cortex, and some prefrontal regions (mainly infralimbic cortex) (Davis, 2006; Maren, 2008; Quirk and Mueller, 2008). These regions have their respective roles in the various components of fear processing such as the perception of threat or of unconditioned stimuli, the pairing of an unconditioned stimulus and conditioned response

(learning/conditioning), the execution of efferent components of fear response, and the modulation of fear responses through potentiation, contextual modulation, or extinction.Some key findings from animal literature, such as the central role of amygdaloid nuclei in the acquisition of fear conditioning and expression of fear responses, the involvement of the hippocampus in contextual processing, and the importance of the infralimbic cortex in extinction recall, have been replicated across different studies and laboratories (Ledoux, 2005). Some of the regions mentioned above are illustrated below:

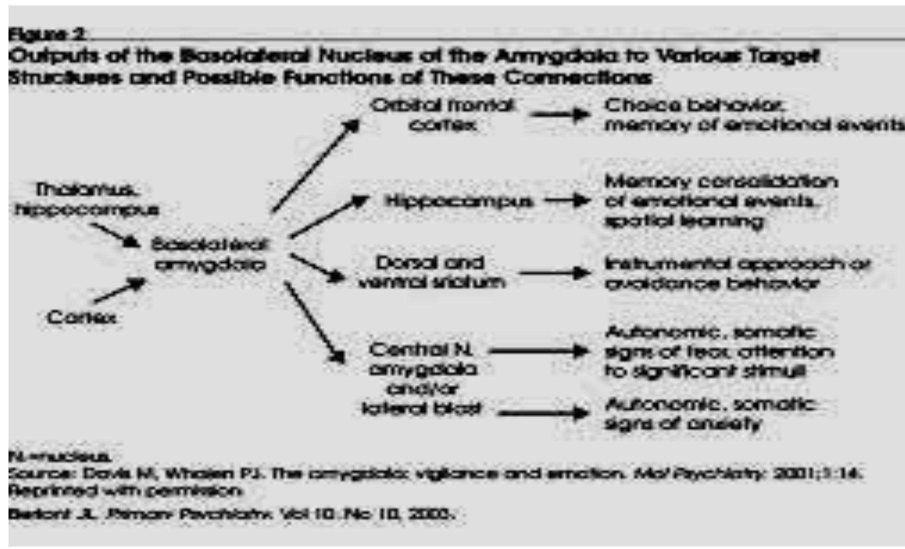

FIG. 2.3 Sensory Inputs Into and Out of the Basolateral Nucleus of the amygdala. Retrived from Davis M., Whalen P. J. (2001). The Amygdala: vigilance and emotion. *Mol Psychiatry* 1:14

2.4 THE AMYGDALOID COMPLEX: ANATOMY

The amygdala is an almond-shaped structure located deep within the temporal lobe and was first identified by Burdach in the early 19th century. The amygdaloid complex, located in the medial temporal lobe, is structurally diverse and comprises approximately 13 nuclei. These are further divided into subdivisions that have extensive internuclear and intranuclear connections.

These nuclei and subnuclei are distinguished on the basis of cytoarchitectonics, histochemistry, and the connections they make (Krettek and Price, 1978; Pitkanen, 2000). The architectonic organization and connectivity of the amygdala have been extensively studied (Alheid *et al.,* 1995; De Olmos *et al.,* 1985; Mc Donald, 1998; Pitkanen, 2000). Although these studies have mostly concentrated on the rat amygdala, other studies have been made in the monkey (Amaral *et al.,*1992), cat (Price *et al.,* 1987), common shrew (Rowniak *et al.,* 2004), rabbit (Rowniak *et al.,* 2007), guinea pig (Rowniak *et al.,* 2005), etc. These studies reveal that while there are many similarities between species, there are also clear differences in the organization and the relative sizes of the different amygdaloid nuclei. Using the nomenclature introduced by Price *et al.,* (1987) with some modifications (Mc Donald, 1998), the amygdala nuclei are divided into three groups:

1) The deep or basolateral group, which includes the lateral nucleus, the basal (basolateral) nucleus, and accessory basal (basomedial) nucleus;

2) The superficial or cortical-like group, which includes the cortical nuclei and nucleus of the lateral olfactory tract (NLOT);

3) The centromedial group composed of the medial and central nuclei.

Another set of nuclei that do not easily fall into any of these three groups and are listed separately. They include:

-The intercalated cell masses and

-The amygdalohippocampal area (AHA) (Sah *et al.,* 2003).

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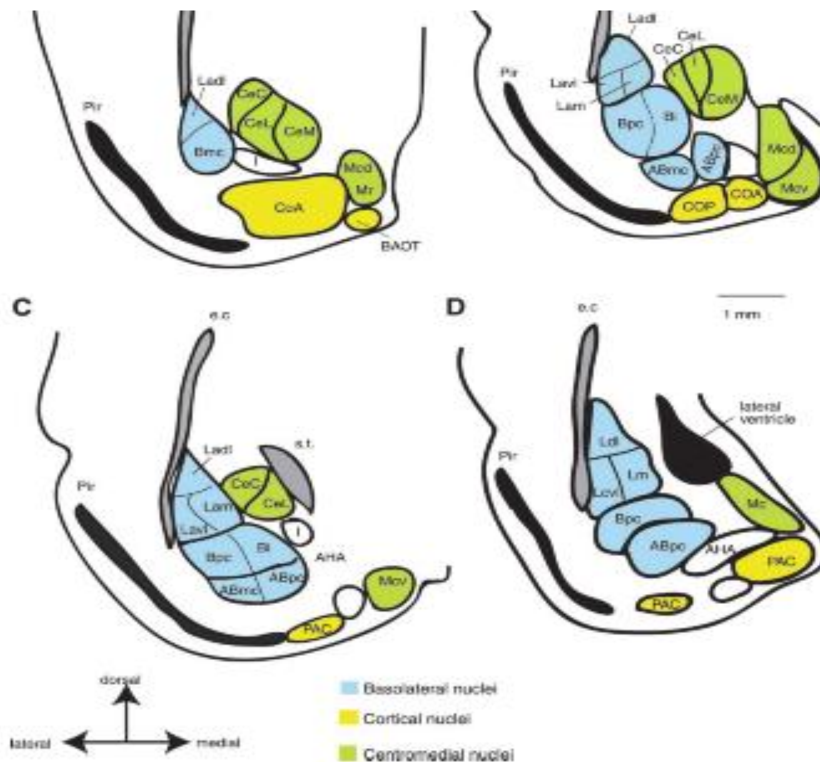

Fig 2.4: Coronal sections through the rat's amygdala. Picture was adapted from Sah *et al.,* (2003). The Amygdaloid Complex: Anatomy and Physiology. *Physiol. Rev*. 83:803-834.

In Fig. 2.4, Coronal sections are drawn from rostral (A) to caudal (D). Areas in blue form part of the basolateral group, areas in yellow are the cortical group, and areas in green form the centromedial group. (Sah *et al.,* 2003). ABmc- accessory basal magnocellular subdivision;Abp- accessory basal parvicellular subdivision; Bpc- basal nucleus magnocellular subdivision; e.c.- external capsule; Ladl- lateral amygdala medial subdivision; Lam- lateral amygdala medial subdivision; Lavl- lateral amygdala ventrolateral subdivision; Mcd-medial amygdala dorsal subdivision; Mcv- medial amygdala ventral subdivision; Mr- medial amygdala rostral subdivision; Pir- piriform cortex; s.t.- stria terminalis**.**

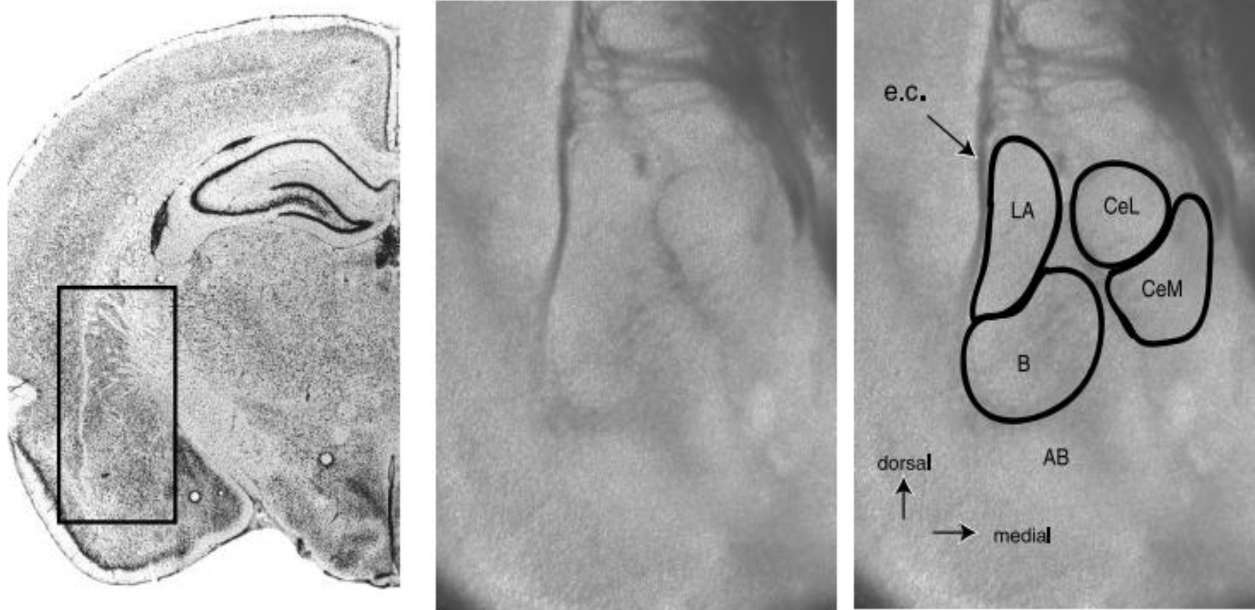

Figure 2.5. An example of the amygdaloid region as it appears in acutely prepared coronal sections. [Retrieved from Paxinos G. and Watson C. (1986). The Rat Brain in Stereotaxic Coordinates (2nd Ed.). Sydney, Australia: Academic.]

In figure 2.5 above, the figure on the left is a Nissl-stained hemisection of a rat brain around bregma-3. The areas shown in the outlined region in the figure above are shown in an acutely prepared coronal brain slice as it appears under brightfield illumination (middle). Shown also is the region containing the basolateral complex and central nucleus. yApproximate regions of the lateral (LA), basal (B), accessory basal (AB) and central nucleus have been outlined (Right). In the central nucleus, the approximate locations of the lateral (CeL) and medial (CeM) subdivisions have also been shown. (Sah *et al.,* 2003).

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2.4.1 THE DEEP OR BASOLATERAL GROUP/COMPLEX

The basolateral or deep nuclei comprise the lateral nucleus (LA), the basal nucleus (B), which is sometimes called the basolateral nucleus (BLA), and the accessory basal nucleus (AB),

which is also known as the basomedial nucleus (see figure 2.4 above). Often, these three nuclei are collectively referred to as the basolateral complex (Johnston, 1923). The LA is located dorsally in the amygdala where it abuts the basal nucleus ventrally. It is bordered laterally by the external capsule and medially by the central nucleus. It has three subdivisions: the smaller celled dorsolateral subdivision, the larger celled ventrolateral subdivision, and the medial subdivision. The basal nucleus is located ventral to the LA and is subdivided into the rostral magnocellular subdivision and the more caudal intermediate and parvicellular subdivisions. The AB is found ventral to the basal nucleus and lies adjacent to the amygdalohippocampal area (AHA). It is comprised of the magnocellular subdivision, the intermediate subdivision, and the parvicellular subdivision (Pitkanen, 2000; Pitkanen *et al.,* 1997).

Two main types of neuron were described based on Golgi studies. The first type is made up of about 70% of the cell population and has been described as pyramidal (Hall, 1972; Millhouse and De Olmos, 1983; Washborn and Moises, 1992), spiny, or class I cells (Mc Donald, 1982; Mc Donald, 1984). Many have pyramidal-like somata with three to seven dendrites emanating from the soma. The secondary and tertiary dendrites of these cells are spiny. One of the dendrites is usually more prominent than the others and thus has been likened to the apical dendrite of cortical neurons (Faber *et al.,* 2001; Hall, 1972). Some neurons appear to have two apical dendrites and are more like the spiny stellate cells of the cortex (Mc Donald, 1992). Unlike pyramidal neurons in the cortex or hippocampus, these cells are not arranged with parallel apical dendrites but are randomly organized, particularly close to the nuclear borders (Faber *et al.,* 2001; Mc Donald, 1992, Pare *et al.,* 1995; Rainnie *et al.,* 1993, Washborn and Moises, 1992). Furthermore, while this cell type has been described as pyramidal, these neurons differ from cortical pyramidal neurons in the following ways: The primary dendrite of

the apical and the basal dendrites are of equivalent length and tapers rapidly, the distal dendrites do not have an elaborate terminal aborization or rigid orientation of the pyramids in one plane (Faber *et al.,* 2001; Larkman and Mason, 1990). Thus, based on these, these cells are called pyramidal-like or projection neurons (Faber, 2001, Pare *et al.,* 1995). The axons of these cells originate either from the soma or from the initial portion of the primary dendrite (Faber *et al.,* 2001; Mc Donald, 1982). They give off several collaterals within the vicinity of the cell before projecting into the efferent bundles of the amygdala, showing that they are projection neurons as so called (Mc Donald, 1982; Smith and Pare, 1994). For neurons within the basolateral complex, cells described as pyramidal comprise a morphological continuum ranging from pyramidal to semi-pyramidal to stellate (Faber *et al.,* 2001; McDonald, 1992; Pare *et al.,* 1995; Rainnie *et al.,* 1993; Washburn and Moises, 1992). However, it should be noted that when reconstructed in coronal sections, cells can sometimes appear stellate because they have a largely rostrocaudal orientation (Faber *et al.,* 2001, Millhouse and DeOlmos, 1983; Pare *et al.,* 1995; Washburn and Moises, 1992). In general, neurons in the B are somewhat larger than in the LA with an average soma diameter of approximately15–20 μm compared with 10–15 μm in the LA (McDonald, 1982b; Millhouse and DeOlmos, 1983). No clear morphological distinctions have been found between neurons in the different subdivisions of the lateral or basal nuclei. As mentioned above, the large dendritic arbors of pyramidal like neurons indicate that the dendritic trees of these cells would cover the boundary between subdivisions (Faber *et al.,* 2001; Pare and Gaudreau, 1996). These considerations call into question the functional parcellation of neurons in the basolateral complex into different subdivisions. The second main group of cells found within the basolateral complex has slightly smaller somata (10–15μm) and resemble nonspiny stellate cells of the cortex. These were

termed “S,” for spiny cells by Hall (1973) and “stellate” or “class II” cells by Millhouse and De Olmos (1983). These cells have two to six primary dendrites that lack spines and form a relatively spherical dendritic field (Lang and Pare, 1998; McDonald, 1982b). There is no apparent apical dendrite and, as with the pyramidal like neurons, they form a heterogeneous population that has been subdivided into multipolar, bitufted, and bipolar cells according to their dendritic trees by McDonald (1982b). These neurons are GABAergic (McDonald and Augustine, 1993) and are local circuit interneurons. Their axons originate from the soma or from the proximal portion of a primary dendrite (McDonald, 1982b). Consistent with local circuit interneurons, the axons branch several times and thus have a “cloud of axonal collaterals and terminals” near the cell body (Millhouse and DeOlmos, 1983). Some of these interneurons form a pericellular basket or axonal cartridge around the perikarya and initial segment of pyramidal cells, respectively, allowing a tight inhibitory control over the output of the cell (Carlsen, 1988; Lang and Pare, 1998; McDonald and Betette, 2001; Smith *et al.,* 1998). Like interneurons in other cortical areas, these cells express several calcium binding proteins (Kemppainen and Pitkanen, 2000; McDonald and Mascagni, 2001). About one-half of the cells express parvalbumin, whereas the other half express calbindin and/or calretinin in their cytosol (Kemppainen and Pitkanen, 2000; McDonald, 1997), suggesting that there are different classes of interneurons in the basolateral complex. However, there is significant overlap between these three markers, while the calretinin and parvalbumin positive neurons form separate populations, a large proportion of the parvalbumin positive cells also express calbindin (Kemppainen and Pitkanen, 2000; McDonald and Mascagni, 2001). The functional relevance, if any, of these different populations of interneurons is currently not known.

In addition, although uncommon, several other types of cells have also been described in the basolateral complex on the basis of distinctive axonal or dendritic patterns. These have been termed extended neurons, cone cells, chandelier cells, and neurogliaform cells (Faulkner and Brown, 1999; Kamal and Tombol,1975; McDonald, 1983b; McDonald,1982b; Millhouse and DeOlmos, 1983). Extended cells are large cells with long thick dendrites with few branches and few spines and are found in the rostral parts of the basal nucleus. Cone cells, which have only been described in the rat, have large cell bodies (20–30 μm) and cone-shaped dendritic trees that are nonspiny and are found in the dorsal angle of the lateral nucleus (Millhouse and DeOlmos, 1983). Chandelier cells resemble cortical chandelier cells and have clustered axon varicosities that form synapses with the initial segment of pyramidal like neurons (McDonald, 1982b).Finally, neurogliaform cells are another type of small nonspiny stellate neuron found in the basolateral complex (Kamal and Tombol, 1975; McDonald, 1984; McDonald, 1982b). These cells are small (10 μm) with a restricted spherical dendritic tree and branching axons that travel little further than the confines of their dendritic trees. They form numerous synaptic connections along the dendrites of pyramidal-like neurons and therefore probably represent inhibitory local circuit neurons (McDonald, 1982b).

2.4.2 CENTROMEDIAL NUCLEI

The centromedial nuclear group is found in the dorsomedial portion of the amygdaloid complex and consists of the central (CeA), medial (M), and the amygdaloid part of the bed nucleus of stria terminalis (BNST). Traditionally these nuclei were pooled with the cortical nuclei. However, it has recently been suggested that the central, medial, and BNST have histochemical and developmental characteristics that are distinct from the cortical nuclei (Sah

et al., 2003). Thus, as initiated by McDonald (1998), this group is separated from cortical nuclei. The CeA is located dorsomedially in the rostral part of the amygdala, bordered laterally by the basolateral complex, dorsally by the globus pallidus, and medially by the stria terminalis. The CeA has four divisions: the capsular subdivision (CeC), lateral subdivision (CeL), intermediate subdivision (CeI), and medial subdivision (CeM) (Jolkkonen E. and Pitkanen A., 1998); McDonald, 1982a). The medial nucleus is found near the surface bounded medially by the optic tract. It begins at the level of the NLOT and extends caudally. It has four subdivisions: rostral, central (dorsal and ventral), and caudal (Sah *et al.,* 2003).

2.4.2.1 CENTRAL NUCLEUS

The morphology of neurons in the central nucleus has been studied using Golgi techniques as well as reconstruction after recording physiological properties in acute brain slices (Martina *et al.,* 1999; Schiess *et al.,* 1999). As with the basolateral complex, the different subdivisions of the central nucleus (Cassell *et al.,* 1986; Krettek and Price, 1978; McDonald, 1982a; Pitkanen, 2000) cannot be easily identified in acute slices maintained in vitro. Thus, while Golgi studies have described neurons in the different subdivisions, results from brain slices have either not discussed subdivisions (Schiess *et al.,* 1999) or divided cells into those in the lateral and medial sectors (Martina *et al.,* 1999; Schiess *et al.,* 1993). Therefore concentration is made on cells in the lateral and medial sectors of the central nucleus. There is general agreement that in both subdivisions there is one predominant cell type that has been called “medium spiny neurons” in the CeL by comparison with neurons in the nearby striatum (Hall, 1972; McDonald, 1982a). These cells have an ovoid or fusiform soma and three to five non spiny primary dendrites from which moderately spiny, sparsely branching secondary and tertiary

dendrites arise (Martina *et al.,* 1999; McDonald, 1982a, Schiess *et al.,* 1999). Axons give off several local collaterals before leaving the nucleus. A second type of neuron has also been described that has a somewhat larger soma and a thick primary aspiny dendrite that tapers into sparsely spiny secondary dendrites (Cassell and Gray, 1989; McDonald, 1982a; Schiess *et al.,* 1999). In addition, a small number of aspiny neurons have also been described (Cassell and Gray, 1989). These three cell types are distributed homogeneously throughout the CeA. Immunohistochemical studies have demonstrated the presence of wide variety of peptides in cells in the CeA as well as in the afferents innervating these neurons (Cassell and Gray, 1989; Cassell *et al.,* 1986; Moga and Grey, 1985). One study has shown that the peptides enkephalin, neurotensin and corticotropin releasing hormone (CRH) is found in GABAergic neurons. There appear to be two populations of these cells: one contains enkephalin and the other CRH (Day *et al.,* 1999)). Both populations have a partial overlap with neurotensin containing neurons (Day *et al.,* 1999). Interestingly, intraperitoneal administration of the cytokine interleukin-1 preferentially activated GABAergic neurons containing enkephalin (Day *et al.,* 1999), suggesting that neurons with different peptide content have different functional roles.

Thus neurons in the central nucleus are morphologically very different from those found in the basolateral complex with basolateral neurons having similar morphology to cortical structures and the central nucleus being more striatal-like (Hall, 1972; McDonald, 1982a). This finding is consistent with the different embryological origins of the two nuclei (Puelles, 2001; Swanson and Petrovich, 1998). Finally, as with the majority of cells in the striatum, projections from the central nuclei are predominantly GABAergic while the basolateral nuclei have glutamatergic projections (Davis *et al.,* 1994; Saha *et al.,* 2000; Swanson and Petrovich, 1998).

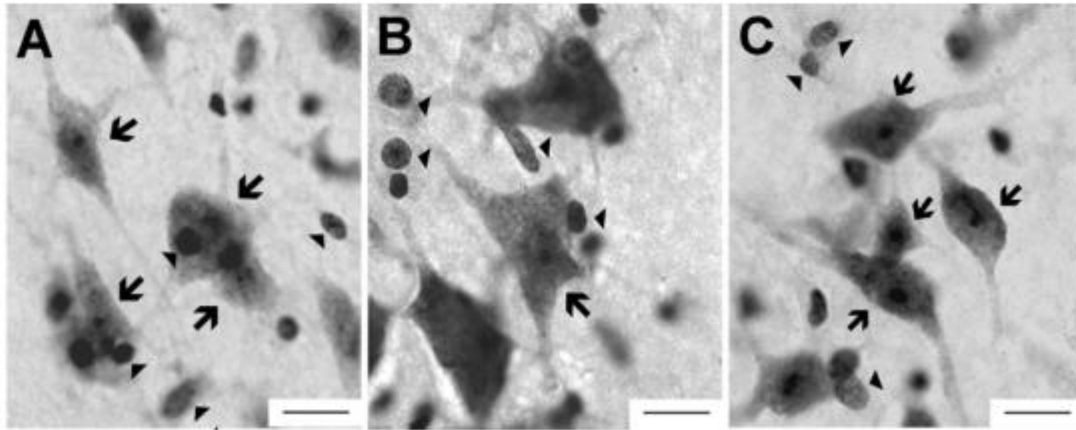


Figure 2.6.Tissue from the chimpanzee. Retrieved from Barger, N., Stenfanacci, L., Semendeferi, K., (2007). A comparative volumetric analysis of the amygdaloid complex and basolateral division in the human and ape brain. ***Am J Phys Anthropol.,*** **134:392-403. (A) lateral nucleus, (B) basal nucleus, and (C) central nucleus as viewed through x100Mg objective. Morphological features of neurons (arrows) and glia (arrowheads) can be distinguished at this magnification.**

2.4.2.2 MEDIAL NUCLEUS

The medial nucleus contains just one cell type that resembles the main neurons located in the CeM. They are small to medium-sized ovoid cells with two to four moderately spiny primary dendrites (McDonald, 1992b). There do not appear to be any local circuit neurons in this structure. Neurons in the bed nucleus of the stria terminalis, which are similar to the main cell types found in the medial and intermediate subdivisions of the central nucleus, have medium-sized somata and multipolar spiny dendrites (Mc Donald, 1983a). The anterior amygdaloid area contains cells that have ovoid somata and three to four primary dendrites that branch sparingly and have few spines (Hall, 1972). Thus they resemble the second class of neuron found in the CeM. The cell types observed in the nucleus of the lateral olfactory tract, the amygdalohippocampal area, and the cortical nuclei are similar to those in the basolateral complex. The majority of the cells are pyramidal-like with smaller stellate cells (which

resemble the local circuit neurons), spiny stellate cells, and neurogliaform cells also present to lesser degrees (McDonald, 1992; McDonald, 1983b). Orientation of neurons in the olfactory areas is more cortical-like with apical dendrites oriented parallel to each other.

2.4.3 CORTICOMEDIAL NUCLEI

This is the superficial or corticomedial nuclei (McDonald, 1982b; Price *et al.,* 1987). Although these superficial structures are called nuclei, many have cortical characteristics since they are located at the surface of the brain and have a layered structure (Price *et al.,* 1987). They comprise the nucleus of the lateral olfactory tract (NLOT), bed nucleus of the accessory olfactory tract (BAOT), the anterior and posterior cortical nucleus (CoA and CoP, respectively), and the periamygdaloid cortex (PAC). The BAOT is at the very rostral part of the amygdala where it is bordered laterally by the CoA. The CoA is a layered structure located lateral to the NLOT rostrally and the medial nucleus caudally. The CoP is also three layered and is located in the most caudal parts of the amygdala where it borders the AHA dorsally and the PAC laterally. The PAC is found ventral to the basal nucleus and is subdivided into three subdivisions: the periamygdaloid cortex, the medial division, and the sulcal division (Pitkanen, 2000; Price *et al.,* 1987).

2.4.4. OTHER AMYGDALOID NUCLEI

The final groups of nuclei comprising the remaining amygdala areas are:

- the anterior amygdala area (AAA),
- the amygdalo-hippocampal area (AHA),
- the intercalated nuclei (Amaral *et al.,* 1992; Pitkanen *et al.,* 1997; Price *et al.,* 1987).

The AHA is the most caudal of the amygdaloid nuclei and is comprised of the medial and lateral subdivisions. The intercalated nuclei are small groups of neurons found in clusters within the fiber bundles that separate the different amygdaloid nuclei (Millhouse, 1986).

2.4.4.1 INTERCALATED CELLS

There are two main types of neuron found in the intercalated cell masses. The first, which accounts for the vast majority of cells, has medium (10–15 μm) ovoid cell bodies with spiny, largely bipolar dendritic trees and axons that send collaterals into the lateral, basal, and central nuclei (Millhouse, 1986). The other type are very large cells (50 μm) with very long thick spiny or aspiny dendrites that travel in parallel to the borders of the basal, lateral, and central nuclei (Millhouse, 1986; Pare and Smith, 1993). These two cell types are very similar to striatal neurons.

2.5 THE HIPPOCAMPUS: ANATOMY

Topologically, the surface of a cerebral hemisphere can be regarded as a sphere with an indentation where it attaches to the midbrain. The structures that line the edge of the hole collectively make up the so-called limbic system (Latin limbus = border), with the hippocampus lining the posterior edge of this hole. These limbic structures include the hippocampus, cingulate cortex, olfactory cortex, and amygdala. Paul MacLean, in 1949, once suggested, as part of his triune brain theory, that the limbic structures comprise the neural basis of emotion. While most neuroscientists no longer believe in the concept of a unified "limbic system", these regions are highly interconnected and do interact with one another (Amaral and

Lavenex, 2006). Starting at the dentate gyrus and working inward along the S-curve of the hippocampus means traversing a series of narrow zones. The first of these, the dentate gyrus (DG), is actually a separate structure, a tightly packed layer of small granule cells wrapped around the end of the hippocampus proper, forming a pointed wedge in some cross-sections, a semicircle in others (Amaral and Lavenex, 2006). Next come a series of Cornu Ammonis (CA) areas: first CA4 (which underlies the dentate gyrus), then CA3, then a very small zone called CA2, then CA1. The CA areas are all filled with densely packed pyramidal cells similar to those found in the neocortex. After CA1 comes an area called the subiculum (Amaral and Lavenex, 2006). After this comes a pair of ill-defined areas called the presubiculum and parasubiculum, then a transition to the cortex proper, mostly the entorhinal area of the cortex (Amaral and Lavenex, 2006). The hippocampus proper is formed of Cornu Ammonis (CA). CA1 and CA2 formed of zone of small pyramidal cells, CA3 and CA4 formed of zone of large pyramidal cells (Amin *et al.,* 2013). CA4 projects into concavity of dentate gyrus that is formed of small granule cells. Subiculum is outward continuation of CA1 region. Areas in between compact zones of cells comprise the molecular layer which consists of neuronal processes (axons and dendrites), glial cells, and scattered nerve cells (Amin *et al.,* 2013). Most anatomists use the term "hippocampus proper" to refer to the four CA fields, and "hippocampal formation" to refer to the hippocampus proper plus dentate gyrus and subiculum (Amaral and Lavenex, 2006).

The major pathways of signal flow through the hippocampus combine to form a loop. Most external input comes from the adjoining entorhinal cortex, via the axons of the so-called perforant path. These axons arise from layer 2 of the entorhinal cortex (EC), and terminate in

the dentate gyrus and CA3 (Anderson *et al.,* 1971).There is also a distinct pathway from layer 3 of the EC directly to CA1, often referred to as the temporoammonic or TA-CA1 pathway. Granule cells of the DG send their axons (called "mossy fibers") to CA3. Pyramidal cells of CA3 send their axons to CA1. Pyramidal cells of CA1 send their axons to the subiculum and deep layers of the EC. Subicular neurons send their axons mainly to the EC. The perforant path-to-dentate gyrus-to-CA3-to-CA1 was called the trisynaptic circuit by Per Andersen, who noted that thin slices could be cut out of the hippocampus perpendicular to its long axis, in a way that preserves all of these connections (Anderson *et al.,* 1971). This observation was the basis of his lamellar hypothesis, which proposed that the hippocampus can be thought of as a series of parallel strips, operating in a functionally independent way (Anderson *et al.,* 1971). The lamellar concept is still sometimes considered to be a useful organizing principle, but more recent data, showing extensive longitudinal connections within the hippocampal system, have required it to be substantially modified (Anderson *et al.,* 2000). Perforant path input from EC layer II enters the dentate gyrus and is relayed to region CA3 (and to mossy cells, located in the hilus of the dentate gyrus, which then send information to distant portions of the dentate gyrus where the cycle is repeated (Anderson *et al.,*1971; 2000). Region CA3 combines this input with signals from EC layer II and sends extensive connections within the region and also sends connections to region CA1 through a set of fibers called the Schaffer collaterals. Region CA1 receives input from the CA3 subfield, EC layer III and the nucleus reuniens of the thalamus (which project only to the terminal apical dendritic tufts in the stratum lacunosum-moleculare). In turn, CA1 projects to the subiculum as well as sending information along the aforementioned output paths of the hippocampus. The subiculum is the final stage in the pathway, combining information from the CA1 projection and EC layer III to also send

information along the output pathways of the hippocampus (Anderson *et al.,* 1971; 2000; Heath and Harper, 1974). The hippocampus also receives a number of subcortical inputs. In Macaca fascicularis, these inputs include the amygdala (specifically the anterior amygdaloid area, the basolateral nucleus, and the periamygdaloid cortex), the medial septum and the diagonal band of Broca, the claustrum, the substantia innominata and the basal nucleus of Meynert, the thalamus (including the anterior nuclear complex, the laterodorsal nucleus, the paraventricular and parataenial nuclei, the nucleus reuniens, and the nucleus centralis medialis), the lateral preoptic and lateral hypothalamic areas, the supramammillary and retromammillary regions, the ventral tegmental area, the tegmental reticular fields, the raphe nuclei (the nucleus centralis superior and the dorsal raphe nucleus), the nucleus reticularis tegementi pontis, the central gray, the dorsal tegmental nucleus, and the locus coeruleus. The hippocampus also receives direct monosynaptic projections from the cerebellar fastigial nucleus (Heath and Harper, 1974).

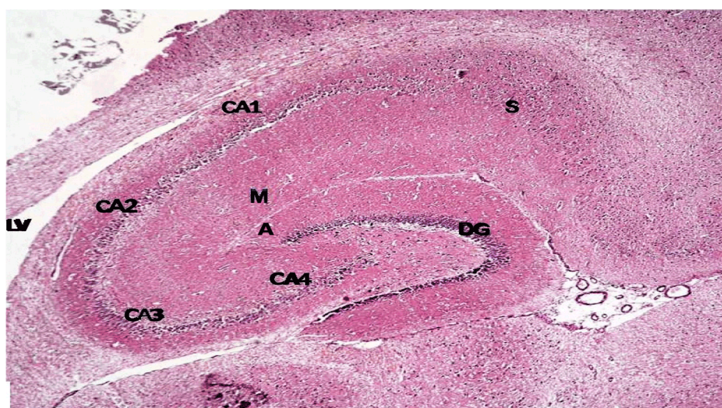


Fig. 2.7. Basic regions of the hippocampal formation. Retrieved with permission from Amin, S. N., Yousssef, M. F. (2013). A Histological and Functional study on Hippocampal formation of normal and diabetic rats. *F1000Research*. 2:151. DG: dentate gyrus. Sub: subiculum. EC: entorhinal cortex, CA 1-4: Regions of the Cornu Ammonis (CA).

2.5.1 HIPPOCAMPUS PROPER

The hippocampus is composed of multiple subfields. Though terminology varies among authors, the terms most frequently used are dentate gyrus and the Cornu ammonis (literally "Amun's horns", abbreviated CA). The dentate gyrus contains the fascia dentata and the hilus, while CA is differentiated into fields CA1, CA2, CA3, and CA4. The hippocampus proper is formed of cornu ammonis: CA1 and CA2 formed of zone of small pyramidal cells, CA3 and CA4 formed of zone of large pyramidal cells (Amin *et al.,* 2013). CA4 projects into concavity of dentate gyrus that is formed of small granule cells. Subiculum is outward continuation of CA1 region (Amin *et al.,* 2013). Areas in between compact zones of cells comprise the molecular layer which consists of neuronal processes (axons and dendrites), glial cells, and scattered nerve cells (Amin *et al.,* 2013). Cut in cross section, the hippocampus is a C-shaped structure that resembles a ram's horns. The name "cornu ammonis" refers to the Egyptian deity Amun, who has the head of a ram. The horned appearance of the hippocampus is caused by cell density differentials and the existence of varying degrees of neuronal fibers (Amaral and Lavenex, 2006). In rodents, the hippocampus is positioned so that, roughly, one end is near the top of the head (the dorsal or septal end) and one end near the bottom of the head (the ventral or temporal end).The structure itself is curved and subfields or regions are defined along the curve, from CA4 through CA1 (only CA3 and CA1 are labeled). The CA regions are also structured depthwise in clearly defined strata or layers (Amaral and Lavenex, 2006).

The **alveus** is the deepest layer and contains the axons from pyramidal neurons, passing on toward the fimbria/fornix, one of the major outputs of the hippocampus.

Stratum oriens (str. oriens) is the next layer superficial to the alveus. The cell bodies of inhibitory basket cells and horizontal trilaminar cells, named for their axons innervating three layers-- the oriens, pyramidal, and radiatum are located in this stratum. The basal dendrites of pyramidal neurons are also found here, where they receive input from other pyramidal cells, septal fibers and commissural fibers from the contralateral hippocampus (usually recurrent connections, especially in CA3 and CA2.) In rodents the two hippocampi are highly connected, but in primates this commissural connection is much sparser.

Stratum pyramidale (str. pyr.) contains the cell bodies of the pyramidal neurons, which are the principal excitatory neurons of the hippocampus. This stratum tends to be one of the more visible strata to the naked eye. In region CA3, this stratum contains synapses from the mossy fibers that course through stratum lucidum. This stratum also contains the cell bodies of many interneurons, including axo-axonic cells, bistratified cells, and radial trilaminar cells.

Stratum lucidum (str. luc.) is one of the thinnest strata in the hippocampus and only found in the CA3 region. Mossy fibers from the dentate gyrus granule cells course through this stratum in CA3, though synapses from these fibers can be found in str. pyr.

Stratum radiatum (str. rad.), like str. oriens, contains septal and commissural fibers. It also contains Schaffer collateral fibers, which are the projection forward from CA3 to CA1. Some interneurons that can be found in more superficial layers can also be found here, including basket cells, bistratified cells, and radial trilaminar cells.

Stratum lacunosum (str. lac.) is a thin stratum that too contains Schaffer collateral fibers, but it also contains perforant path fibers from the superficial layers of entorhinal cortex. Due to its

small size, it is often grouped together with stratum moleculare into a single stratum called stratum lacunosum-moleculare (str. l-m.).

Stratum moleculare (str. mol.) is the most superficial stratum in the hippocampus. Here the perforant path fibers form synapses onto the distal, apical dendrites of pyramidal cells.

The **hippocampal sulcus** (sulc.) or **fissure** is a cell-free region that separates the CA1 field from the dentate gyrus. Because the phase of recorded theta rhythm varies systematically through the strata, the fissure is often used as a fixed reference point for recording EEG as it is easily identifiable (Amaral and Lavenex, 2006).

2.5.2 DENTATE GYRUS

The dentate gyrus is composed of a similar series of strata:

The **polymorphic layer** (poly. lay.) is the most superficial layer of the dentate gyrus and is often considered a separate subfield (see CA4/hilus below). This layer contains many interneurons, and the axons of the dentate granule cells pass through this stratum on the way to CA3.

Stratum granulosum (str. gr.) contains the cell bodies of the dentate granule cells.

Stratum moleculare, inner third (str. mol. 1/3) is where both commissural fibers from the contralateral dentate gyrus run and form synapses as well as where inputs from the medial septum terminate, both on the proximal dendrites of the granule cells.

Stratum moleculare, external two thirds (str. mol. 2/3) is the deepest of the strata, sitting just superficial to the hippocampal fissure across from stratum moleculare in the CA fields. The perforant path fibers run through this strata, making excitatory synapses onto the distal apical dendrites of granule cells (Amaral and Lavenex, 2006).

2.6 THE PREFRONTAL CORTEX: ANATOMY

The prefrontal cortex is located rostral to the motor and premotor cortices. It is also called the frontal association cortex or the frontal granular cortex, referring to its functional and structural attributes, respectively. Like the rest of the cortex, it has been subdivided qualitatively into smaller architectonic regions on the basis of their distinct neuronal organization, such as the number and size of the cortical layers, the size, shape, and density of the neurons, and the degree of axon myelination (Damasio, 1985). In addition, support for this more refined cortical parcellation comes from the distinct connections of each cortical area with the various subdivisions of the mediodorsal nucleus of the thalamus and other cortical and subcortical structures (like the temporal and parietal lobes, the hypothalamus, the amygdala, and the hippocampal formation). In humans, lesions on the dorsolateral portion of the prefrontal cortex, including area 10, are associated with impairment in higher-cognitive abilities that facilitate extraction of meaning from ongoing experiences, the organization of mental contents that control creative thinking and language, and the artistic expression and planning of future actions (Damasio, 1985).

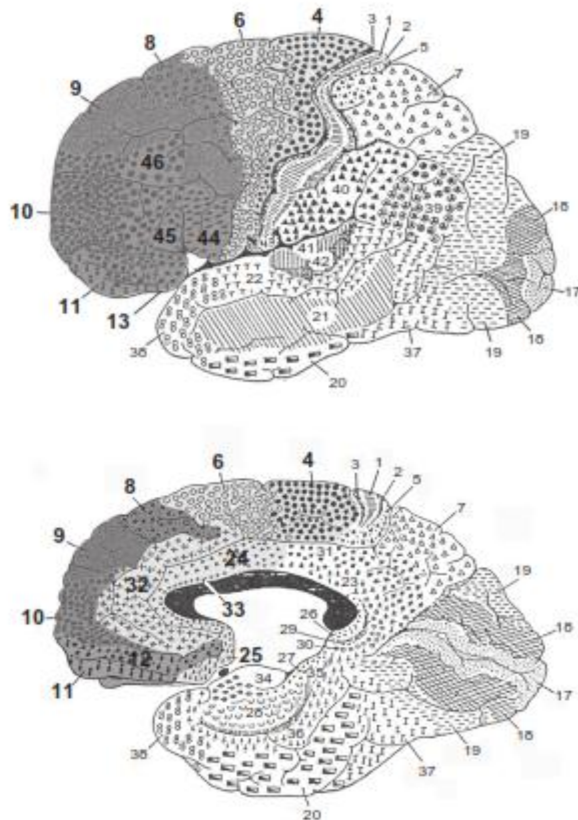


Fig. 2.5. Diagram of the human brain modified from Brodmann (1909) Vergleichende localisationslehre der grosshirnrinde in ihren prinzipien dargestellt auf grund des zellenbaues. Leipzig: Barth. Retrieved from Kate Teffer and Katerina Semendeferi (2012) M. A. Hofman and D. Falk (Eds.) Progress in Brain Research, Vol. 195 ISSN: 0079-6123. Illustrations show the frontal lobe (all shaded regions rostral to the central sulcus), including the prefrontal cortex (only the darker gray shaded regions) and the anterior cingulate.

Frontal lobe impairment produces the delayed response defcit that has been related to the lack of initiative or, in other words, the impairment of [a]interest and hence sustained attention and initiative. According to Brodmann’s classification scheme, “frontal region” included areas 8, 9, 10, 11, 12, 44, 45, 46, and 47 (of which 44, 45, and 47 he termed “subfrontal”); “precentral region” included areas 4 and 6; and anterior “cingulate region” included areas 24, 32, 33, and 25. Area 13 was at first identified by Brodmann only in nonhuman primates, not humans. It

has subsequently been identified in humans as part of the orbital prefrontal. Interestingly, the term "prefrontal" was used by Brodmann (1909) only for orbitofrontal area 11 located in the rostroventral part of the frontal lobe. Contemporary use of the term prefrontal cortex refers usually either to all areas demarcated here as part of the frontal lobe, with the exception of the motor/premotor cortex (BA 4 and 6), BA 44, and BA 24, or to areas located only in the dorsolateral frontal lobe, mostly BA 9, 10, and 46. Another use of the term prefrontal is increasingly found in the imaging literature, where the term usually refers to areas "anterior to the genu of the corpus callosum" (Semendeferi *et al.,* 2001).

2.5.1 Cytoarchitecture of area 10 of the prefrontal cortex in humans

Based on a qualitative description, Semendeferi and other scientists (2001) evaluated the cytoarchitectonic of area 10. The size of cortical layers, the stain intensity, and the size of cells were described in relation to each other (e.g., when a cortical layer was identifed as thin, this means that it was thin in relation to the size of the other layers). In humans, six cortical layers were easily distinguished (Semendeferi *et al.,* 2001). In comparison to the rest of the layers, layer I was thin to medium in width. Layer II was thin and, although not prominent, it was easily identifable. It included small granular and pyramidal cells that had a medium to dark staining. Layer III was the widest layer in the frontal pole, and its cells had a small, but gradual change in size; the pyramids close to layer II were smaller than those close to layer IV, and had a medium to dark staining. Layer IV was thin, but continuous with pale to medium stained granular and pyramidal cells. Its borders with layers III and V were clear and regular. Layer V was wide and includes large pyramids, though their sizes were only minimally larger than those of the pyramidal cells of layer III. Two sublayers, Va and Vb, were seen clearly. The

density of cells in Vb was less than that of Va, and many of the neurons have a pale staining. Layer VI included dark pyramidal and fusiform cells. Its bordered with layer V and the white matter were regular. Caudally, area 10 borders with other cortical areas on the dorsolateral, mesial, and orbital surfaces. The cortex of the superior frontal gyrus (on the dorsomesial and dorsolateral surfaces), which constituted Brodmann's area 9, was characterized by a more distinct layer Vb that had a much lower cell-packing density than Va or VI. Also, layer II became particularly prominent. It had a higher cell-packing density than layer II in area 10, and the cells were darkly stained. On the orbital surface, Brodmann's area 11 that neighbors area 10 included a thinner layer IV. Layers Va and VIb become more prominent in this cortical area, as does layer II (Semendeferi *et al.,* 2001).

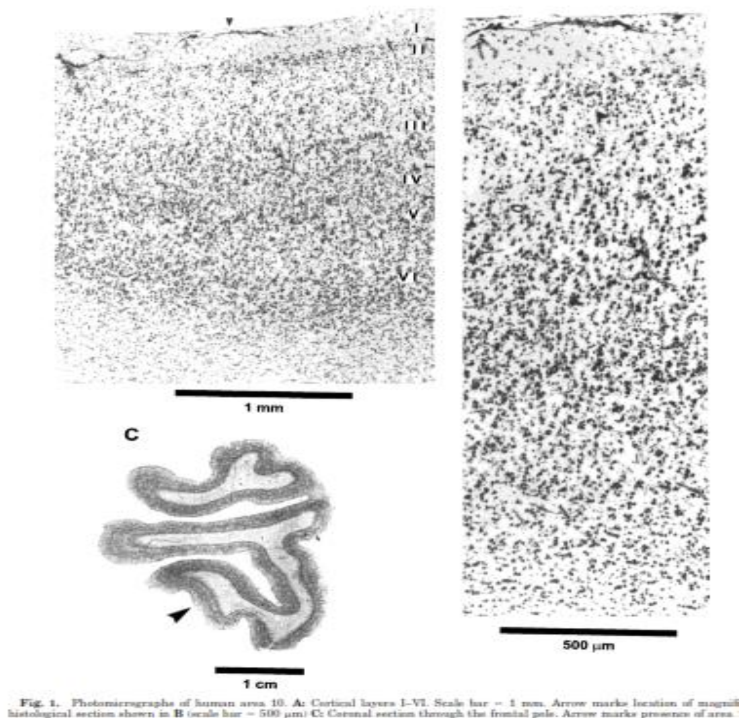


Fig. 2.6 Photomicrographs of Human Area 10 of the prefrontal cortex. Retrieved from Semendeferi *et al., (*2001). Prefrontal cortex in Humans and Apes: A comparative Study of Area 10. *American Journal of Phys Anthropol*. 114:224-241

2.7. A MORPHOMETRIC STUDY OF THE AMYGDALA IN THE COMMON SHREW AND GUINEA PIG

A previous morphometric studies in the common shrew (Rowniak *et al*, 2004) and guinea pig (Rowniak *et al.,* 2005) have demonstrated some interesting differences in the organization of the amygdala (CA) in the two species. These include the following:

1. Volumetric differences in the relative size of particular nuclei in CA of the common shrew (Rowniak *et al.,* 2004) and guinea pig (Rowniak *et al.,* 2005). These were the most interesting of the differences observed. For example, the lateral (LA) and basomedial (BM) nuclei, which were poorly developed in the common shrew (Rowniak *et al.,* 2004) showed a distinct size increase in the guinea pig (Rowniak *et al.,* 2005). On the other hand, basolateral (BL) and lateral olfactory tract (NLOT) nuclei, which were particularly well represented in the common shrew (Rowniak *et al.,* 2004), become distinctly smaller in the guinea pig (Rowniak *et al.,* 2005). The central (CE), cortical (CO) and medial (ME) nuclei seemed to be relatively stable in size in both the species studied (Rowniak *et al.,* 2004, Rowniak *et al.,* 2005). It is noteworthy that a comparison of the total numbers of neurons in the individual CA nuclei in the common shrew (Rowniak et el, 2004) and guinea pig (Rowniak *et al.,* 2005) respectively confirmed all the changes presented by the volumetric measurements and made them even clearer. For example, the percentages of LA and BM neurons in the guinea pig CA (Rowniak *et al.,* 2005) were distinctly higher than in the common shrew (Rowniak *et al.,* 2004) Moreover, the increases in neuronal population size in both these nuclei were even greater than their volume increases. The marked volume reduction of BL and NLOT seen in the guinea pig (Rowniak *et al.,* 2005) was accompanied by an even more marked reduction in the total number of neurons in both these nuclei. A clear reduction in density in CA and in all its

subdivisions in the guinea pig was revealed by a comparison of the density of neurons in CA of this species (Rowniak *et al.,* 2005) and the common shrew (Rowniak *et al.,* 2004) When the difference in the size of CA in the two species was taken into account, the result suggested a negative correlation between the size of CA and the density of cells in it. An increase, however, in the size of the neurons in the guinea pig CA and its subdivisions was revealed (Rowniak *et al.,* 2005) when compared with the common shrew (Rowniak *et al.,* 2004).

2.8. THE AMYGDALA AND FEAR CONDITIONING

In the years following Weiskrantz's publication in 1956, a number of studies pursued the role of the amygdala in fear by using a variety of different approaches. However, no consistent conclusions emerged, in large part because complex behavioral tasks that varied considerably from study to study were used. In short, there was little appreciation that different emotional tasks would be mediated by the brain in unique ways. Then, in the late 1970s and early 80s, researchers began using a simple behavioral task, Pavlovian fear conditioning/ Classical conditioning, to study fear networks. This made all the difference. It involves the creation of response to stimuli or the teaching of a new stimulus by pairing it with a stimulus for which there is biological reflex (LeDoux, 2003).

In Pavlovian fear conditioning, an emotionally neutral conditioned stimulus (CS), usually a tone, is presented in conjunction with an aversive unconditioned stimulus (US), often footshock. After one or several pairings, the CS acquires the capacity to elicit responses that typically occur in the presence of danger, such as defensive behavior (freezing or escape responses), autonomic nervous system responses (changes in blood pressure and heart rate), neuroendocrine responses (release of hormones from the pituitary and adrenal glands), etc. The

responses are not learned and are not voluntary. They are innate, species-typical responses to threats and are expressed automatically in the presence of appropriate stimuli. Fear conditioning thus allows new or learned threats to automatically activate evolutionarily tuned ways of responding to danger. The ease of establishment, rapidity of learning, long duration of the memory, and stereotyped nature of the responses all speak to the value of the Pavlovian learning as an approach to the study of fear mechanisms and account for the success achieved with this procedure (LeDoux, 2003).

Studies from many labs have led to the conclusion that damage to the amygdala interferes with the acquisition and expression of conditioned fear (LeDoux, 2000; Maren, 2001). Sensory inputs to the amygdala terminate mainly in the lateral nucleus (LA) (Amaral *et al.,* 1992; LeDoux *et al.,* 1990a; Mascagni *et al.,*. 1993; McDonald, 1998; Romanski and LeDoux, 1993; Turner *et al.,* 1980; Turner and Herkenham, 1991), and damage to LA interferes with fear conditioning (Campeau and Davis, 1995b; LeDoux *et al.,* 1990b). Auditory inputs to LA come from both the auditory thalamus and auditory cortex (see LeDoux *et al.,* 1990a; Mascagni *et al.,* 1993; McDonald, 1998; Romanski and LeDoux, 1993), and fear conditioning to a simple auditory CS can be mediated by either of these pathways (Romanski and LeDoux, 1992). It appears that the projection to LA from the auditory cortex is involved with a more complex auditory stimulus pattern (Jarrell *et al.,* 1987), but the exact conditions that require the cortex are poorly understood (Armony *et al.,* 1997). Although some lesion studies have questioned the ability of the thalamic pathway to mediate conditioning (Campeau and Davis, 1995b; Shi and Davis, 1999), single unit recordings show that the cortical pathway conditions slower over trials than the thalamic pathway (Quirk *et al.,* 1995, 1997; Repa *et al.,* 2001), thus indicating that plasticity in the amygdala occurs initially through the thalamic pathway (fig. 2.7).

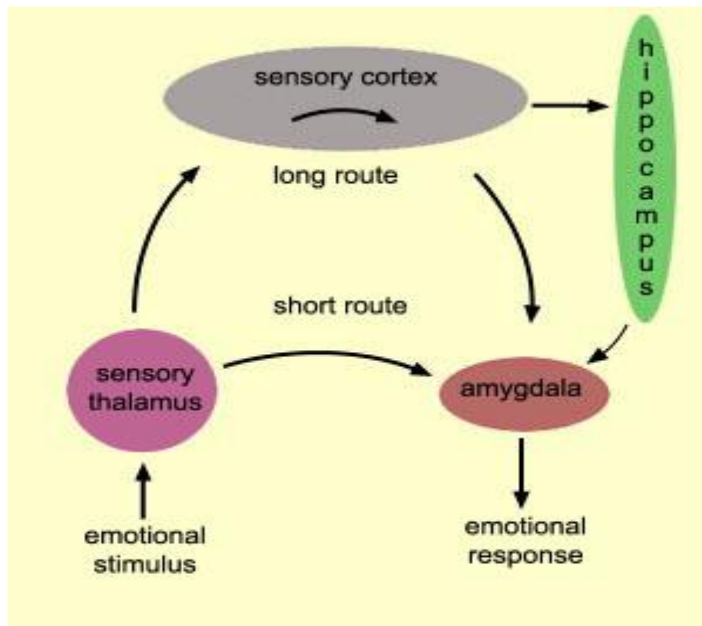


Fig. 2.7. The two pathways of fear: short route (Thalamoamygdala pathway) and long route (Thalamocorticoamygdala pathway)). Picture was adapted from Bruno Dubuc (2001) From Top to Bottom: The Amygdala and its allies-. (Canadian Institute of Neurosciences, Mental health, and Addiction. Canadian Institute of Health Research. www.thebrain.mcgill.ca

Recent fMRI studies in humans have found that the human amygdala shows activity changes during conditioning (LaBar *et al.,* 1998; Morris, 1998) and these correlate with activity in the thalamus but not the cortex (Morris *et al.,* 1999). Animals also exhibit fear responses when returned to the chamber in which the tone and shock were paired, or a chamber in which shocks occur alone. The chamber thus becomes a CS. This is called contextual fear conditioning and requires both the amygdala and hippocampus (Anagnostaras *et al.,* 2001; Blanchard *et al.,* 1970; Frankland *et al.,* 1997; Kim and Fanselow, 1992; Maren *et al.,* 1997; Phillips and LeDoux, 1992). Areas of the ventral hippocampus (CA1 and subiculum) project to the basal (B) and accesory basal (AB) nuclei of the amygdala (Canteras and Swanson, 1992), which are also known as the basolateral and basomedial nuclei (Pitkanen *et al.,* 1997). Damage

to these areas interferes with contextual conditioning (Majidishad *et al.,* 1996; Maren and Fanselow, 1995). Hippocampal projections to B and AB thus seem to be involved in contextual conditioning.

The central nucleus of the amygdala (CE) is the interface with motor systems. Damage to CE interferes with the expression of conditioned fear responses (Gentile *et al.,* 1986; Hitchcock and Davis, 1986; Iwata *et al.,* 1986; Kapp *et al.,* 1979; Van de Kar *et al.,* 1991), while damage to areas that CE projects to selectively interrupts the expression of individual responses. For example, damage to the lateral hypothalamus affects blood pressure but not freezing responses, and damage to the peraqueductal gray interferes with freezing but not blood pressure responses (LeDoux *et al.,* 1988). Similarly, damage to the bed nucleus of the stria terminalis has no effect on either blood pressure or freezing responses (LeDoux *et al.,* 1988) but disrupts the conditioned release of pituitary-adrenal stress hormones (Van de Kar *et al.,* 1991). Because CE receives inputs from LA, B, and AB (Pitkanen *et al.,* 1997), it is in a position to mediate the expression of conditioned fear responses elicited by both acoustic and contextual CSs. The direct projection from LA to CE seems to be sufficient for conditioning to an auditory CS, since lesions of B and AB have no effect on fear conditioning to a tone (Majidishad *et al.,* 1996). The exact manner in which LA and CE communicate is not clear (Royer *et al.,* 1999), but the intercalated cell mass located between LA and CE may be involved (Royer *et al.,* 1999).

2.8.1. MEMORY VS. MODULATION

In spite of a wealth of data implicating the amygdala in fear conditioning, some authors have recently suggested that the amygdala is not a site of plasticity or storage during fear

conditioning (e.g., Cahill and McGaugh, 1998; Vazdarjanova and McGaugh, 1998). They argue instead that the amygdala modulates memories that are formed elsewhere. It is clear that there are multiple memory systems in the brain (Eichenbaum, 1994; McDonald and White, 1993; Squire *et al.,* 1993), and that the amygdala does indeed modulate memories formed in other systems, such as declarative or explicit memories formed through hippocampal circuits or habit memories formed through striatal circuits (Packard *et al.,* 1994). However, evidence for a role of the amygdala in modulation should not be confused with evidence against a role in plasticity. That the amygdala is indeed important for learning is suggested by studies showing that inactivation of the amygdala during learning prevents learning from taking place (e.g., Helmstetter and Bellgowan, 1994; Muller *et al.,* 1997). Further, if the inactivation occurs immediately after training, then there is no effect on subsequent memory (Wilensky *et al.,* 1999), showing that the effects of pre-training treatment is on learning and not on processes that occur after learning. The amygdala thus seems to be essential for fear learning, and does not modulate its own learning.

2.8.2 The Amygdala, Prefrontal cortex, Hippocampus and fear

The brain structure that is the center of most neurobiological events associated with fear is the amygdala, located behind the pituitary gland. The role of the amygdala in fear is best understood as part of a circuitry of fear learning (Olsson and Phelps, 2007). It is essential for proper adaptation to stress and specific modulation of emotional learning and memory. In the presence of a threatening stimulus, the amygdala generates the secretion of hormones that influence fear and aggression (Ben, 2004). Once response to the stimulus in the form of fear or aggression commences, the amygdala may elicit the release of hormones into the body to put

the person into a state of alertness, in which they are ready to move, run, fight, etc. This defensive response is generally referred to in physiology as the fight-or-flight response regulated by the hypothalamus (Henry *et al.,* 2004). Once the person is in safe mode, meaning that there are no longer any potential threats surrounding them, the amygdala will send this information to the medial prefrontal cortex (mPFC) where it is stored for similar future situations. The storing of memory in the mPFC is known as memory consolidation (John, 2004). Some of the hormones involved during the state of fight-or-flight include epinephrine and norepinephrine and cortisol. Epinephrine regulates heart rate and metabolism as well as dilating blood vessels and air passages. Norepinephrine increases heart rate, blood flow to skeletal muscles and the release of glucose from energy stores (von Bohlen und and Dermietzel, 2006). Cortisol increases blood sugar and helps with metabolism (Hoehn and Marieb, 2010).

After a situation which incites fear occurs, the amygdala and hippocampus record the event through synaptic plasticity (Amunts *et al.,* 2005). The stimulation to the hippocampus will cause the individual to remember many details surrounding the situation. Plasticity and memory formation in the amygdala are generated by activation of the neurons in the region. Experimental data supports the notion that synaptic plasiticity of the neurons leading to the lateral amygdala occurs with fear conditioning (LeDoux, 2003). In some cases, this forms permanent fear responses such as post-traumatic stress disorder (PTSD) or a phobia. MRI and fMRI scans have shown that the amygdala in individuals diagnosed with such disorders including bipolar or panic disorder is larger and wired for a higher level of fear (Cheng *et al.,* 2003).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 MATERIALS

3.1.1 Animal Heads: Seven (7) heads of dogs (Mongrel breed), goats (Red sokoto breed), and sheep (Uda breed) respectively were obtained from slaughterhouses in Zaria, Kaduna state. They were sacrificed by decapitation. Only adult animals were used and they were aged using dentition as previously described by Dyce *et al.* (1996) after being adjudged clinically healthy.

3.1.2 Reagents: 10% formal saline solution, Heamatoxylin and Eosin stain , Phosphotungstic acid (Sigma Chemical Company, St. Louis, U.S.A.) , Toludine blue, Graded Alcohol solution - 70%, 80%, 95%, 100%(BDH Chemicals Ltd, Poole, England), Xylene (BDH Chemicals Ltd, Poole, England), Silver nitrate (Hopkin & Williams, Searle Company, Chadwell Health, Essex, England)

3.1.3 Equipments and instruments: Dissection Kit, specimen containers, glass slides, cover slips, Vernier caliper, Graduated measuring cylinder and beaker, Digital weighing balance (Mettler balance P 1210, Mettler instrument AG, Switzerland; sensitivity: 0.001

3.2 METHOD

3.2.1 Removal of Brain specimen from the skull

Freshly slaughtered animals were used and heads were obtained immediately after decapitation. The scalp tissue layers were removed using forceps thereby exposing the skull (Fig. 3.1). The skulls were immediately opened up using a butcher's knife in order to facilitate penetration of the fixative into the brain tissue. Also, a syringe was used to inject some of the

fixative into the brain through the cracked skull to ensure deep penetration of 10% formal saline solution. Then the opened skulls were fixed in a bucket containing ten (10) litres of 10% formal saline solution and transported to the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Kaduna State. They were washed so as to remove debris and blood, and allowed fix for one (1) week in ten (10) litres of freshly prepared 10% formal saline solution. All the animal skulls were subjected to the same condition of fixation to reduce tissue shrinkages resulting from use of fixatives. Afterwards, the fixed brain tissues were carefully removed from the skull by sawing the calvaria manually, starting from the frontal bone anteriorly, proceeding to the parietal bone, then to the occipital bone posteriorly. Skull bones of the basicranium were removed gradually using a forcep until the brains were completely exposed. The brains were detached from skulls by cutting through the meninges and cranial nerves at the base of the skull when they were encountered for ease of removal of the intact brain from the cranial cavity. The dural covering of the intact brains were removed completely, thereby exposing the brains. Then morphological and morphometric studies involving observation of differences and similarities on brain shapes and sizes, measurement of brain weight and volume, cerebral and cerebellar length and width measurement, and counting of number of gyri and sulci were carried out on the brains.

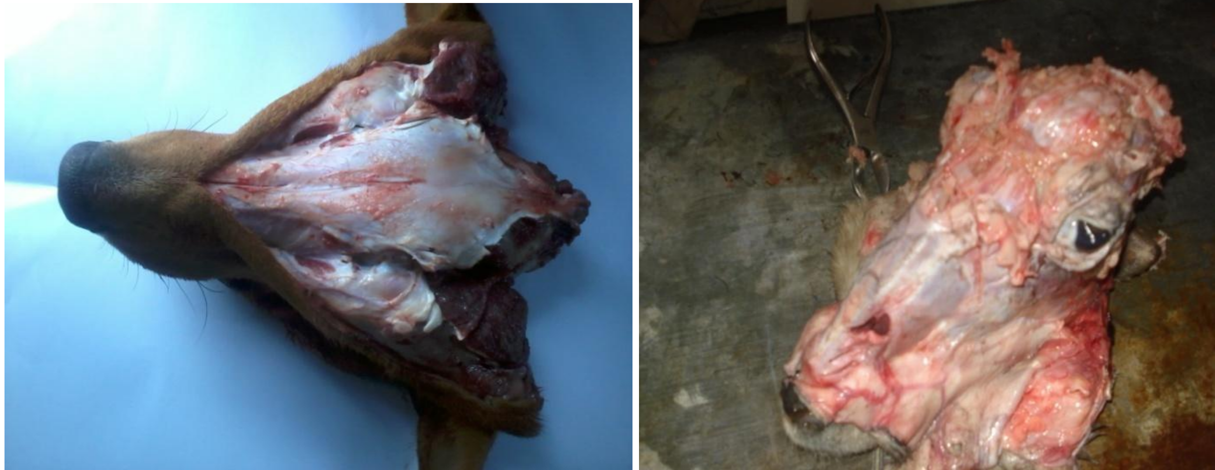


Fig 3.1 Removal of scalp tissue layers of dog and sheep heads respectively using forceps

3.2.2 Gross morphological studies

Each brain was observed grossly for cranial topography and malformation among the species under study (Fig. 4.1- 4.6). The nature of cerebral gyrifications, including the intricacy of cerebral folding, was studied in the three animals and differences and similarities observed were reported.

3.2.3 Gross morphometric studies

This included the following:

-Counting of number of gyri and sulci

-Measurement of brain weight, brain volume, cerebral length and width, cerebellar length and width.

1) Gyrification: A gyrus is identified as a fold surrounded by one or more sulci. The number of gyri and sulci were counted and recorded for each brain. A coloured marker was used to mark each sulci immediately after being counted so as to prevent repetition of count (Fig. 3.2).

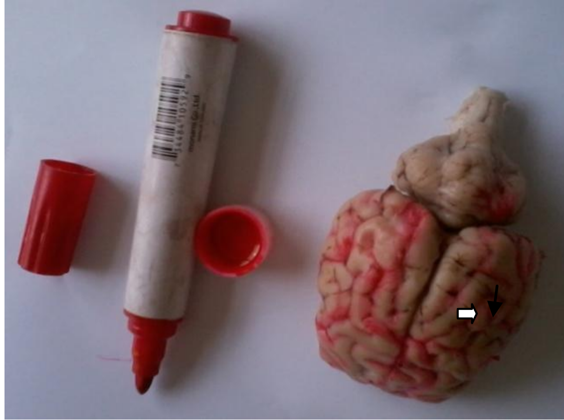


Fig. 3.2 Counting of number of sulci and gyri on each brain specimen using a coloured marker. ⇨ Indicates gyrus (elevation) on the brain while ↓ shows sulcus (depression) on the brain.

2) **Weight of brain**: Measurement was done using a digital weighing scale (Fig. 3.3). Readings were taken three (3) times for each brain specimen for accuracy and the average value obtained were recorded (Kavoi and Jameela, 2011)..

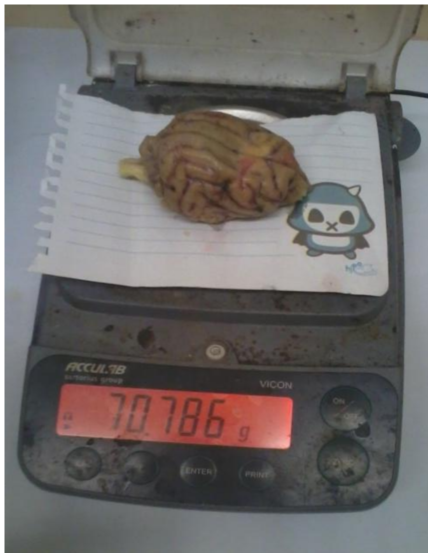


Fig. 3.3. Weighing the brain using a digital weighing scale

3) **Volume of brain**: Volumes of brain specimens were determined using Displacement Method (Fig. 3.4). This involved taking the volume of 10% formal saline solution displaced by

the brain after immersion in the fluid as equivalent to the volume of brain. Readings were taken three (3) times and the average was recorded (Kavoi and Jameela, 2011).

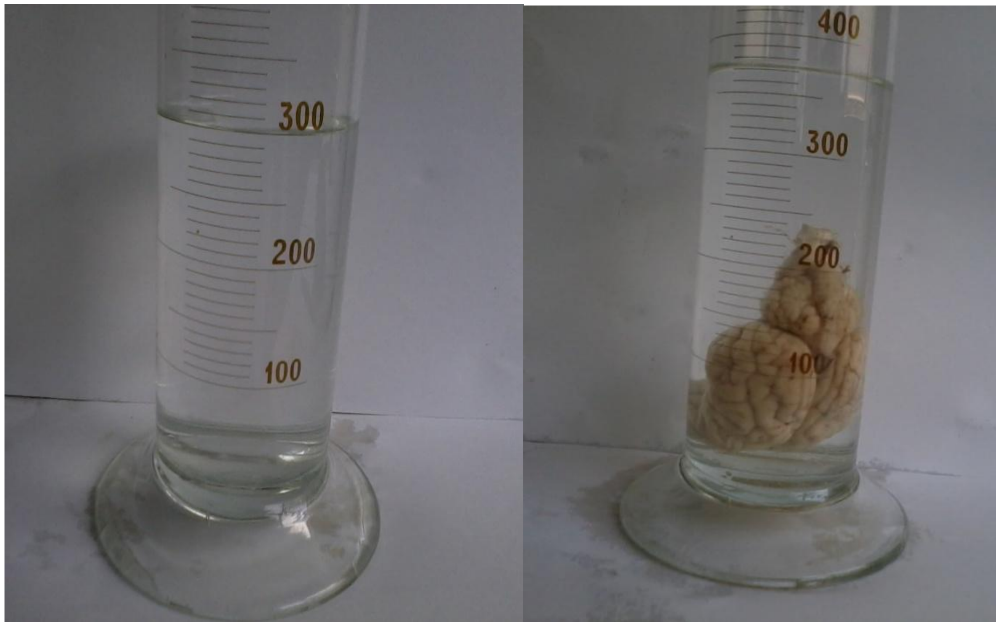


Fig 3.4. Volume displaced by brain= Final volume – Initial volume where Initial volume is the volume of 10% normal saline solution in measuring cylinder before immersion of the brain and Final volume is the volume of 10% normal saline solution in the cylinder after immersion of the brain into the fixative (Water Displacement Method).

4**) Linear measurements**: These were carried out on whole brain tissue using a vernier caliper (fig 3.5- 3.11). The following measurements were taken:

a Length of the Cerebrum (Right and Left) (Fig. 3.5)

b Width of the Cerebrum (Right and Left, and Full) (Fig. 3.6 and 3.7 respectively)

c Length of the Cerebellum (Fig. 3.8)

d Width of the Cerebellum (Fig 3.9)

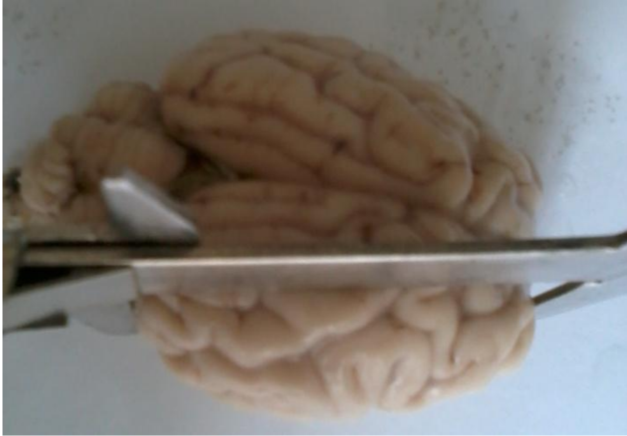


Fig. 3.5 Right cerebral length

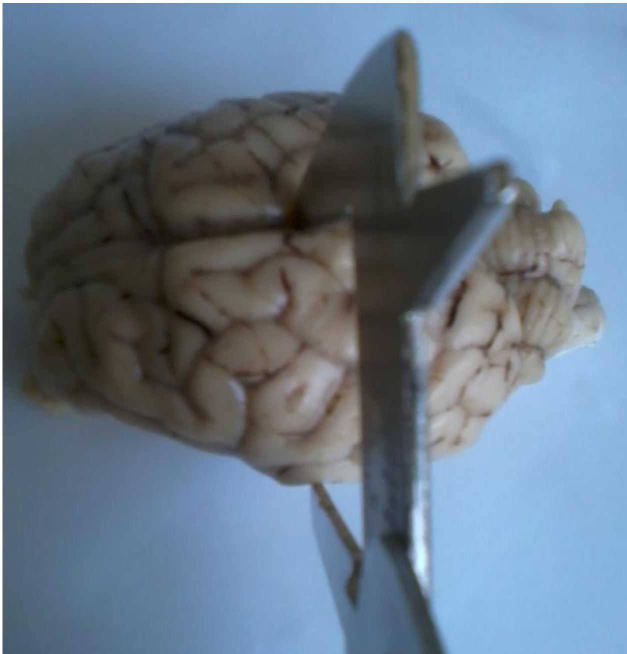


Fig. 3.6 Right cerebral width

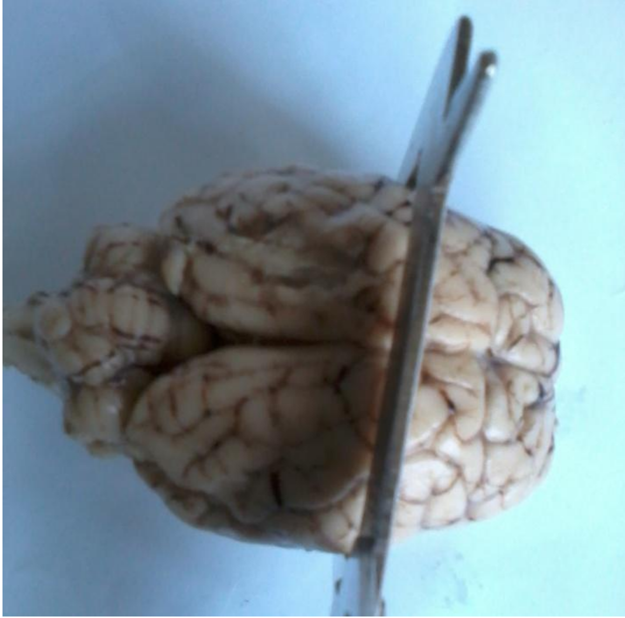


Fig. 3.7 Full cerebral width

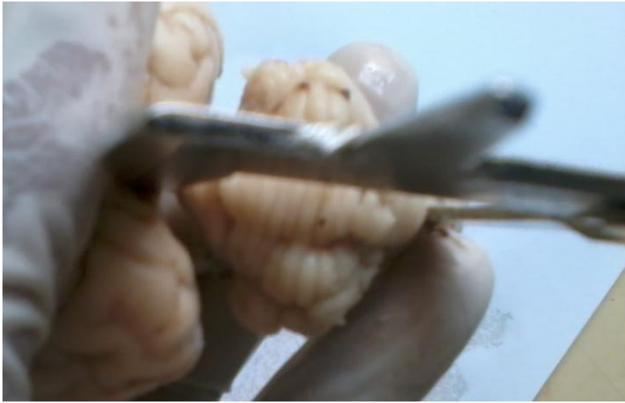


Fig. 3.8 Cerebellar length

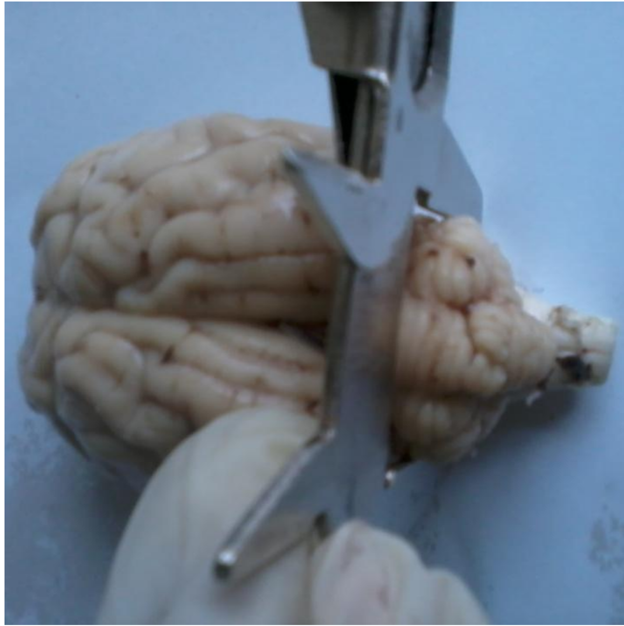


Fig. 3.9 Cerebellar width

3.2.4 Dissection of brain specimens

Using a dissecting blade, each brain was sectioned coronally through the frontal region of the brain to expose the prefrontal cortex. The prefrontal cortex of the brain was dissected out and kept in a labeled specimen container. Also, on the ventral aspect of the brain, coronal section passing close to the mamillary body, cutting through the left and right temporal lobes of the cerebrum was made. Ventromedially on each temporal lobe, the left and the right amygdala of each brain were located. The amygdale is an almond-shaped mass of nuclei in the corresponding temporal lobe lying close to the enlarged end of the hippocampus anteriorly. Tha amygdala and the hippocampus were dissected out. Dissected sections of the hippocampus, amygdala, and prefrontal cortex were each kept seperately in well labeled specimen containers having 10% formal saline solution. The brain tissues were processed for routine histological and histochemical studies.

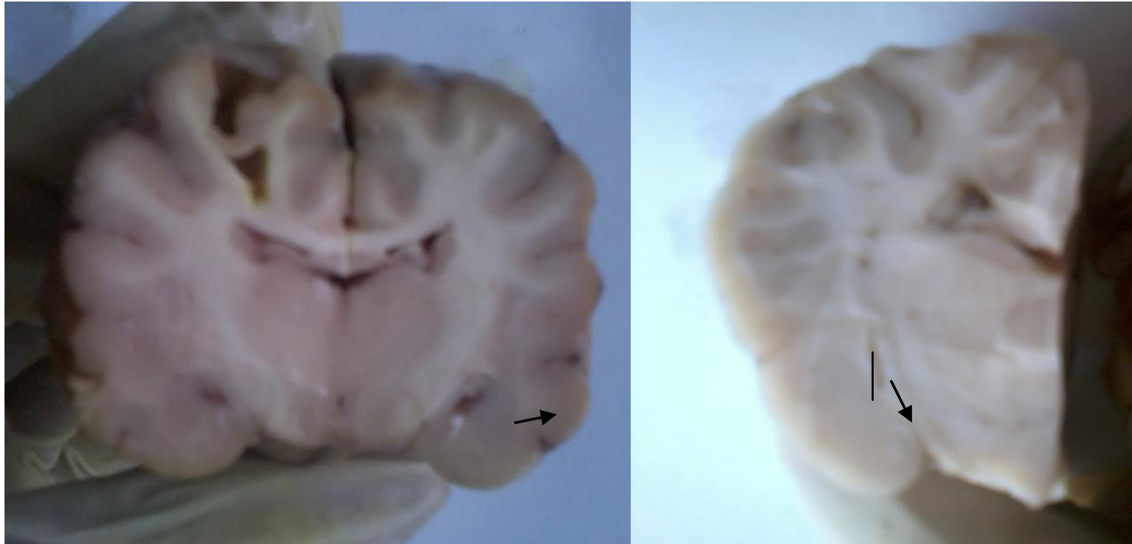


Fig. 3.10. Different coronal sections through the brain. The amygdala is structure labeled with an arrow. The hippocampus is located posterior to the amygdala. The black line indicates the location of the external capsule which is a helpful land mark in identifying the lateral nucleus of the amygdala.

3.2.5 Tissue processing

The dissected sections of hippocampus, amygdala, and prefrontal cortex of goat, sheep, and dog brain tissues were fixed in 10% formal saline solution for one (1) more day. Dehydration of tissues was done in ascending grades of alcohol: 70% alcohol, 80% alcohol, 95% alcohol, absolute alcohol. The dehydrated tissues were then cleared in xylene in order to remove opacity from dehydrated tissue, making them transparent. The cleared tissues were infiltrated by immersion in molten wax using a Shandon Southern Duplex Processor (Shandon Southern Products Limited, England), and manually embedded in paraffin wax using L-shaped moulds. Tissue moulds were labeled and blocks of tissues were trimmed and sectioned at 5 microns using a rotary microtome. The ribbons of sections were floated out on a water bath at 55ºC. The brain sections were picked on slides by immersing the slide lightly smeared with adhesive (albumen) vertically in water bath to three quarters of its length and maneuvering the section

into contact with slide. On lifting the slide vertically, from the water, the section was flattened onto the slide and labeled accordingly. The Sections were correctly positioned on the slides and drained in the vertical position for 60 minutes and then dried in oven at 37ºC overnight. Some sections of tissues were stained with Phosphotungsic Acid and Heamatoxylin (PTAH), Heamatoxylin and Eosin (H and E), and Toludine Blue Stains repectively.

3.2.5.1 Staining by H and E method

The H and E staining was done using modified Bancroft and Gamble procedure (2008).

3.2.5.2 Staining by PTAH Method

Tissues were deparaffinized and hydrated with distilled water. Tissues were put in Zenker's fixative and allowed to stand for 5 minutes. Then they were washed with tap water. Tissues were allowed to stand in Lugol's iodine for 5 minutes. They were again washed in tap water. For glial fibers, iodine was bleached out in 95% alcohol. Tissues were again washed in tap water 10 minutes. Oxidation was done in 0.25% potassium permanganate for 5 minutes. Tissues were again washed in tap water. Bleaching was in 5% oxalic acid until white. Washing was done in tap water, rinsing in distilled water. Tissues were placed in PTAH stain overnight at room temperature. Dehydration was done rapidly through two changes each of 95%, 100% alcohol, and two changes of Xylene. Coversliping was done. Slides were viewed under light microscope (Modified Mallory's Method).

3.2.5.3 Staining by Toludine Blue Method

The tissues were stained with toludine blue to show the cell bodies of neurons in terms of amount. The sections were de-waxed and brought to water. They were then covered with

toludine stained for 10 – 20 minutes. The sections were rinsed in distilled water and differentiated in 0.25% acetic alcohol until most of the stain was removed. Sections were then rinsed well and then mounted in DPX (Bancroft and Gamble, 2008). Slides were viewed using a light microscope. Then ten (10) separate photomicrographs of each tissue section (hippocampus, prefrontal cortex, and basal amygdala nucleus, lateral amygdala nucleus, and central amygdala nucleus) taken with a digital camera (Amscope MA 500) at a magnification of X250

3.2.5.4 Golgi Method

In this work, a modified protocol for golgi (silver) technique according to method of Raju T. R. *et al.,* (2004). Whole brain tissues were fixed in 10% formalin for about a week. The hippocampus, prefrontal cortex, and the amygdala were dissected. The tissues were then placed in 3% potassium dichromate for 5 days. The solution was changed every day. The tissues were transferred into 2% silver nitrate solution for 2 days at room temperature. Filter paper was used to absorb excess solution and the silver nitrate solution was changed several times until brown precipitates disappeared. Sections were cut at 50 µm thickness and allowed to float on distilled water in a water bath. The sections were mounted on superfrost plus slides, air-dried for 10 minutes and dehydrated through 95% alcohol and cleared in xylene and cover slipped. Slides were viewed using a light microscope. Photomicrographs of each tissue section (hippocampus, prefrontal cortex, lateral amygdala nucleus, and central amygdala nucleus) were taken with a digital camera (Amscope MA 500) connected to a light microscope and computer

3.2.6 Cell count and Cell measurements (Histomorphometry)

In this study, neuronal count was carried out on the basolateral and central nuclei of the amygdala, the CA3 cells of the hippocampus, and the ganglionic (layer V) cells of the prefrontal cortex using Digimizer image analysis 4.2.2. MedCalc. Software (Ostend, Belgium). Measurement of the cell sizes and counting of the number of nerve cells in tissue sections at X250 magnification were done. Areas, perimeters, lengths, and diameters of the neuronal cells were obtained. The neuronal types were classified based on anatomical parameters such as somatic shape and size, and dendritic arborization. To measure a variable, the cell to be measured was traced manually with a path pointer and the software automatically gave the mean value of the perimeter, area, and length of that cell. Nerve cells at the borders of the photomicrographs were not measured or counted. Nerve cells in ten (10) separate golgi-stained photomicrographs were measured and the average taken for measurement of dendritic aborization of cells. Determination of the density of neurons using cell count and measurement of the diameters of nerve cell bodies were done using toludine blue-stained sections. In this case also, cells in ten (10) separate photomicrographs of a slide were counted and the average taken.

The variables measured were:

- Area of nerve cells (μm^2)
- Perimeter of nerve cells (µm).
- Diameter of nerve cells (µm).
- Number of cells

Photomicrographs were taken with a X250 objective lens. The eyepiece was constant at a magnification of X10. Measurements were originally obtained in pixel value and then

converted to micrometer using University of Geneva Bioimaging microscope calibration for Axiocam microscope. At a magnification of X5, 1 pixel is equal to 1.3441333µm. Therefore, at a magnification of X250, 1 pixel would be equivalent to 0.26882666 µm.

3.2.7 Statistical analysis

Data obtained from gross morphometric and histomorphometric studies were expressed as mean value ± Standard Error of Mean (SEM). Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 18. LSD post-hoc test was used to determine the level significance of the values obtained. Values of $P \leq 0.05$ were considered significant.

4.0 RESULTS

4.1 GROSS MORPHOLOGICAL STUDIES

Gross comparison of adult brains of sheep, goat, and dog revealed many distinctions in their brain morphology. However, sheep and goat brains were very similar in morphology but different when compared with dog. Figures 4.1- 4.6 show some intact whole brains of these domestic animals. The sheep brains (fig 4.1 and 4.2) and goat brains(fig. 4.3 and fig. 4.4) showed lateral expansions of their cerebral cortex from rostral to caudal region of the cortex while the brains of the dog demonstrated linear neuraxis from rostral to caudal without prominent rostrolateral expansions but caudal expansions (fig. 4.5 and 4.6). Most notable difference was seen in the frontal view of the brain specimen. The dog displayed a prominent enlongated, sharp frontal contour (Fig. 4.5-4.6) while the sheep and goat had rounded, blunt, and smooth frontal contour of cerebrum (Fig. 4.1- 4.4). The cerebral topography of the dog showed less intricate folding of the gyri as they were longer than gyri in sheep and goat which showed shorter and more intricately folded gyri, hence increased surface area of the brain. Also dorsally, there was observed an overlapping of dog cerebellum by its cerebral hemispheres posteriorly. In sheep and goat, there was no overlap of the cerebellum by the cerebral hemispheres. Their cerebellum could easily be observed when viewed dorsally (fig. 4.1, 4.3, 4.5). On the ventral surface of the brain, the temporal lobe of dog showed pointed and elevated lobe while those of the goat and sheep were round, smooth, and slightly elevated in comparison (fig.4.2, 4.4, 4.6). Coronal section through the amygdala revealed showed that it is an oval shaped structure (fig. 4.7). The hippocampus was located posterior to the amygdala. The external capsule served as a helpful land mark in identifying the lateral nucleus of the

amygdala. Lateral nucleus was bordered laterally by the external capsule and medially by the central nucleus. The basal nucleus was located ventral to the Lateral nucleus (Sah et. al, 2003).

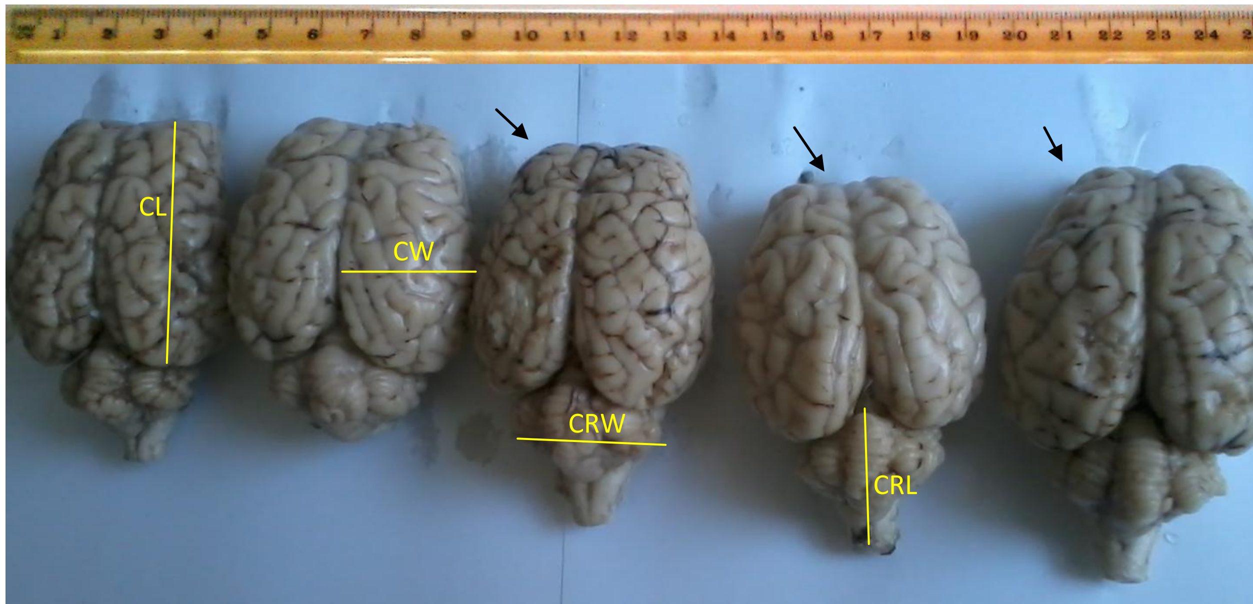


Fig. 4.1 Dorsal view of five (5) sheep brains. ↑arrow shows rounded frontal lobe of sheep brains which is absent in dog. Rostral to caudal expansion of the cerebral cortex was observed sheep. Morphometric studies revealed sheep as having the least cerebral length and the most cerebral width (Table 4.1). Therefore, the rostocaudal expansion of sheep was more than goat and dog. There were intricate foldings of gyri and sulci on the cerebrum of sheep than that of dog, hence larger surface area. The cerebellum of sheep was not overlapped by their cerebral hemispheres. They were easily seen when viewed dorsally. Average Measurement obtained: CL- Cerebral length~ 4.65cm, CW-Cerebral width~ 2.08cm, CRW-Cerebellar Width~1.99cm, CRL-Cerebellar Length~1.59cm

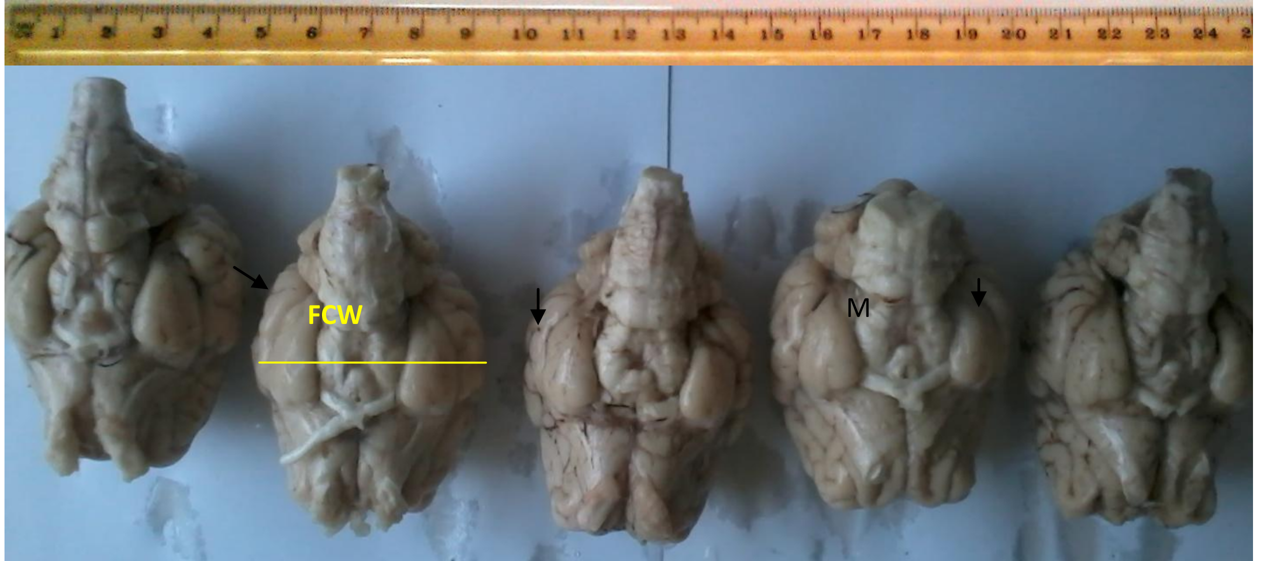


Fig. 4.2 Ventral view of five (5) sheep brains. Arrows indicate temporal lobe of the brain. The hippocampus is located posterior to the amygdala in the temporal lobe. The temporal lobes of sheep is not as elevated as dog, but is rounded and smooth. Average measurement obtained: FCW- Full Cerebral Width~ 4.16cm

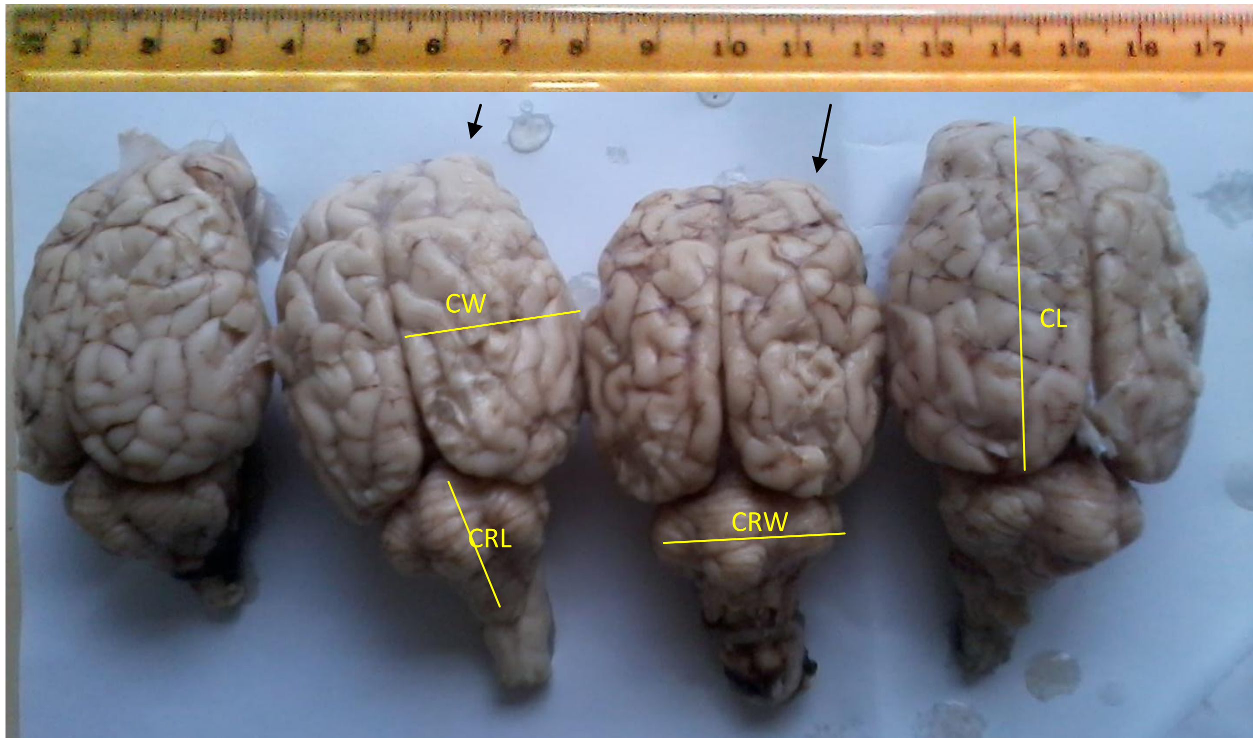


fig 4.3 Dorsal view of four (4) goat brains. Arrows point toward rounded frontal lobe. Rostral to caudal expansion of the cerebral cortex was observed goat also. CL- Cerebral length~ 4.87cm, CW-Cerebral width~ 2.40cm, CRW-Cerebellar Width~2.01cm, CRL-Cerebellar Length~1.86cm

fig 4.4 Ventral view of four (4) goat brains. Black arrow heads indicate the mamillary bodies which serve as a landmark for the dissection of the amygdala. Temporal lobe is rounded, smooth, and slightly elevated as in sheep, but not as elevated as dog. FCW- Full Cerebral Width~ 4.00cm

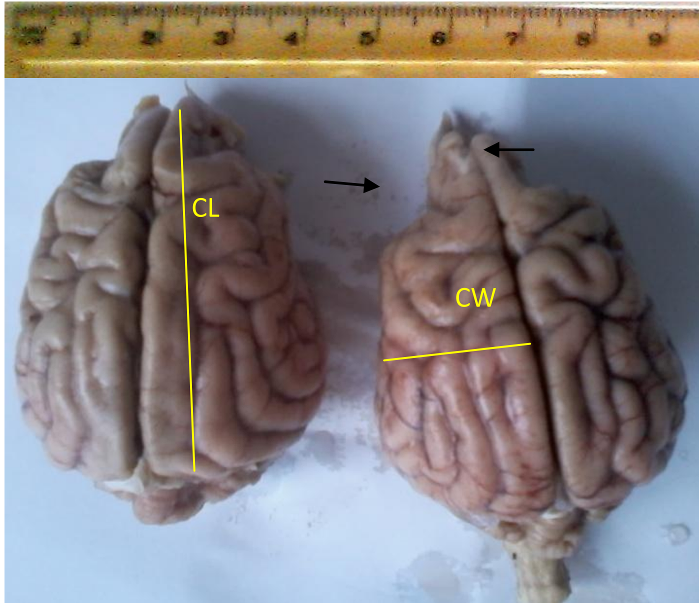


Fig. 4.5 Dorsal view of two (2) dog brains. Black arrow shows indentation of frontal lobe in dog which is absent in sheep and goat brains. The cerebrum is not as intricately folded when compared with sheep and goat. Dog had the most cerebral length and the least cerebral width (Table 4.1). The cerebellar width of dog is more than those of sheep and goat. Average measurement obtained: CL-Cerebral length-5.32cm, CW-Cerebral Width-1.74cm. The cerebellum is overlapped by the cerebrum. CRL- Cerebellar Length-1.35cm, CRW-Cerebellar Width-2.44cm.

Fig 4.6 Ventral view of two(2) dog brains. Arrows identify the temporal lobe of the dog which is elevated and pointed in contrast with the smooth and rounded temporal lobe of sheep and goat. White triangle indicates the location of the mamillary bodies which serve as a useful landmark in dissection of the amygdala and hippocampus. FCW- Full Cerebellar Width-3.71cm

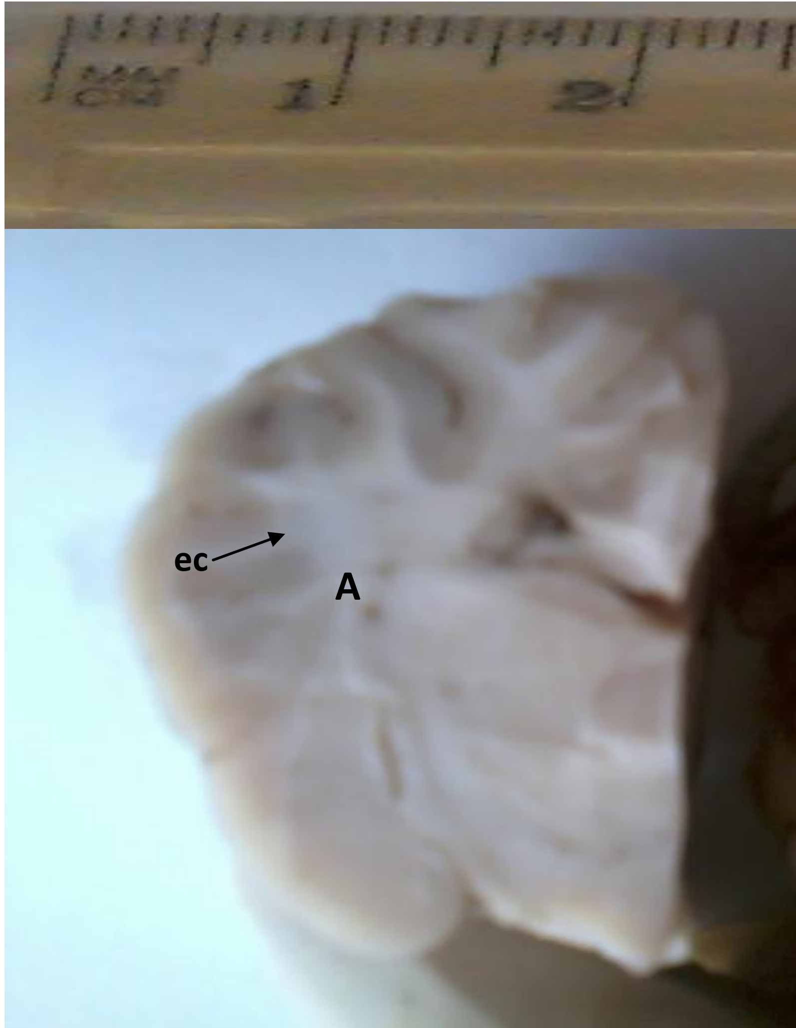


Fig.4.7. Coronal section through the brain. A- amygdala, ec- external capsule. The hippocampus is located posterior to the amygdala. ec is a helpful land mark in identifying the lateral nucleus of the amygdala. It is made up of nerve fibres and seen as a white stripe. Lateral nucleus is bordered laterally by the external capsule and medially by the central nucleus. The basal nucleus is located ventral to the Lateral nucleus.

4.2 GROSS MORPHOMETRIC STUDIES

Table 4.1 revealed significant differences in the brain weight, brain volume, cerebral and cerebellar length and width, number of gyri and sulci of the brains of sheep, goat, and dog. The mean weight of brains was greatest in sheep (82.00±1.71g), followed by goat (79.38±3.13g), and was least in dog (72.79±1.03g). Similar trend was observed in the volume of brain while the cerebral lengths (right and left cerebrum respectively) were greatest in dog (5.32±0.50cm, 5.37±0.05cm) followed by goat (4.87±0.91cm, 4.79±0.11cm), and sheep had the least cerebral length (4.65±0.69cm, 4.74±0.56cm). The full cerebral width (Fig. 3.7) was greatest in sheep (4.16±0.04cm), followed by goat (4.00±0.65cm), and then dog (3.71±0.11cm). Goat had the longest cerebellar length (1.86±0.11cm), followed by sheep (1.59±0.06cm), and then dog (1.35±0.20cm). Dog had the most cerebellar width (2.44±0.20cm), followed by goat (2.01±0.07cm). Sheep had the least (1.99±0.59cm).

Table 4.1 Gross morphometric differences in variables obtained of sheep, goat, and dog brains

Variable	DG	GT	SH
Mean weight (g)	72.79±1.03**	79.36±3.33	82.00±1.71
Mean volume(cm^3)	67.53±1.58**	75.54±3.30	76.00±1.79
Total gyri number	50.15±1.01**	48.80±0.60	63.63±0.58***
Total sulci number	48.34±1.01**	47.81±0.60	54.62±0.56***
Right cerebral length cm	5.32±0.50***	4.87±0.91***	4.65±0.69
Left cerebral length cm	5.37±0.05***	4.39±0.11***	4.34±0.56
Right cerebral width cm	1.80±0.15	2.47±0.35	2.08±0.05
Left cerebral width (cm)	1.74±0.14	2.40±0.36	2.04±0.30
Full cerebral width(cm)	3.71±0.11**	4.00±0.65	4.16±0.04
Cerebellar length (cm)	1.35±0.20	1.86±0.11*	1.59±0.06
Cerebellar width (cm)	2.44±0.20*	2.01±0.07	1.99±0.59

Data are expressed as mean ± standard error of the mean (Mean ± SEM) of values collected. Analysis of Variance (ANOVA) and LSD post hoc test were done to compare the groups and determine level of significance. p signifies level of significance, * represents p≤0.05, ** represents p ≤ 0.01, * represents p ≤ 0.001. *=level of significance between sheep and goat, *=level of significance between goat and dog, *= level of significance between dog and sheep.**

4.3 HISTOMORPHOLOGICAL AND HISTOMORPHOMETRIC STUDIES

4.3.1. Histomorphology of Layer V cells of the Prefrontal cortex of sheep, goat, and dog

The prefrontal cortex of the cerebrum had six histological layers and was compared in dog, goat, and sheep using Heamatoxylin and eosin, toludine blue, and golgi stained brain tissue sections. In haematoxylin and eosin stained photomicrographs, layer V/ ganglionlic/ internal pyramidal cells of dog neurons showed oval shaped cell bodies, with some having very tiny nuclei (Plate I). Goat neurons were more pyramidal in shape and larger in size (plate II) while sheep neurons had cells that were more oval in shape than dog with few rounded neurons (plate III). The nuclei of goat neurons, together with their cytoplasm, stained more darkly than those of sheep and dog. Numerous glia cells were found in the prefrontal cortex. These were mostly oligodendrocytes (blue arrow) as Identified by Alan and Jones in 2005. The oligodendrocytes adjacent to the neurons are called satellite cells (yellow arrow) (Alan and James, 2005).

The soma sizes and morphology were greatly highlighted in toludine blue stained photomicrographs. Layer V cells of dog showed most cells to be pyramidal in shape with all the neurons oriented in one plane. The dendrites of the neurons were also well arranged (plate IV). The same features were observed in sheep. However, the density of neurons was most in dog and least in sheep. Layer V neurons in goat were more ovoid in shape and larger in size than in sheep and dog. The neurons stained more deeply when compared with those of sheep and dog. There were more glia cells in goat layer V of the prefrontal cortex (plate V). Neurons and glia cells were fewer in the sheep and most in dog.

Golgi silver impregnations revealed the pyramidal cells in dog and goat (black arrow) to have thick apical dendrites with larger soma diameter compared to sheep. Some of the dendrites appeared spinous in goat (yellow arrow). Apical dendrites were also shorter in dog compared to sheep and goat (plate VII). Hence, goat had the most dendritic aborization of layer V cells of the prefrontal cortex than dog and sheep. Staining intensity of neurons was more in goat. Therefore, spines were not obvious on their dendrites (plate VIII). Sheep had smaller-sized pyramidal cells in this layer of the prefrontal cortex and numerous networks of processes (plate IX).

4.3.2 Histomorphometry of layer V cells of the prefrontal cortex of dog, goat, and sheep

In studying layer V cells of the prefrontal cortex specifically, goat had the largest soma size with a diameter of 57.60±9.65µm (table 4.2, plates II, V, VIII) based on results from cell count of toludine blue stained tissue sections. Soma diameters of sheep and dog were 46.74±12.10µm and 54.72±12.60µm respectively. Golgi techniques revealed that goat had the largest dendritic aborization (153.79±35.27µm), followed by sheep (134.05±35.12µm), then dog (108.77±15.71µm) (plates VII -IX). Variations were also observed in the density of pyramidal cells in this layer when studied using toludine blue stained sections (plates 4.4-4.6). Dog had the greatest density of pyramidal cells in this layer, followed by goat, then sheep (Table 4.2)

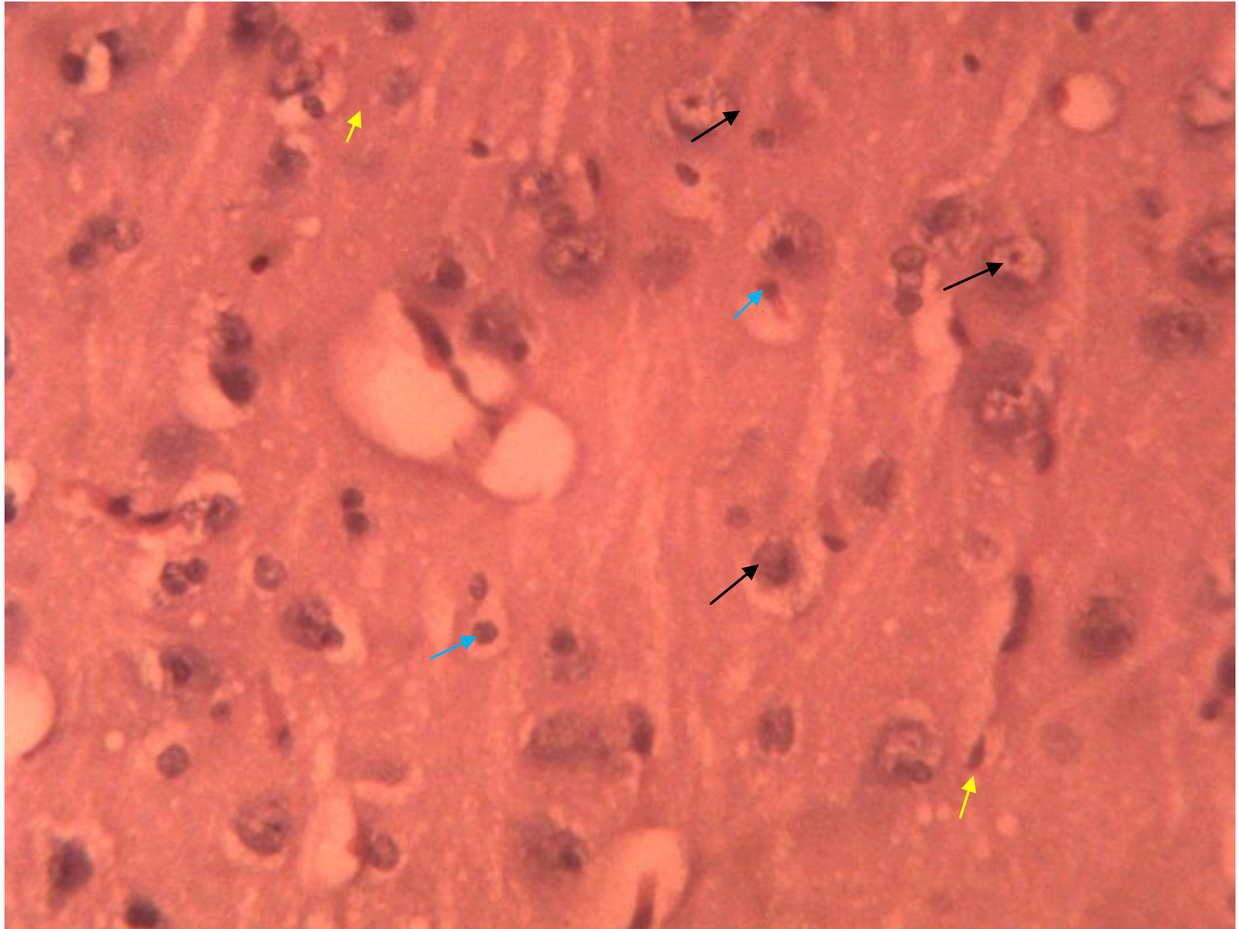


Plate I: Photomicrograph of coronal section through layer V cells of the prefrontal cortex indicating layer oval shaped cells in dog (H & E, X250). Neuron- black arrow, glia cell- blue arrow. Oligodendrocytes are the most prominent glia cells found in the cerebral cortex. The oligodendrocytes adjacent to neurons are Satellite cells (yellow arrow) (Alan and James, 2005). Layer V neurons of dog showed oval to round- shaped cell bodies with some having very tiny nuclei.

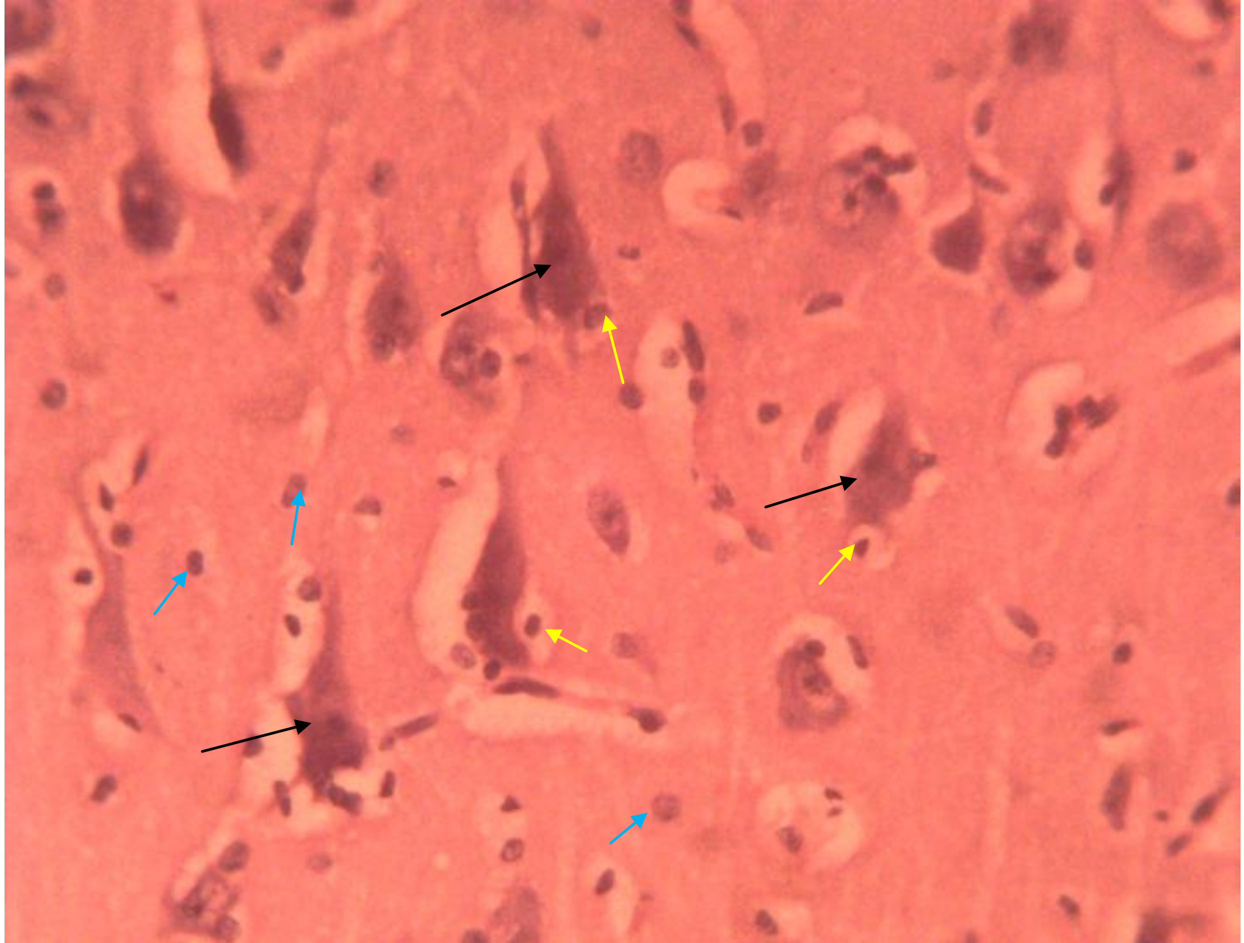


Plate II: Photomicrograph of coronal section through layer V cells of the prefrontal cortex indicating pyramidal cells in goat (H & E, X250). Neuron- black arrow, glia cell- blue arrow. Oligodendrocytes are the most prominent glia cells found in the cerebral cortex. The oligodendrocytes adjacent to neurons are Satellite cells (yellow arrow) (Alan and James, 2005). Layer V neurons of goat showed pyramidal-shaped soma that were larger than those of dog and sheep.

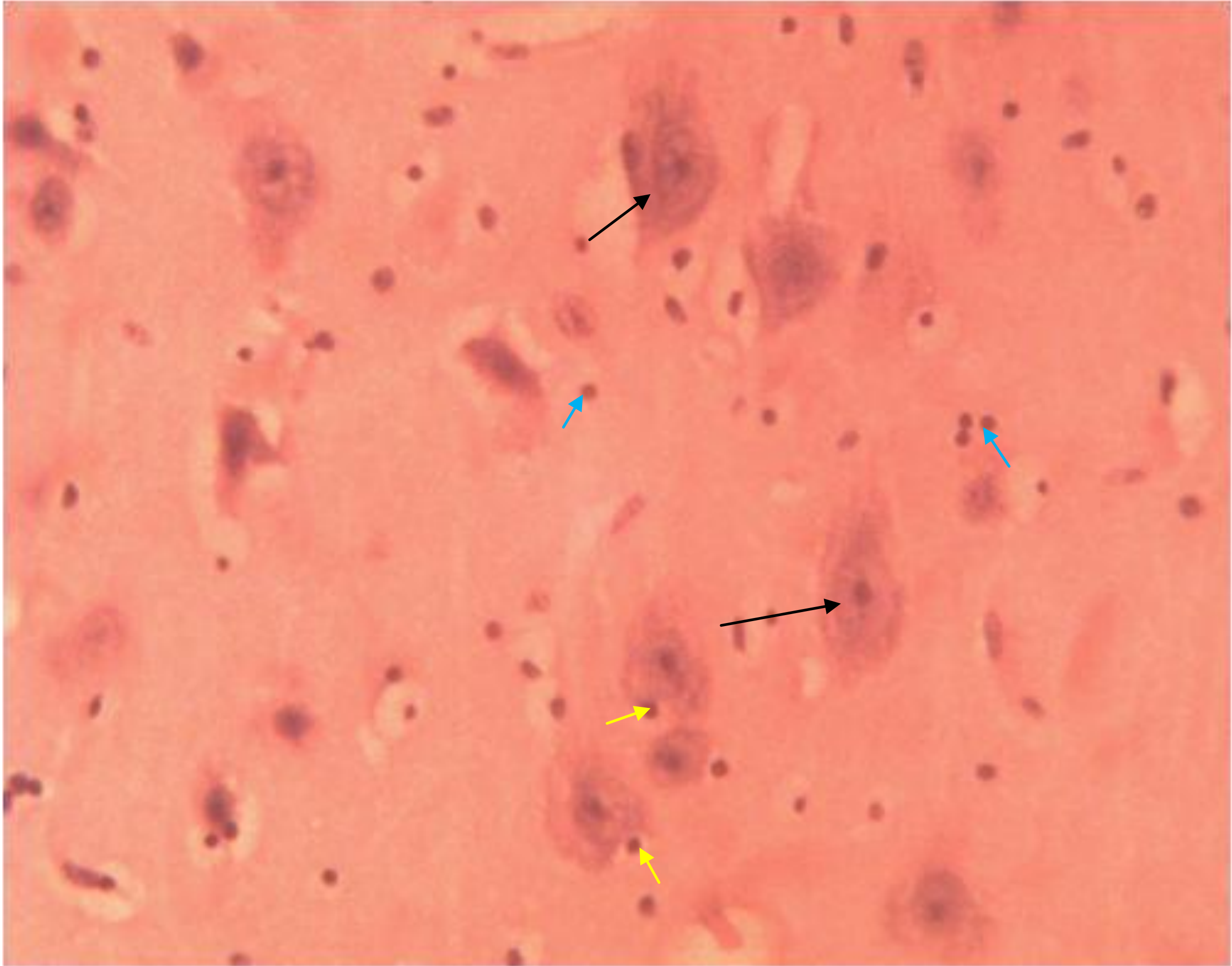


Plate III: Photomicrograph of coronal section through layer V cells of the prefrontal cortex of dog indicating pyramidal cells in sheep (H & E, X250). Neuron- black arrow, glia cell- blue arrow. Oligodendrocytes are the most prominent glia cells found in the cerebral cortex. The oligodendrocytes adjacent to neurons are Satellite cells (yellow arrow) (Alan and James, 2005). Layer V neurons of sheep showed cells that were more oval in shape with few rounded ones.

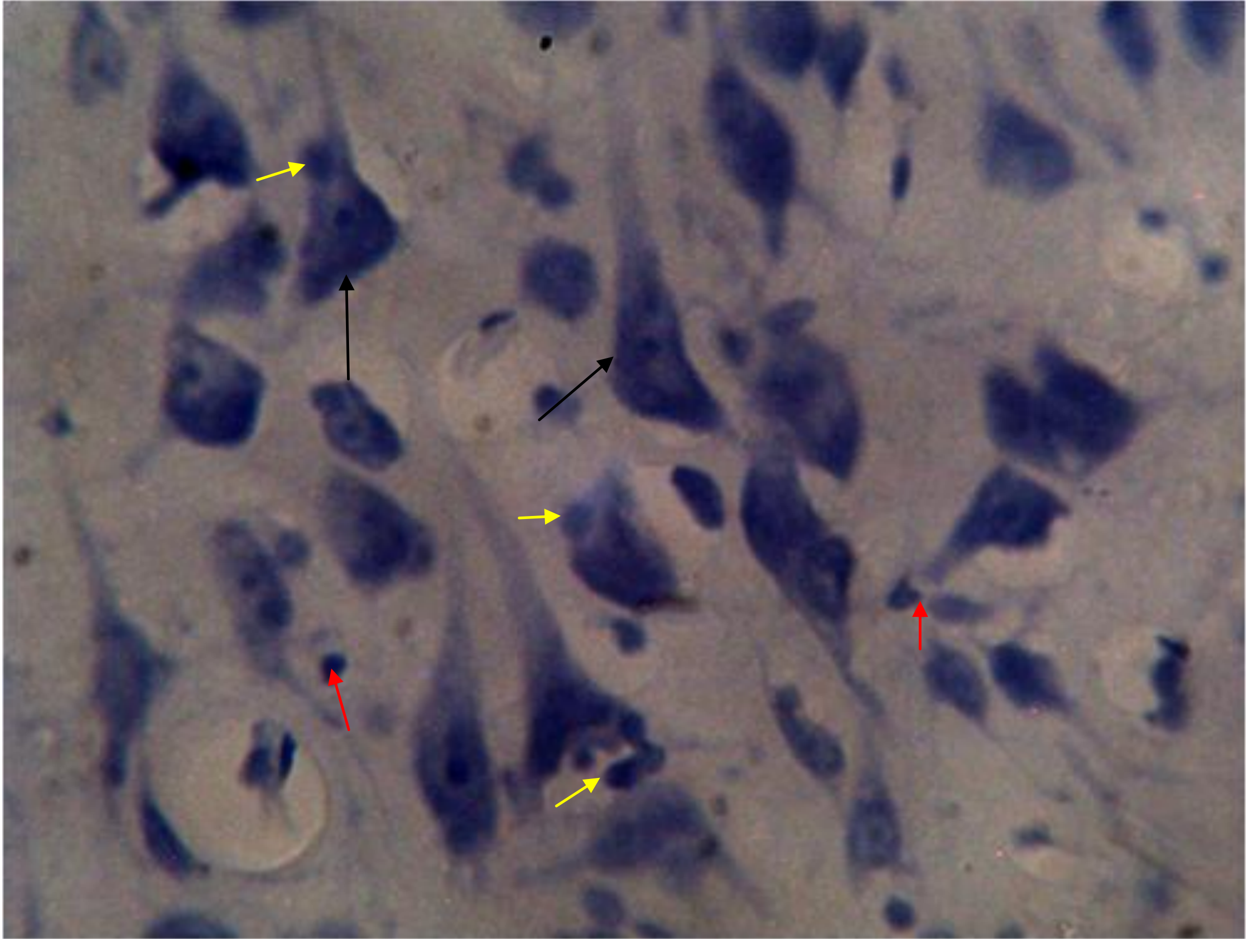


Plate 4: Photomicrograph of coronal section through layer V of prefrontal cortex in dog (Toludine blue x400). Oligodendrocytes (red arrow) are the most prominent glia fibres in the cerebral cortex. Those adjacent to neurons are called Satelite cells (yellow arrow) (Alan and James, 2005). Neurons are more pyramidal in shape. Also, fewer glia cells are present when compared with that of the goat.

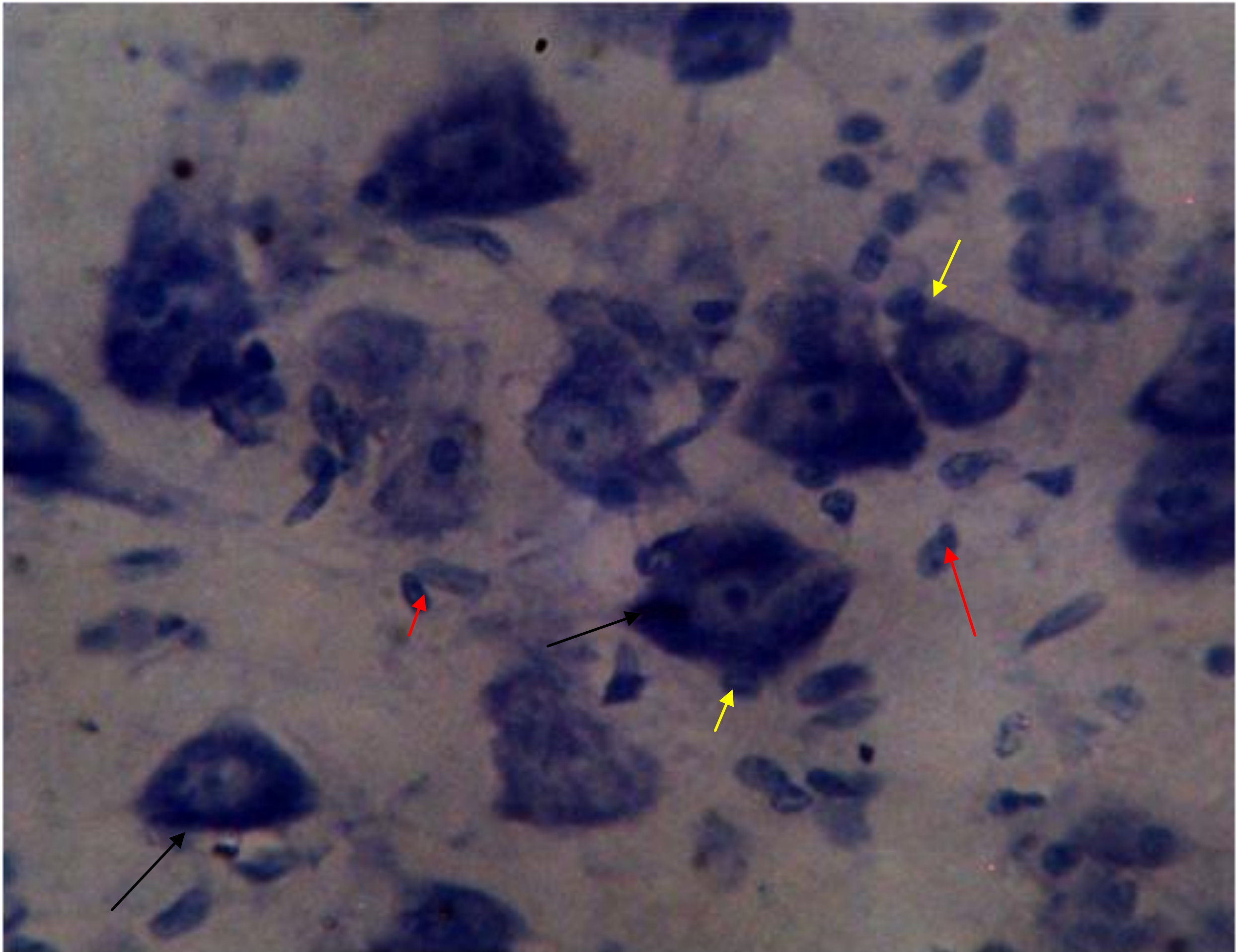


Plate V: Photomicrograph of coronal section through layer V of prefrontal cortex in goat (Toludine blue X400). Oligodendrocytes (red arrow) are the most prominent glia fibres in the cerebral cortex. Those adjacent to neurons are called Satelite cells (yellow arrow) (Alan and James, 2005). Neurons are more ovoid in shape. Also, more glia cells are present in goat when compared with that of sheep and dog.

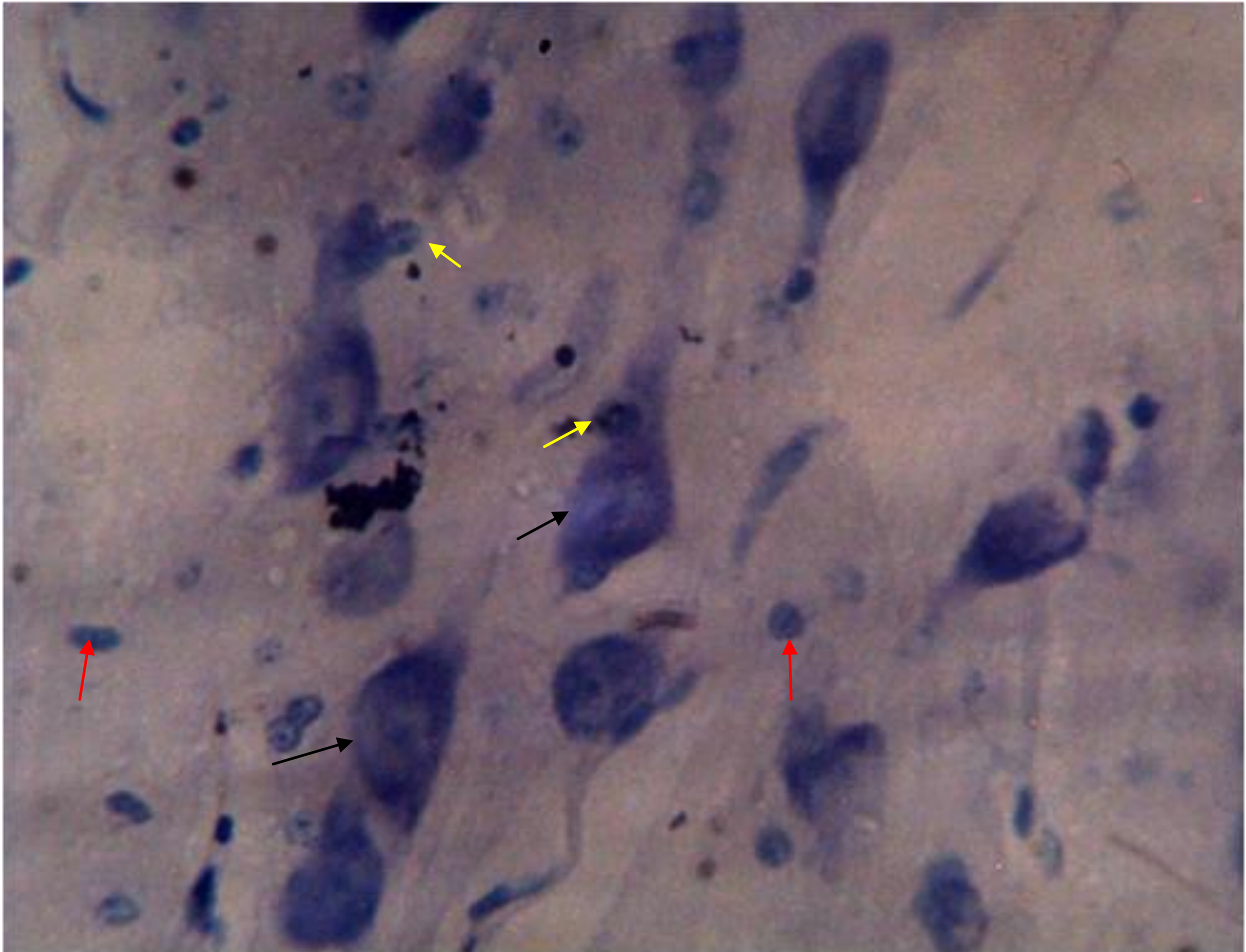


Plate VI: Photomicrograph of layer V of prefrontal cortex indicating pyramidal and oval shaped neurons in sheep (Toludine blue x400). Oligodendrocytes (red arrow) are the most prominent glia fibres in the cerebral cortex. Those adjacent to neurons are called Satelite cells (yellow arrow) (Alan and James, 2005). Neurons are fewer in the sheep, also there are fewer glia cells.

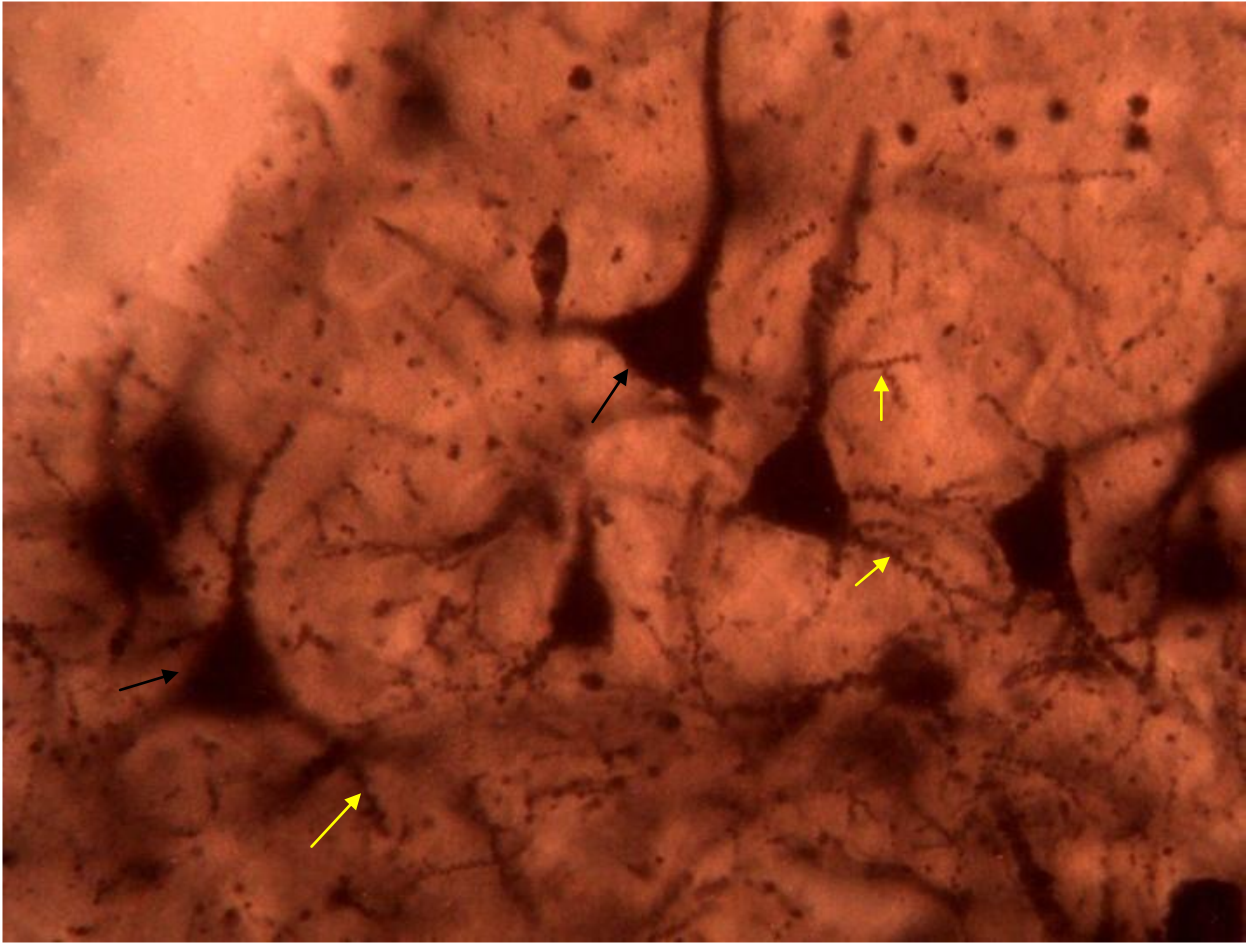


Plate VII: Photomicrograph of coronal section through layer V cells of prefrontal cortex indicating pyramidal cells in dog (Golgi silver Stain, X250). The pyramidal cells (black arrow) have thicker apical dendrites with numerous dendritic spines when compare with those of sheep (yellow arrow).

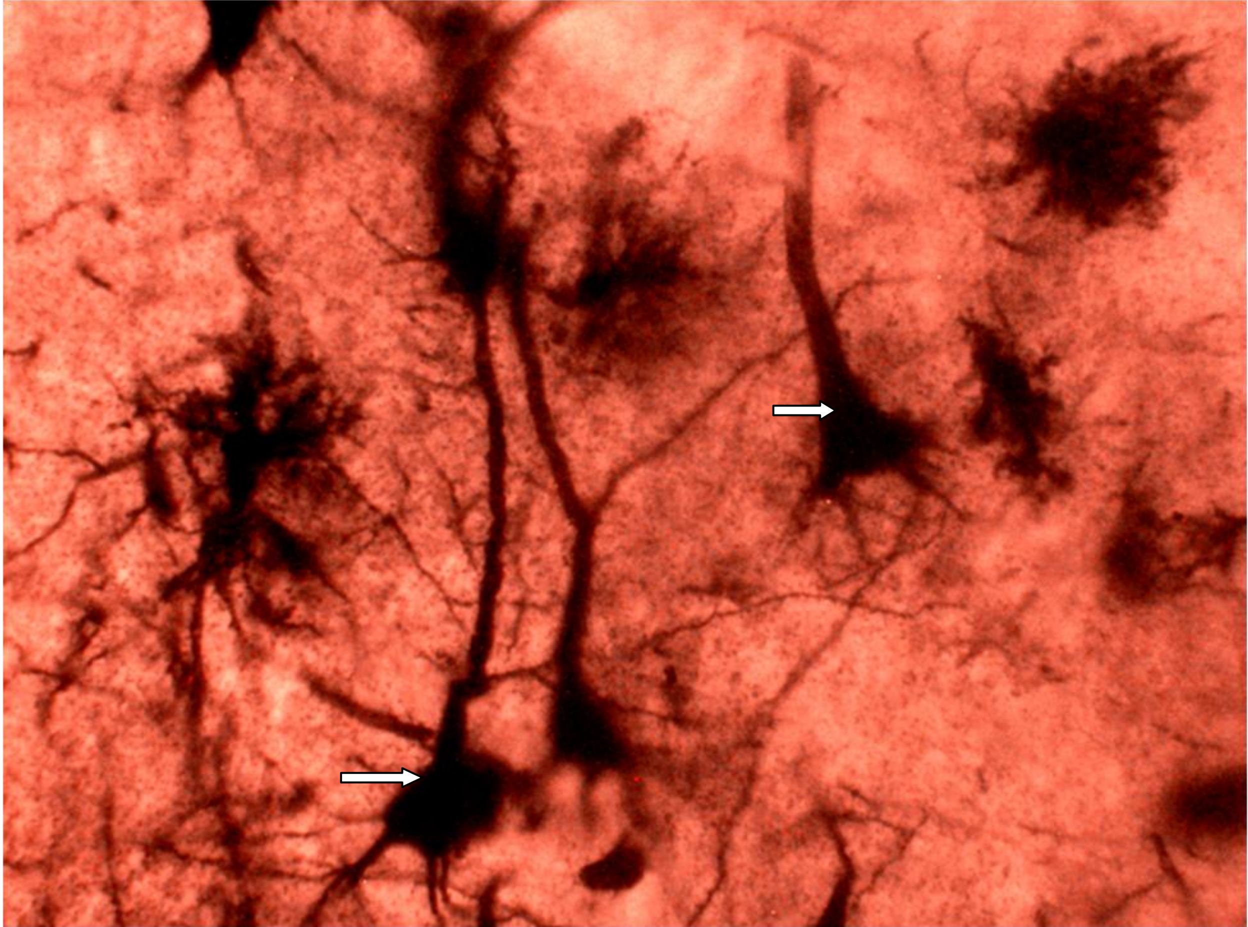
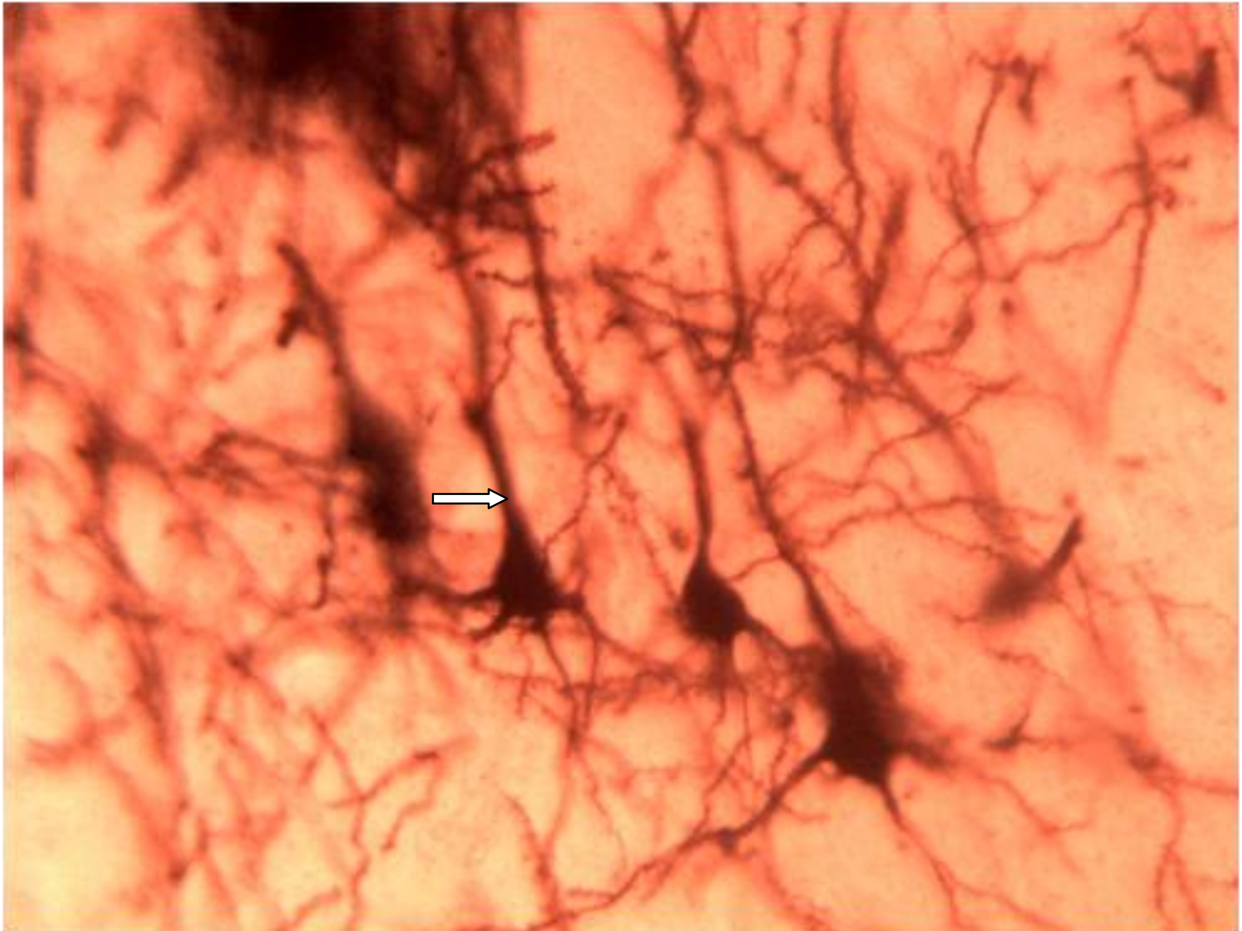


Plate VIII: Photomicrograph of coronal section through layer V cells of prefrontal cortex indicating pyramidal cells in goat (Golgi silver Stain, X250). The pyramidal cells (white arrow) have longer dendritic processes of apical dendrite when compare with those of dog and sheep. The somata are larger in appearance. Dendrites are aspiny and branch to secondary dendrites. Neurons stain deeply.



Plates IX: Photomicrograph of the histomophology of the prefrontal cortex sheep (Golgi method, X250mg) showing the ganglionic cell layer (Layer V) which is characterized by few pyramidal cells indicated by white arrows. The pyramidal cells are smaller than those of goat and dog. It has several primary dendrites which branch into secondary and tertiary dendrites.

Table 4.2 Histomorphometry of the layer V cells of the prefrontal cortex of dog, goat, and sheep

Stain	Variable	DG	GT	SH
Toludine Blue	Area (µm²)	2080.50±423.32*	2562.89±532.38*	2023.731±462.27*
	Perimeter(µm)	142.28±24.69*	137.91±29.26*	116.3707±26.42*
	Diameter (µm)	54.72±12.60*	57.60±9.65*	46.73963±12.10*
	Cell count	54*	39*	37*
Golgi	Area(µm²)	4048.513±558.36*	5243.838±1626.20*	5269.331±1606.19*
	Perimeter(µm)	296.7884±42.46*	390.7248±86.16*	482.2861±102.39*
	Diameter(µm)	108.7701±15.71*	153.7881±35.27*	134.0528±35.12*
	Cells measured	6	6	5

Data are expressed as mean ±standard deviation (Mean±SD). DG- dog, GT-goat, SH-sheep. The first row shows the mean soma diameter of layer V of the prefrontal cortex of dog, goat, and sheep. Goat had the largest soma diameter while dog had the most density of neurons. The second row shows the dendritic aborization of the pyramidal cells in layer V of the prefrontal cortex. Goat had the most dendritic aborisation. This was followed by sheep. Values in first row were obtained from analysis of cells in toludine blue stained photomicrographs while values in second row were obtained by analysis of photomicrographs prepared using golgi silver techniques. *= p≤0.05

4.3.3. Histomorphology of CA3 cells of the hippocampus of sheep, goat, and dog

In heamatoxylin and eosin stained sections, several features were observed in dog CA3 cells (plate X). Neurons were more ovoid in shape than pyramidal (yellow arrow) and they were clustered into several layers in this region. Nuclei could be observed as darkly staining tiny spots. Glia cells were also interspersed between the neurons (black arrow). Goat CA3 cells had a higher staining intensity than sheep and dog. In goat CA3 cells (plate XI), neurons were more fusiform in shape and pyramidal. The cluster of cells in this region was not as much as in dog. Neurons stained deeply that most nuclei of neurons were not seen. Glia cells were also interspersed between the neurons. In sheep CA3 cells, Neurons were more ovoid in shape and their cluster was fewer. Nuclei were seen in some of the cells as darker staining tiny spots. Glia cells were interspersed between the neurons. Neurons were larger in sheep than in dog (plate XII).

Toludine blue stained sections revealed more details about the cell bodies of the CA3 cells. Dog's CA3 cells stained deeply also but not as intense as goat's. The nuclei of the neurons were seen as tiny blue dots. There were more ovoid-shaped cells and very few pyramidal cells. The neurons formed clusters of several layers of cells as the cells were more in dog. There were more glia cells in dog when compared with goat (plate XIV). In goat, staining intensity of cells was high such that nuclei of neurons were not seen. Few glia cells were interspersed among the neurons. The neurons in goat were more than those of sheep but less than those of dog (plate XIII). Neurons were very few in sheep when compared with goat and dog. There was high staining intensity of cells such that nuclei were not obvious. Glia cells were few in this region. The neurons, however, were larger than those of goat and dog (plate XV).

4.3.4. Histomorphometry of CA3 cells of the hippocampus of dog, goat, and sheep.

In the present study, dog had the greatest density of CA3 cells in its hippocampus, followed by goat. This was revealed by neuronal cell count of toludine blue stained tissue sections (table 4.3). Sheep had the largest soma diameter (41.61±11.46μm), followed by dog (22.07±12.56μm), then goat (21.56893±8.59μm) whjle the dendritic aborizations of the sheep, goat, and dog were not obvious in the golgi tissue section prepared. However, both animals- goat and dog- had more cells in their CA3 cells than sheep.

4.3.5. Dentate gyrus

In some coronal sections of the dog hippocampus, the dentate gyrus was observed to form a diffused region of densely packed cluster of granular cells which tapered gradually towards the upper and the lower limb of the dentate gyrus (plate XVIa). The diffused granular cells were seen at the region of the loop of the dentate gyrus. The upper and lower limbs of the ends of the dentate gyrus were of approximately equal length at this coronal section. This was not observed in the sheep (plate XVIb) and goat (plate XVIc) at any coronal section through the hippocampus.

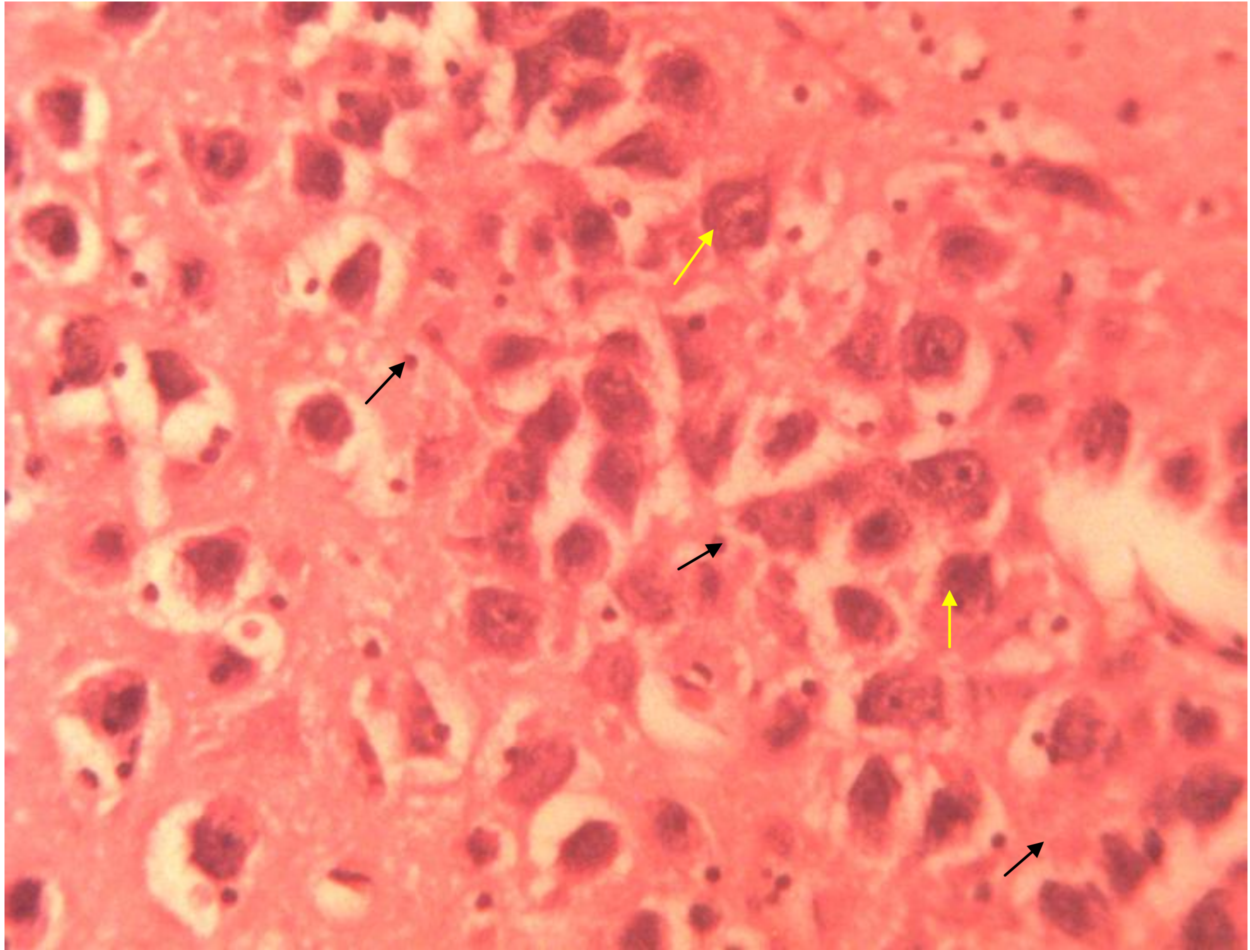


Plate X: Photomicrograph of CA3 cells of the hippocampus of dog (Haematoxylin and eosin stain, X250). Neurons are more ovoid in shape than pyramidal (yellow arrow) and they are clustered in this region. Nuclei could be observed as darkly staining tiny spots. Glia cells are also interspersed between the neurons (black arrow).

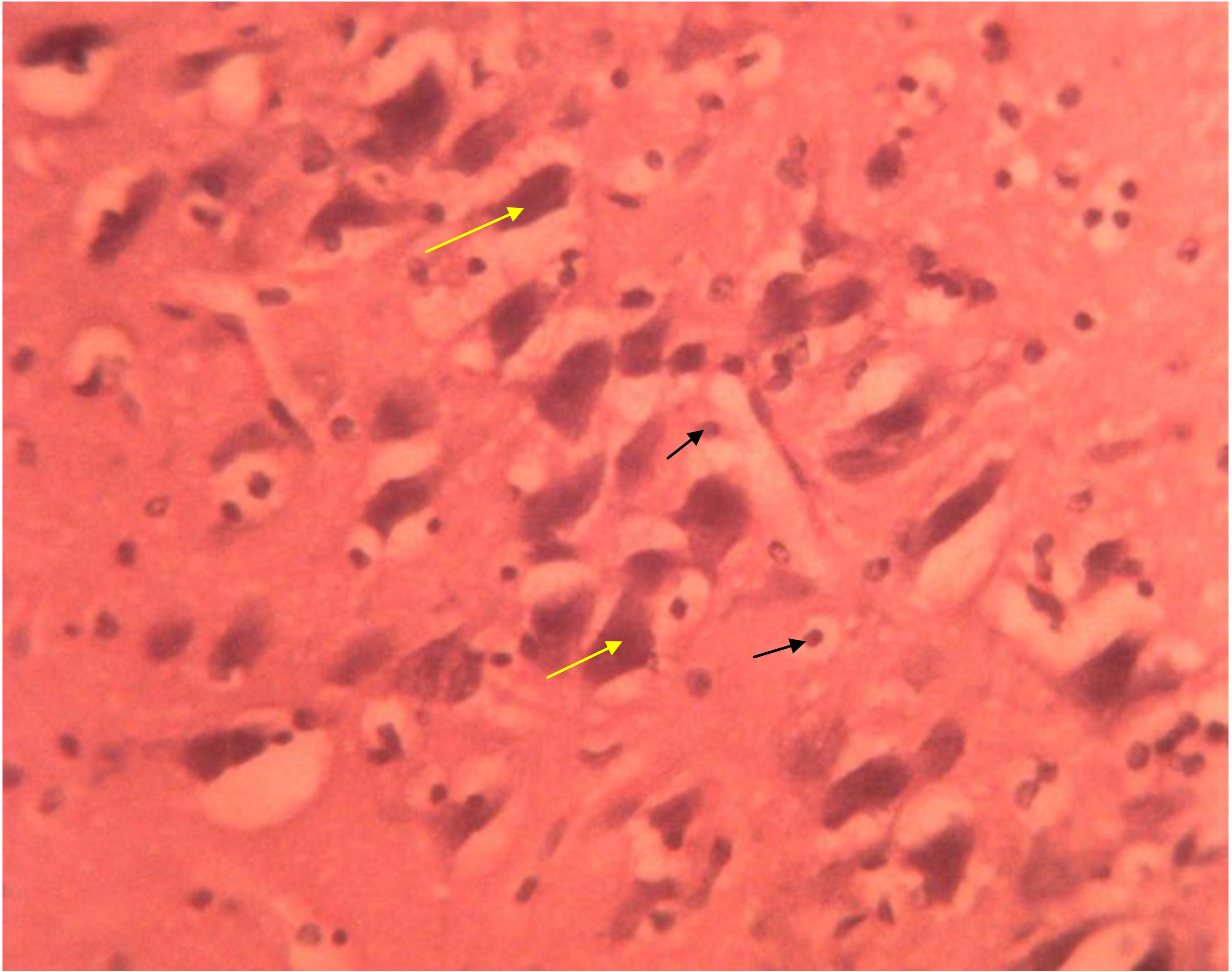


Plate XI: Photomicrograph of CA3 cells of the hippocampus of goat (Haematoxylin and eosin stain, x250mg). Neurons are more fusiform in shape and pyramidal (yellow arrow). The cluster of cells in this region is not as much as in dog. Neurons stained deeply that most nuclei are not seen. Glia cells are also interspersed between the neurons (black arrow).

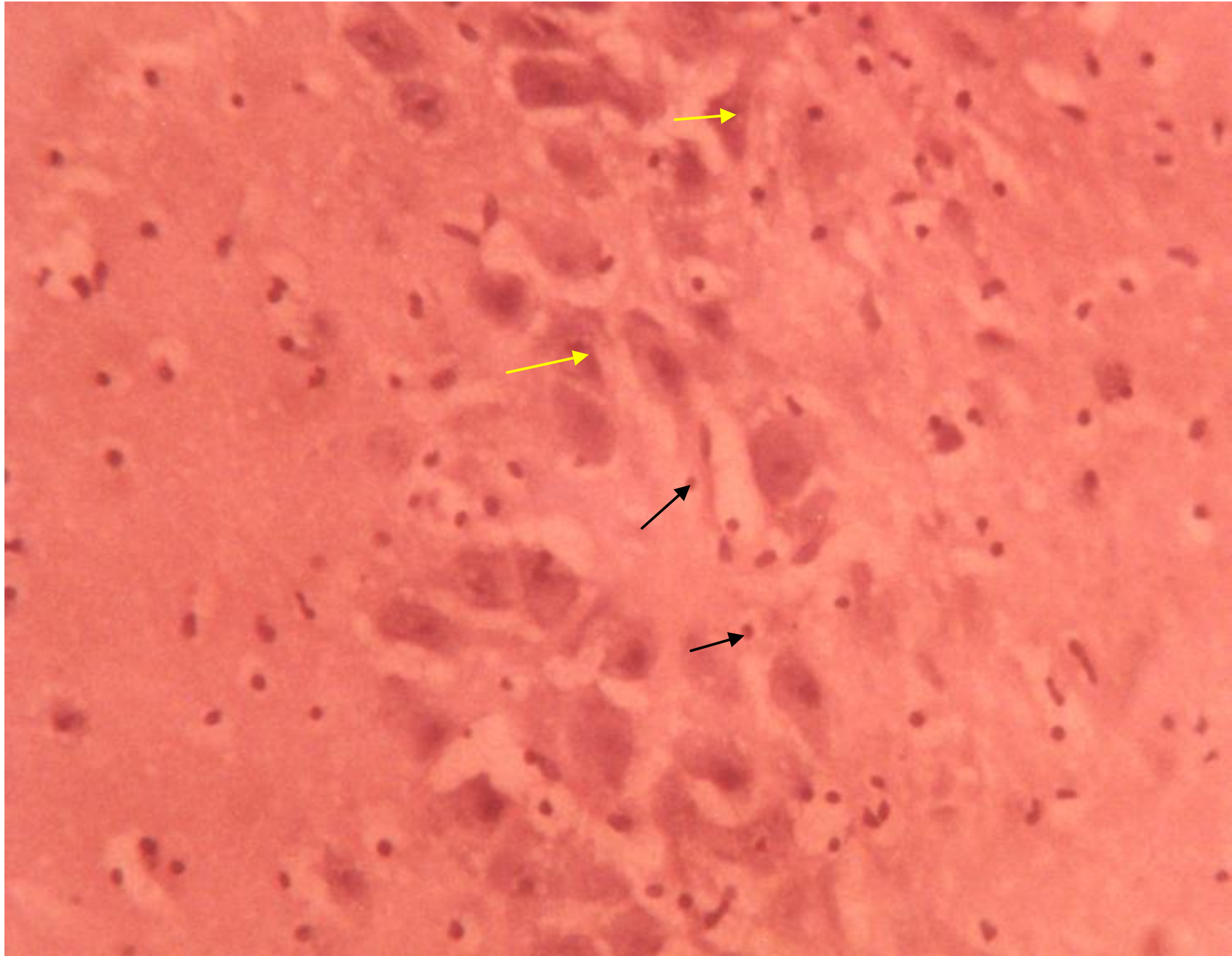


Plate XII: Photomicrograph of CA3 cells of the hippocampus of sheep (Haematoxylin and eosin stain, X250). Neurons are more ovoid in shape (yellow arrow) and their cluster is fewer. Nuclei ase seen in some of the cells as darker staining tiny spots. Glia cells are interspersed between the neurons (black arrow). Neurons are larger than dog's.

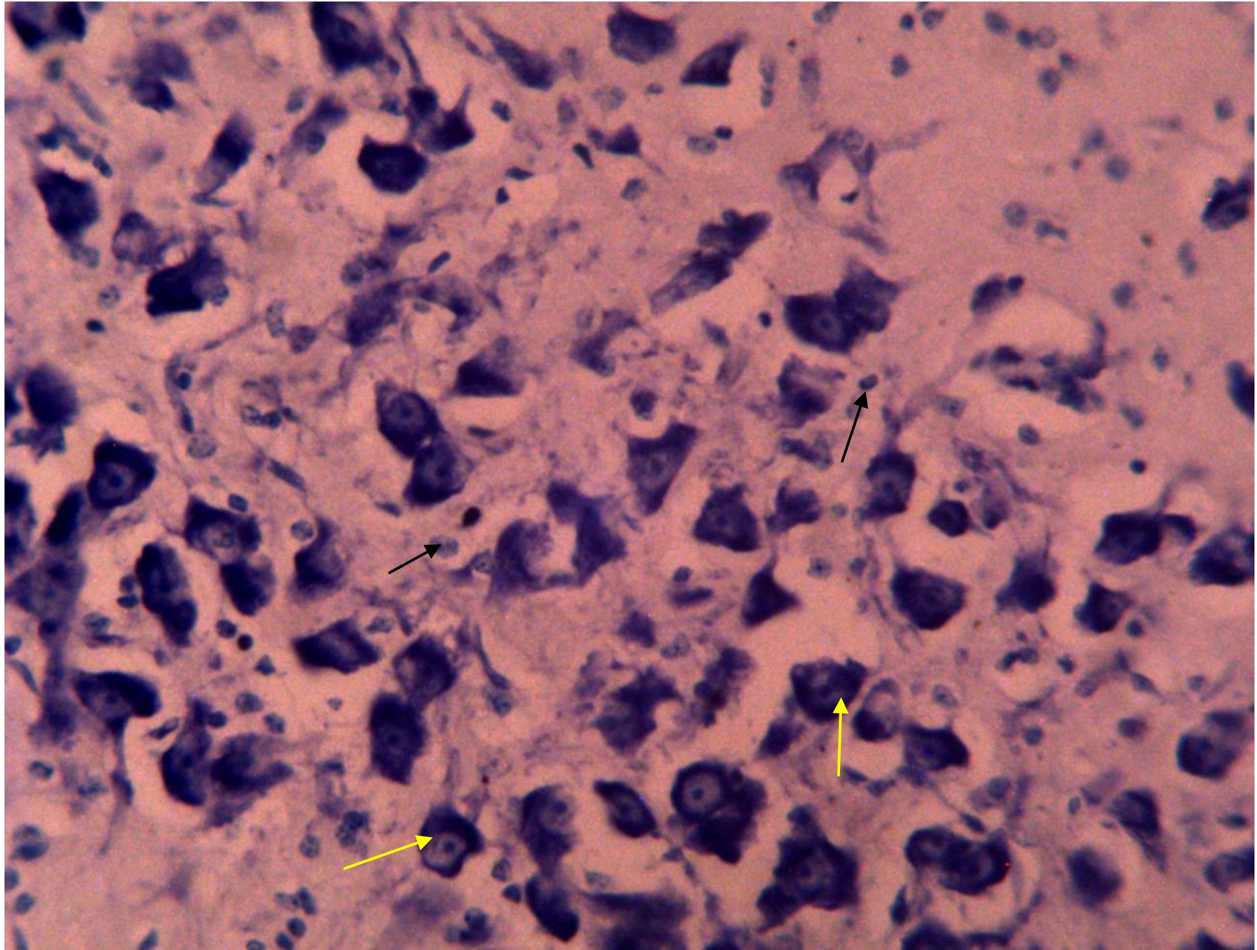


Plate XIII: Photomicrograph of CA3 cells of the hippocampus of Dog (Toludine blue, X250). Dog's CA3 cells stained deeply also but not as intense as goat's. The nuclei of the neurons were seen as tiny blue dots in the center of the neurons. There are more ovoid-shaped cells and very few pyramidal cells. The neurons (yellow arrow) form clusters of several layers of cells as the cells are more in dog. More Glia cells(black arrow) are scattered among the neurons in dog than in goat.

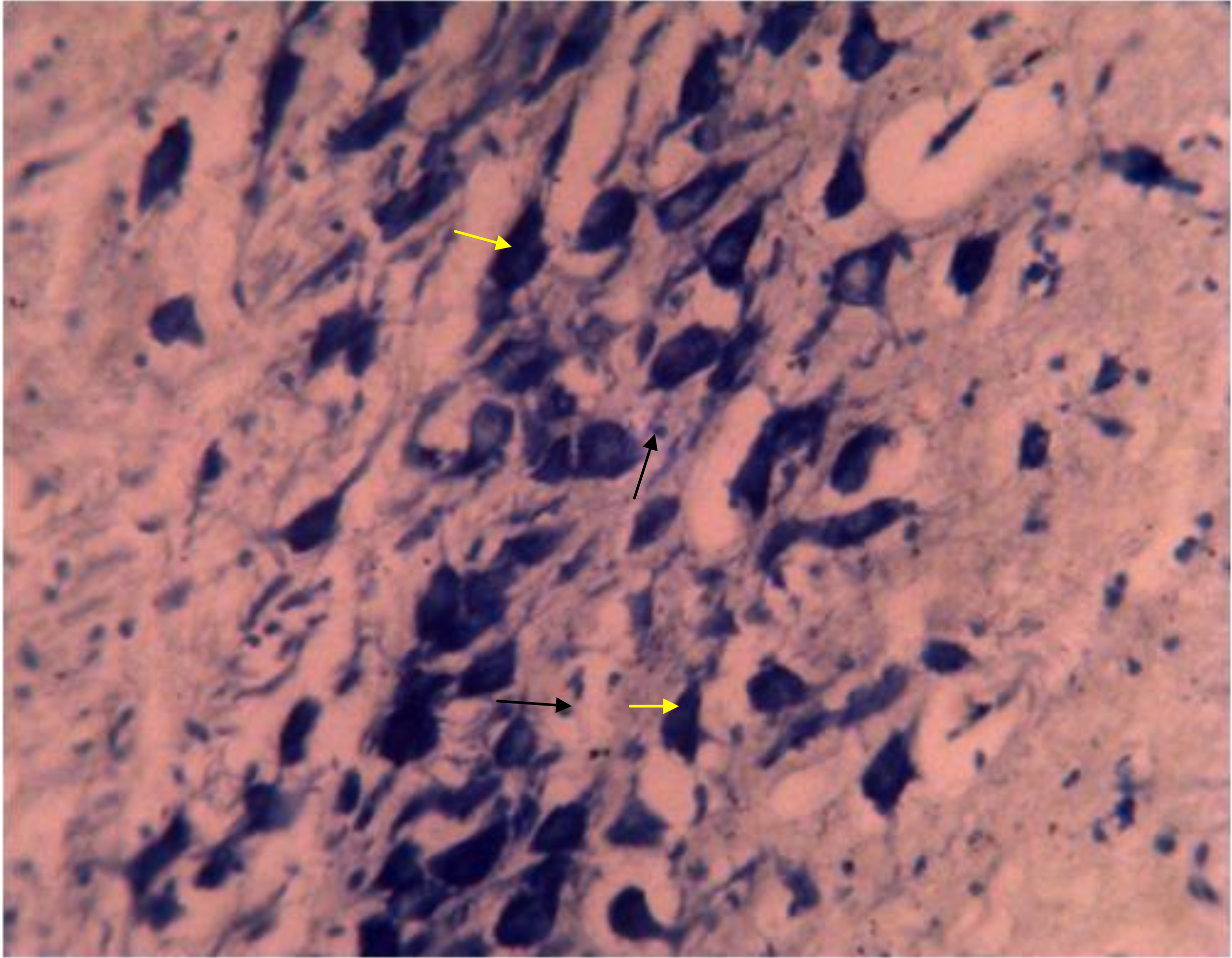


Plate XIV: Photomicrograph of CA3 cells of the hippocampus of goat (Toludine blue, x250mg). Neurons (yellow arrow) ranged from pyramidal to ovoid in shape. Staining intensity of cells high. Few glia cells are interspersed (black arrow). The neurons are more than those of sheep but less than those of dog.

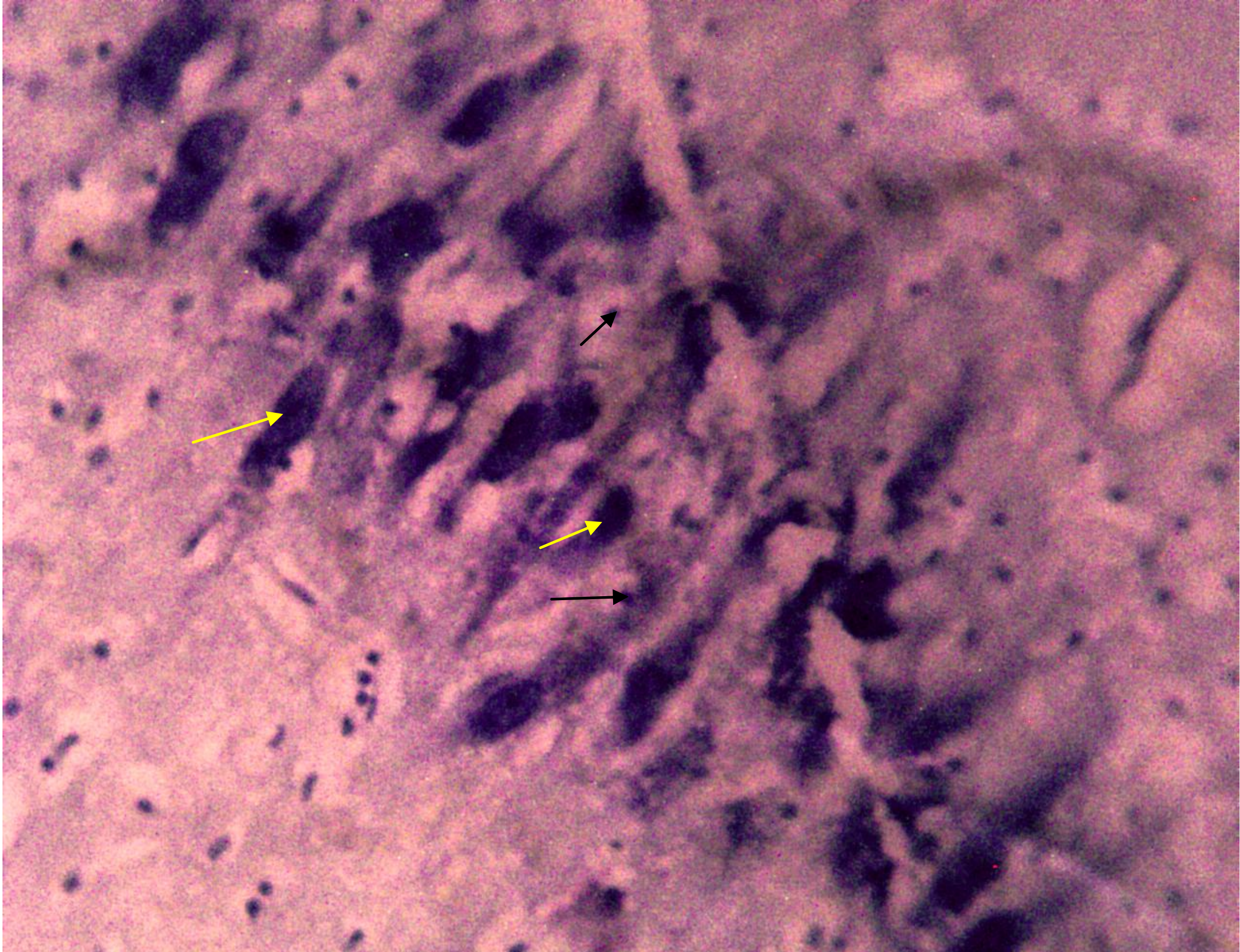


Plate XV: Photomicrograph of CA3 cells of the hippocampus of sheep, goat, and dog (Toludine blue, x250mg). Neurons are very few in sheep when compared with sheep and dog. There is high staining intensity of cells such that nuclei are not obvious. Glia cells are few. The neurons, however, are larger than those of goat and dog.

Table 4.3. Histomorphometry of CA3 cells of the hippocampus of dog, goat, and sheep.

Stain	Variable	DG	GT	SH
	Area(µm²)	886.8363±413.47*	719.72±1644.89*	1662.39±583.23*
Toludine	**Perimeter(µm)**	57.91378±22.68*	54.34±110.90*	98.19±25.13*
Blue	**Diameter(µm)**	22.07±12.56*	21.57±8.59*	41.61±11.46*
	cell count	185*	146*	62*

Data are expressed as mean ±standard deviation (Mean±SD). The first row shows the mean soma diameters of CA3 cells of dog (DG), goat (GT), and sheep(SH). Dog had the highest number of CA3 cells in its hippocampus. Sheep had the largest soma diameter. Values were obtained from analysis of CA3 cells of toludine blue stained photomicrographs. *= p≤0.05

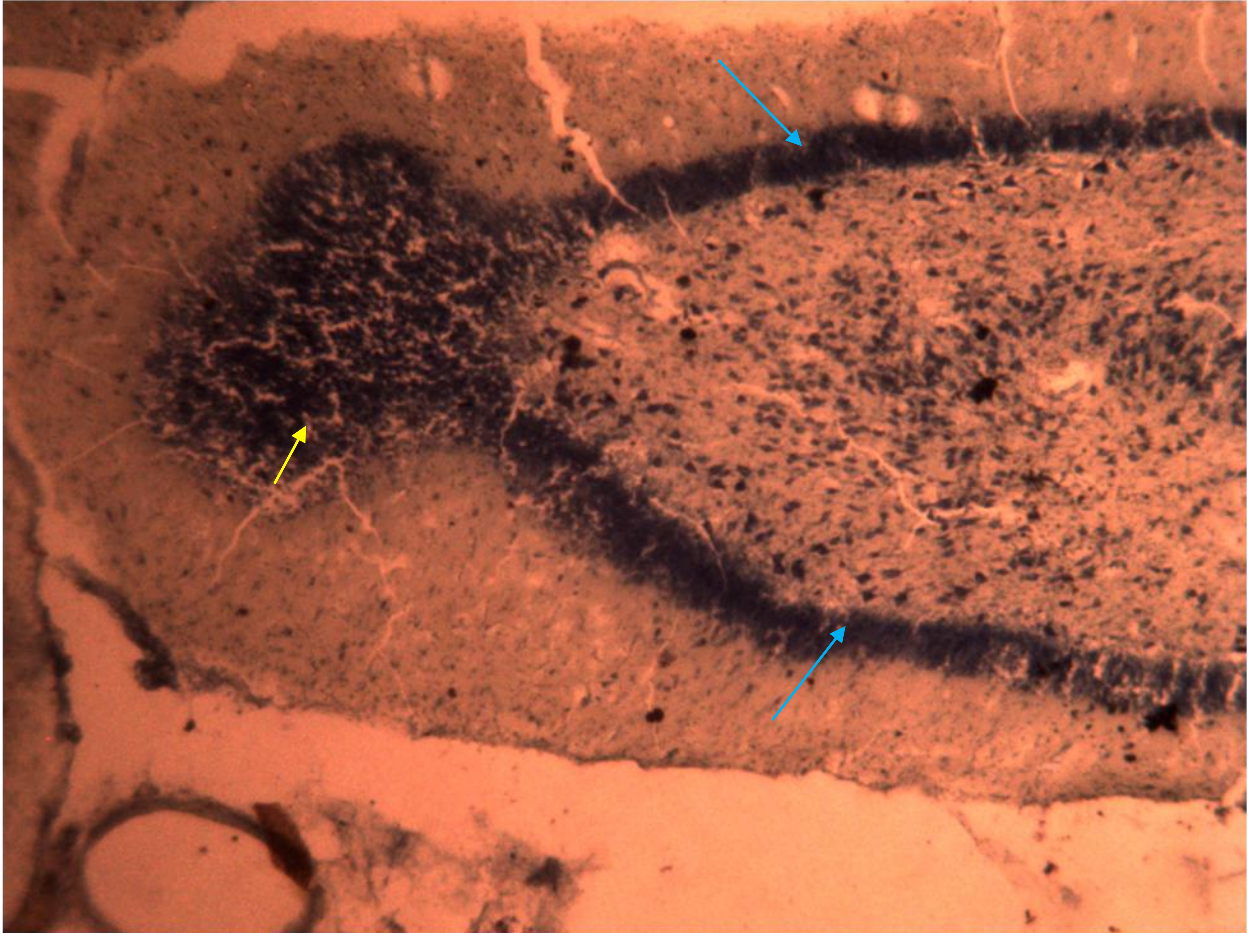


Plate XVIa. Dentate gyrus of dog (toludine blue, X40). Clusters of granular cells are seen in the region where the dentate gyrus forms a loop (yellow arrow). This is not seen in sheep or goat. Blue arrow indicates limb of dentate gyrus

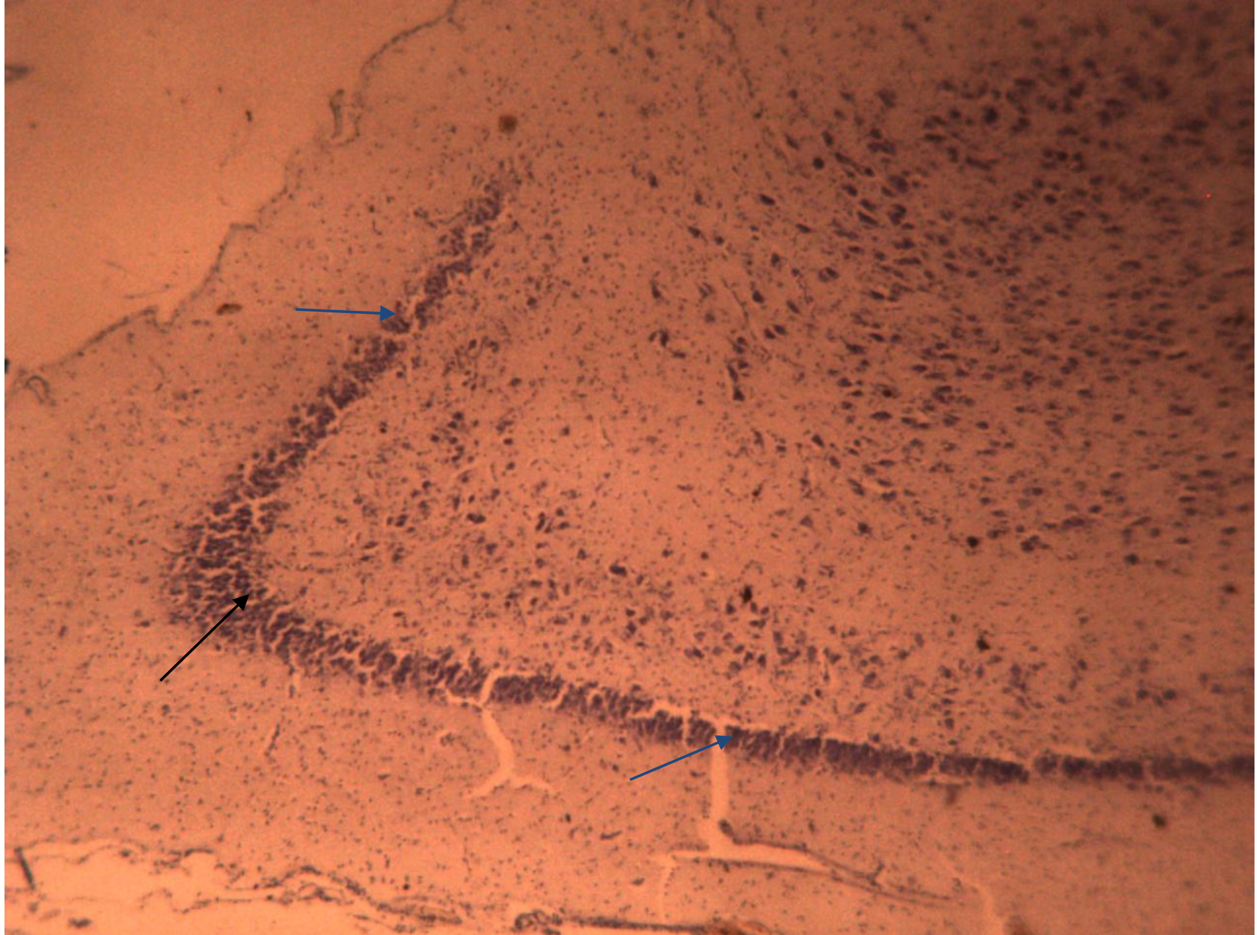


Plate XVIb. Dentate gyrus of goat (toludine blue, X40). Clusters of granular cells are not seen in the region where the dentate gyrus forms a loop (Black arrow). Blue arrow indicates limb of dentate gyrus

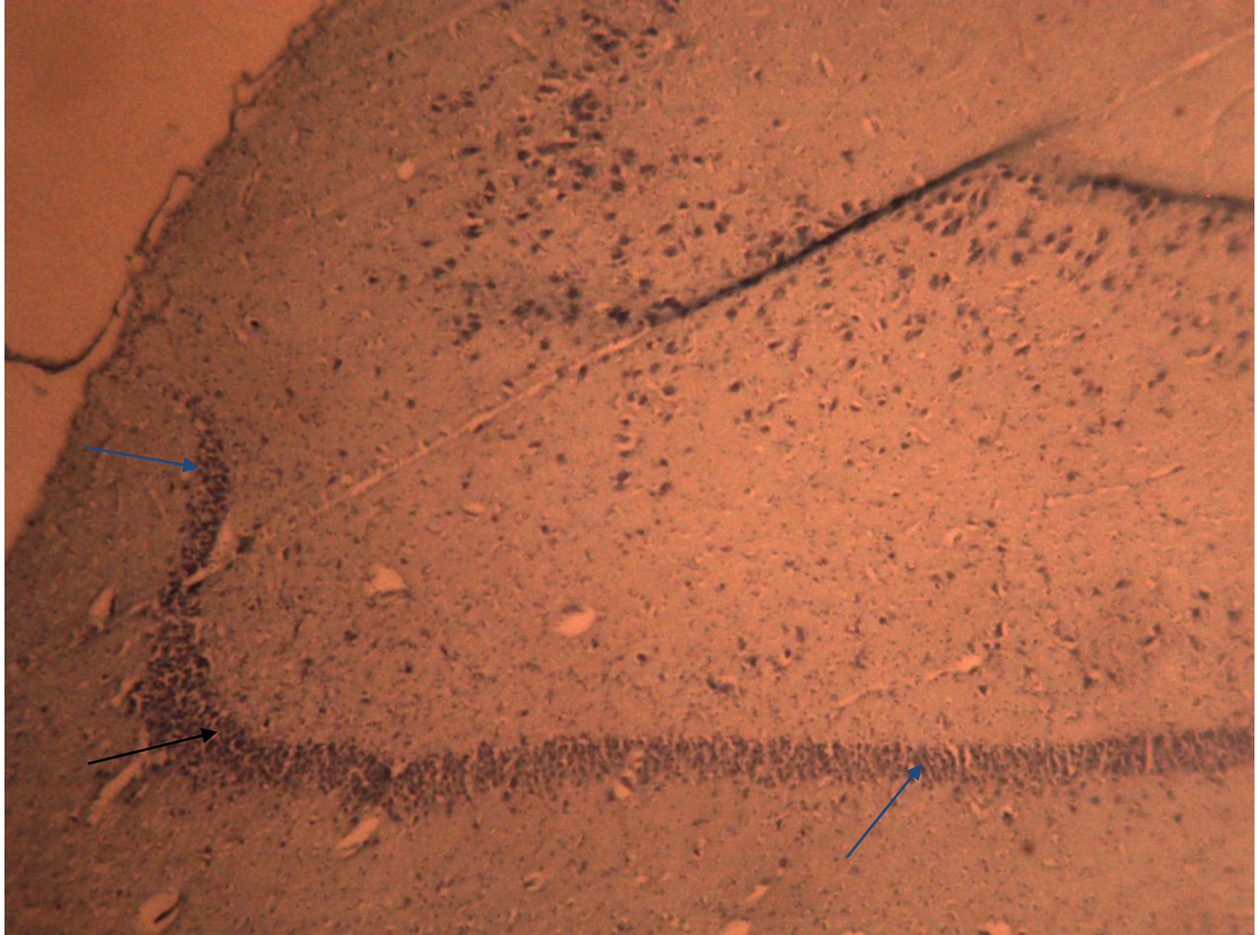


Plate XVIc. Dentate gyrus of sheep (toludine blue, X40). Clusters of granular cells are not seen in the region where the dentate gyrus forms a loop (Black arrow). Blue arrow indicates limb of dentate gyrus

4.3.6. Histomorphology of neurons in the basolateral complex of the amygdala of sheep, goat, and dog

In the basolateral complex, heamatoxylin and eosin tissue sections showed that neuronal density was more in dog than in goat and sheep. Neurons were more ovoid in shape in the three animals (plate XVII- XIX).

Toludine blue sections revealed more details about the morphology of somata in this region of the amygdala. Neurons in dog basolateral complex were identified by the presence of a nucleolus and they had somata which were pyramidal-like or ovoid in shape. Glia cells were much smaller and more in number and had darkly staining intensity. Several glia cells were in close association with the neurons (plate XX). In goat lateral nucleus, neurons were pyramidal-like or ovoid in shape. Neurons were identified by the presence of a nucleolus which was more apparent than in dog. Hence the staining intensity of the neurons in goat was not as much as in dog. Glia cells were also present (Plate XXI). In sheep, some neurons were pyramidal-like, while others were more ovoid in shape. Neurons here were as large as those in dog but larger than those in goat. Also, numerous glia cells were interspersed inbetween the neurons. They had a high staining intensity (plate XXII).

Golgi silver techniques revealed more about the shape as well as the dendritic aborization of neurons in this region of the amygdala. At first glance, a low magnification of dog lateral cells showed it to have larger dendritic trees. This is followed by sheep, then goat (plates XXIII-XXV). The same result, however, was observed at higher magnification (plates XXVI-XXVIII). Neurons of the basolateral complex of dog were the largest. The neurons were either pyramidal-like or stellate in shape. Stellate neurons had complex aborization and their staining

intensity was deep such that their cell bodies were almost completely obscured. Spines were not obvious on the dendrites of these neurons, possibly due to their great staining intensity (plate XXVI). Neurons of the basolateral complex of goat were not as large as those of dog. Numerous stellate neurons which had well ramified dendritic aborization were present as well as pyramidal like neurons. Their staining intensity was high but not as much as in dog. Stellate neurons in goat were spinous. The presence of spines in goat stellate-shaped basolateral complex neurons served as a point of synapes with other neurons within the amygdala, thereby increasing connectivity in goat (plate XXVII). This is a unique feature of goat basolateral complex. Neurons of the basolateral complex of sheep were also large but not as in goat. More stellate than pyramidal-like neurons were found in sheep which had well-ramified and complex dendritic aborization that was not ramified as in dog but more than in goat. Their staining intensity was deep such that the cell bodies were almost completely obscured as in dog. Dendrites appeared sparsely spiny (plate XXVIII).

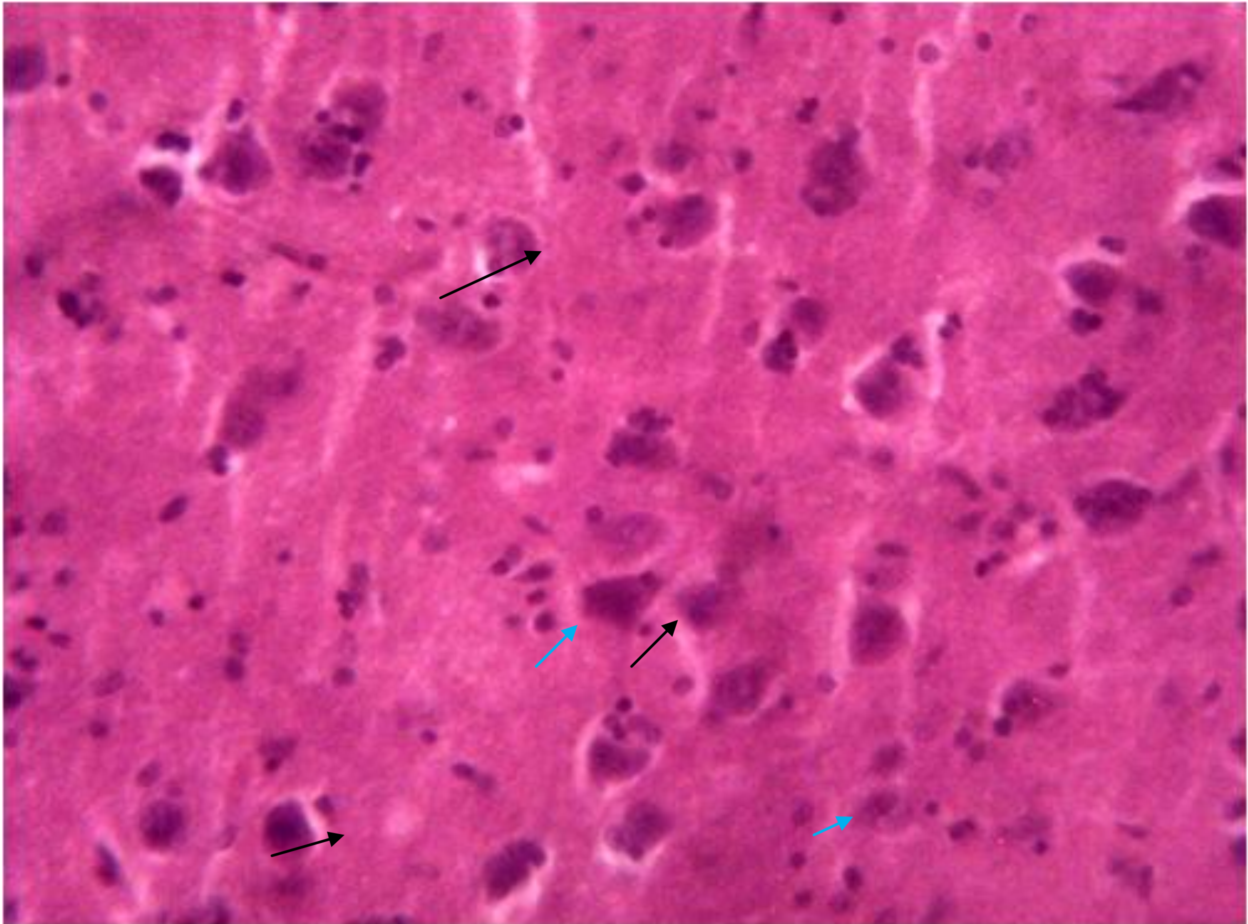


Plate XVII: Photomicrograph showing cells the basolateral complex of dog (Haematoxylin and eosin, X250). Neurons were identified by the presence of a nucleolus. Glia cells (blue arrow) are much smaller and have darkly stained punctuation. Neurons are more ovoid in shape (black arrow).

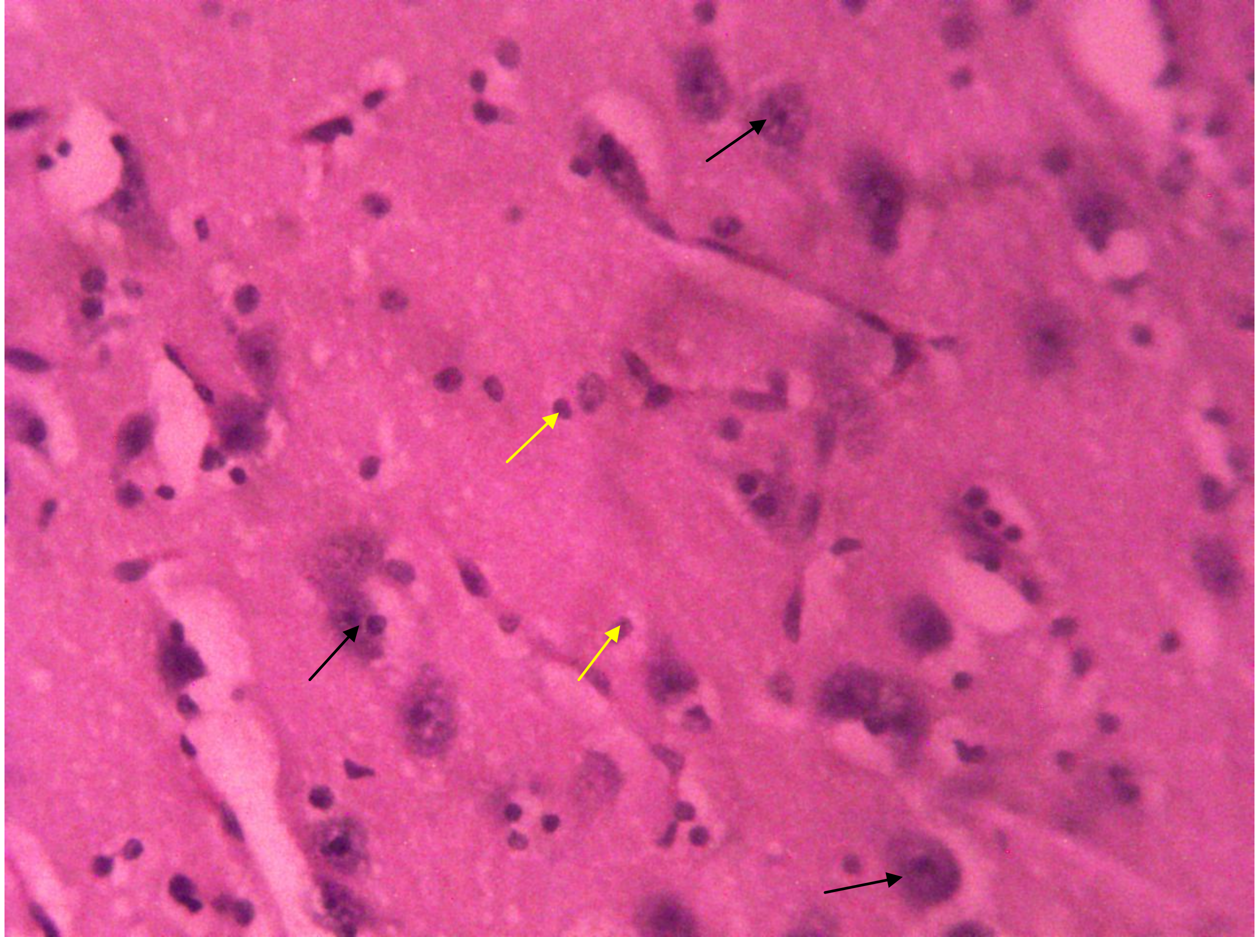


Plate XVIII: Photomicrograph showing cells the basolateral complex of goat (Haematoxylin and eosin, X250). Neurons (black arrows) were identified by the presence of a nucleolus and are ovoid in shape. They are fewer in goat. Glia cells (yellow arrow) are much smaller and have darkly stained punctuation.

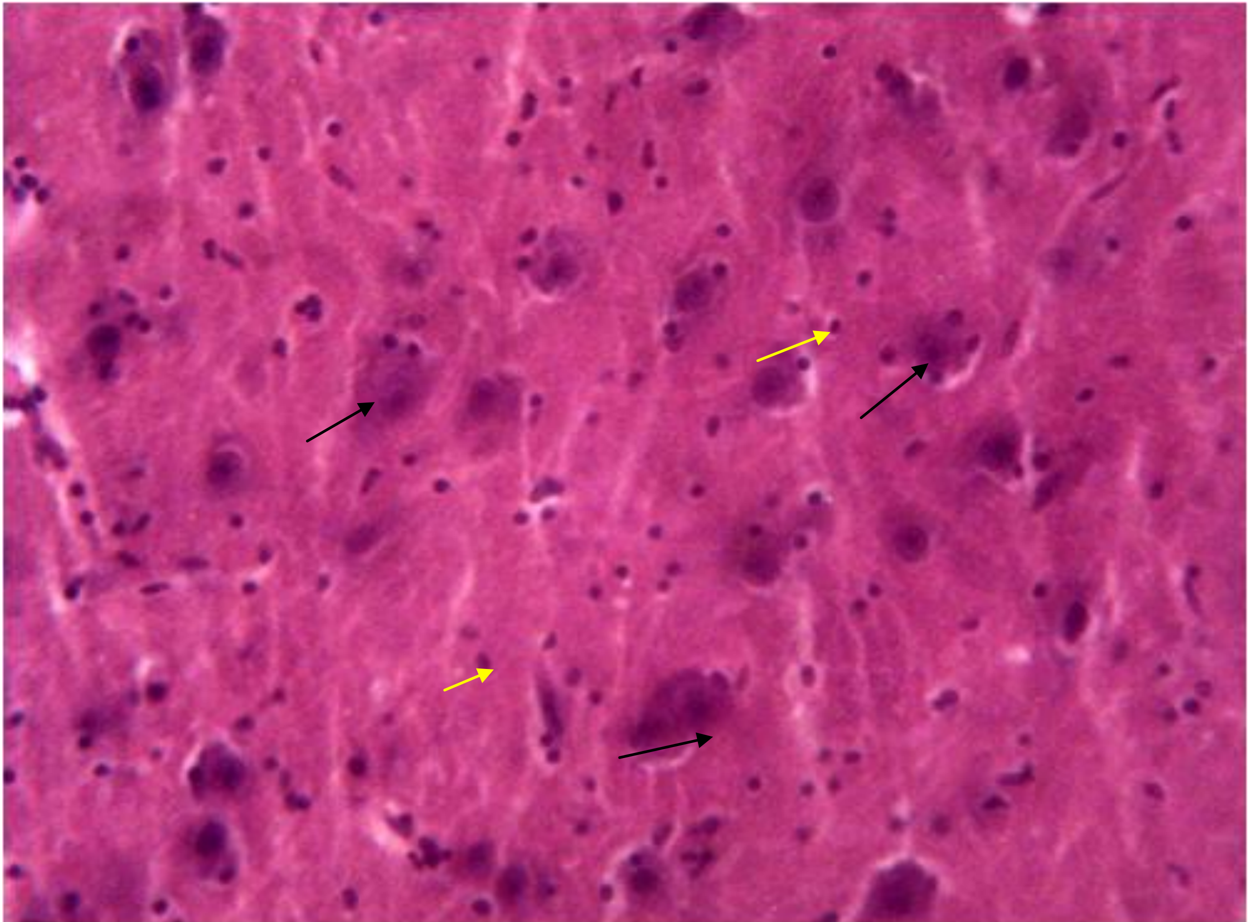


Plate XIX: Photomicrograph showing cells the basolateral complex of sheep (Haematoxylin and eosin, X250). Neurons (black arrows) were identified by the presence of a nucleolus. Glia cells (yellow arrow) are much smaller and more and have darkly stained punctuation. Neurons are more ovoid in shape.

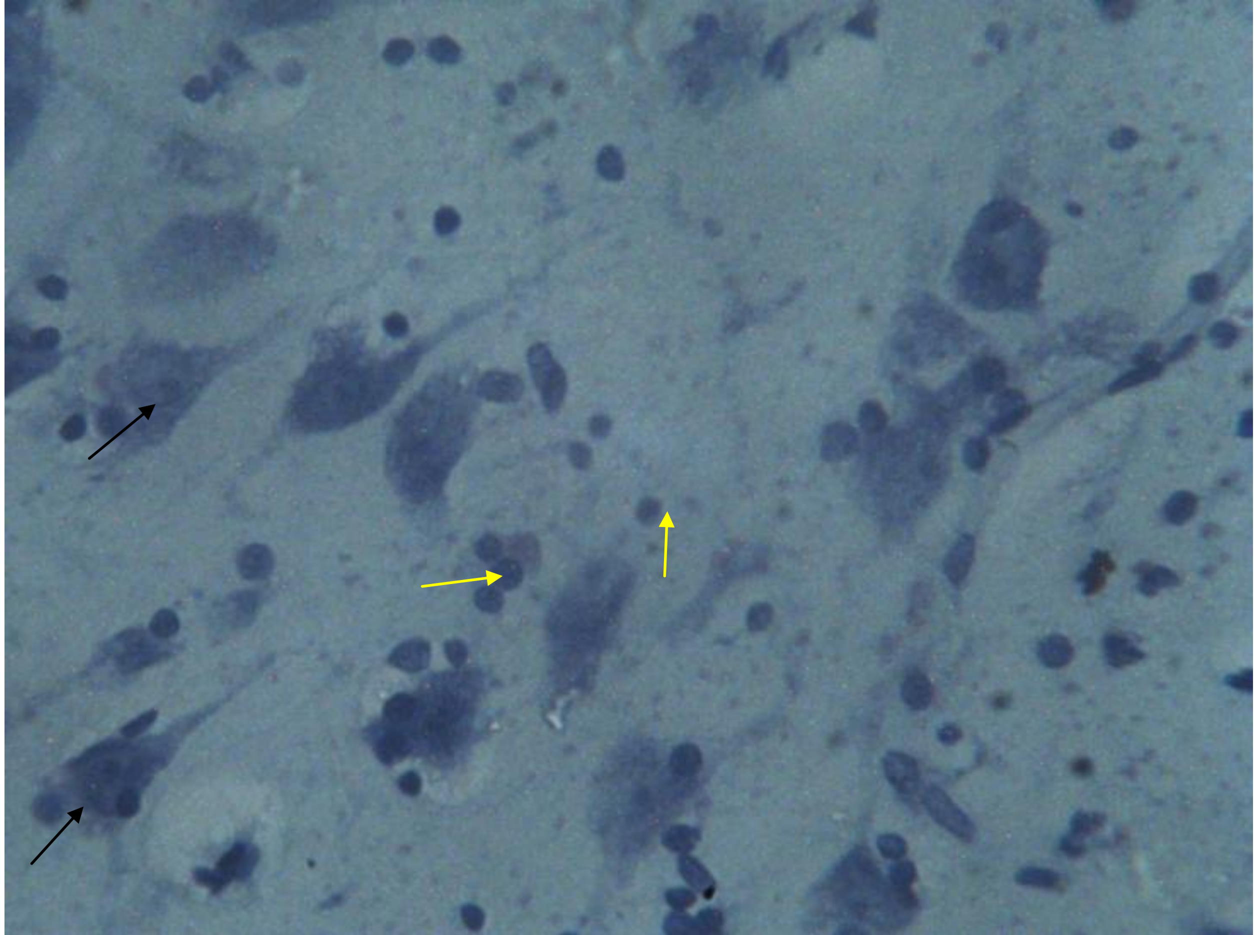


Plate XX: Photomicrograph showing cells in the basolateral complex of dog (Toludine blue, X400). Neurons (black arrows) were identified by the presence of a nucleolus and are pyramidal-like or ovoid in shape. Glia cells (yellow arrow) are much smaller and more and have darkly staining intensity.

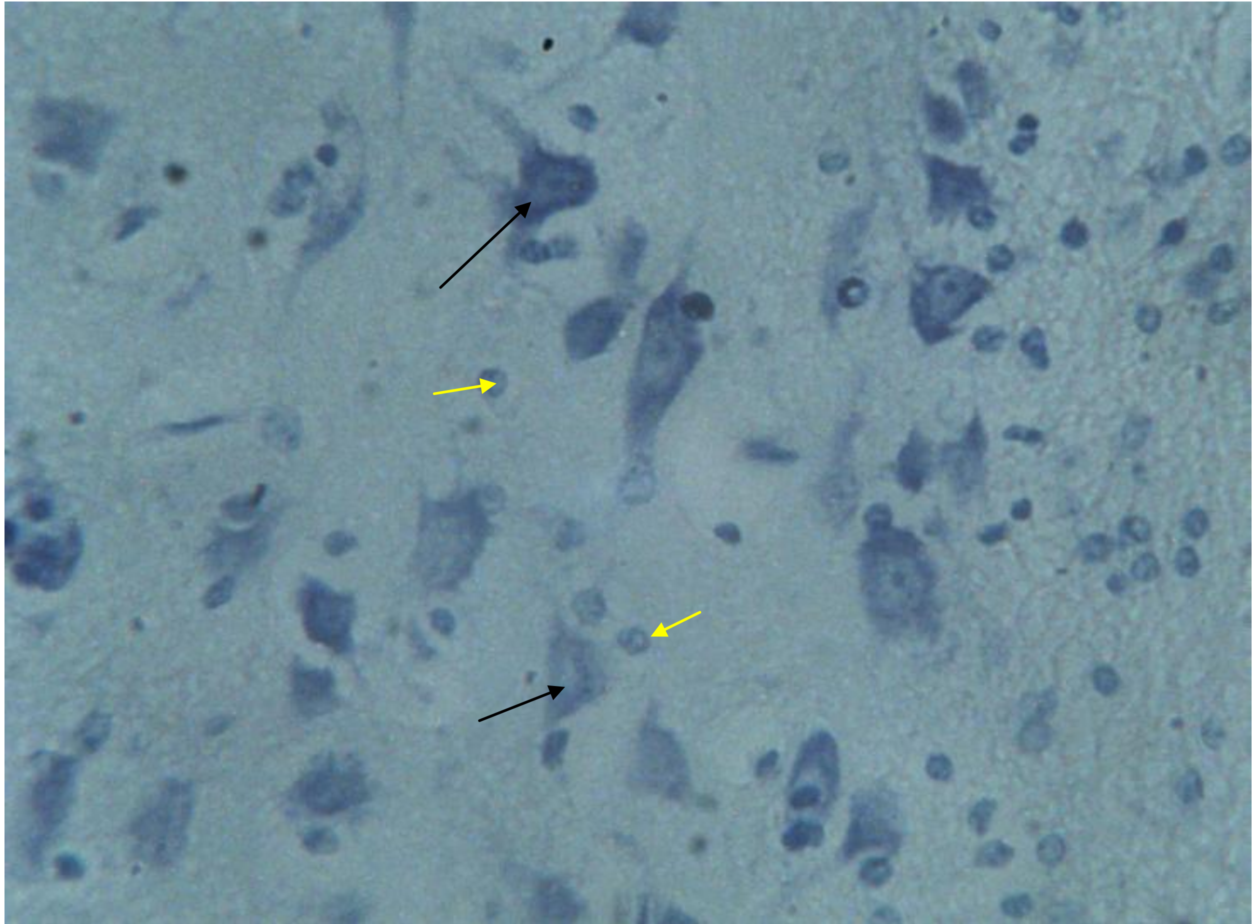


Plate XXI: Photomicrograph showing cells in the basolateral complex of goat (Toludine blue, X400). Neurons were pyramidal-like or ovoid in shape. Neurons (black arrows) were identified by the presence of a nucleolus which is more apparent than in dog. Hence the staining intensity of the neurons in goat is not as much as in dog. Glia cells (yellow arrow) are also present.

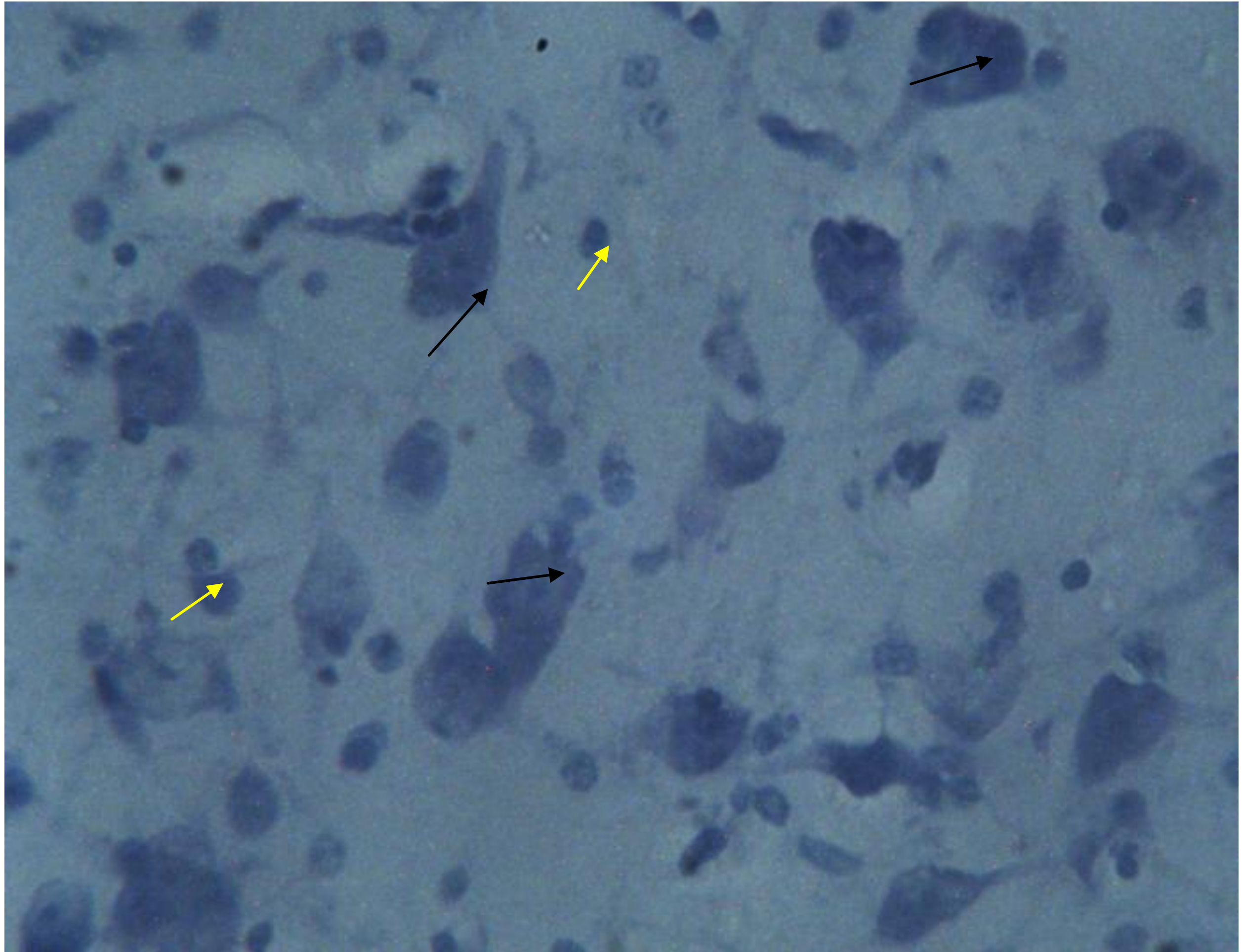


Plate XXII: Photomicrograph showing cells of the basolateral complex of sheep (Toludine blue, X250). Neurons (black arrows) were identified by the presence of a nucleolus. Neurons here were as large as those in dog but larger than those in goat. Also, numerous glia cells (yellow arrow) were interspersed in among the neurons. They have a high staining intensity also.

Plate XXIII. Photomicrograph showing cells of the basolateral complex of dog (Golgi silver, X40). Basolateral cells of the dog's amygdala are the largest. Their dendritic aborizations are the most elaborate when compared with sheep and goat.

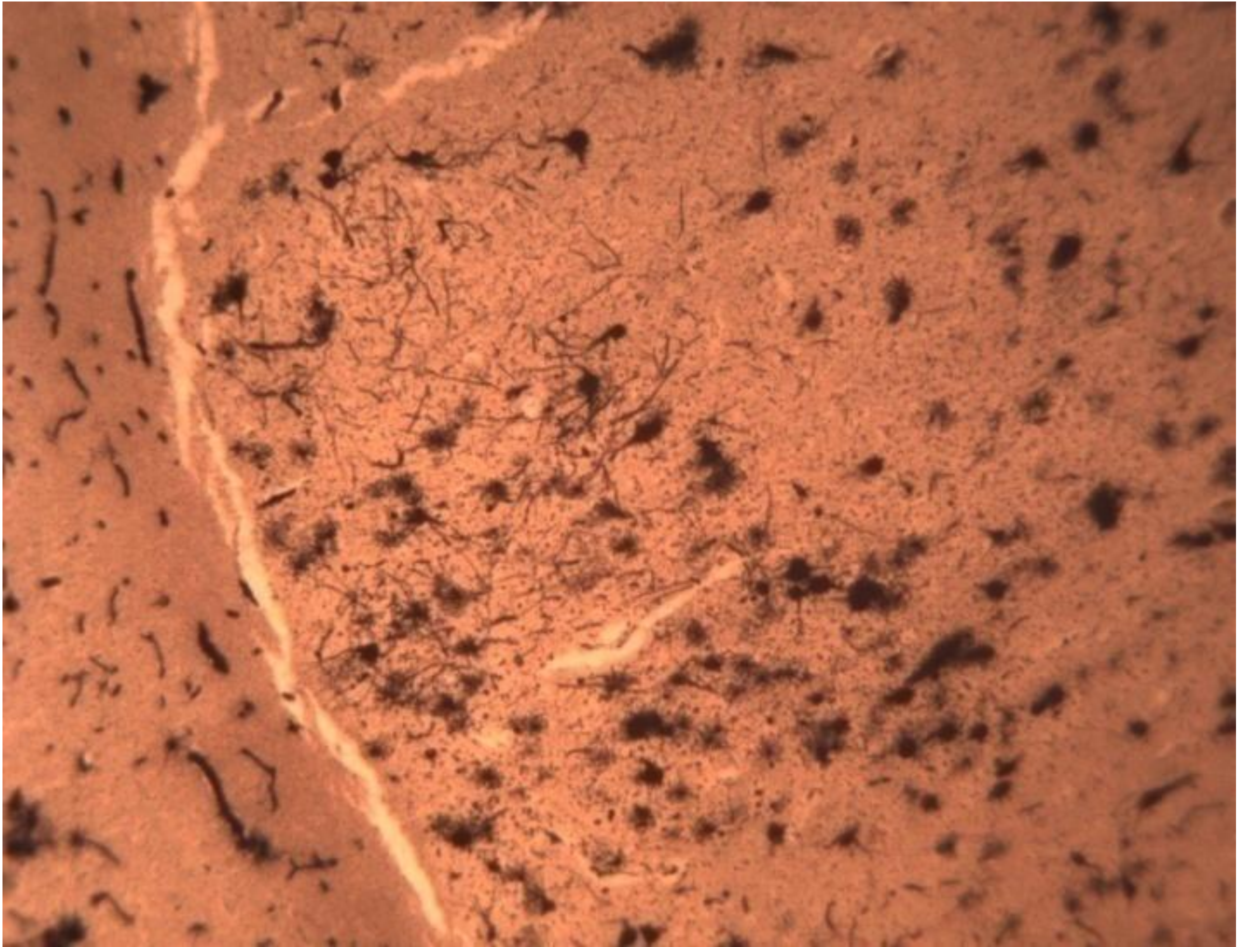


Plate XXIV. Photomicrograph showing cells of the basolateral complex of goat (Golgi silver, X40). Basolateral cells of sheep amygdala are smaller than those of sheep and dog. Their dendritic aborizations were not as elaborate those of sheep and goat.

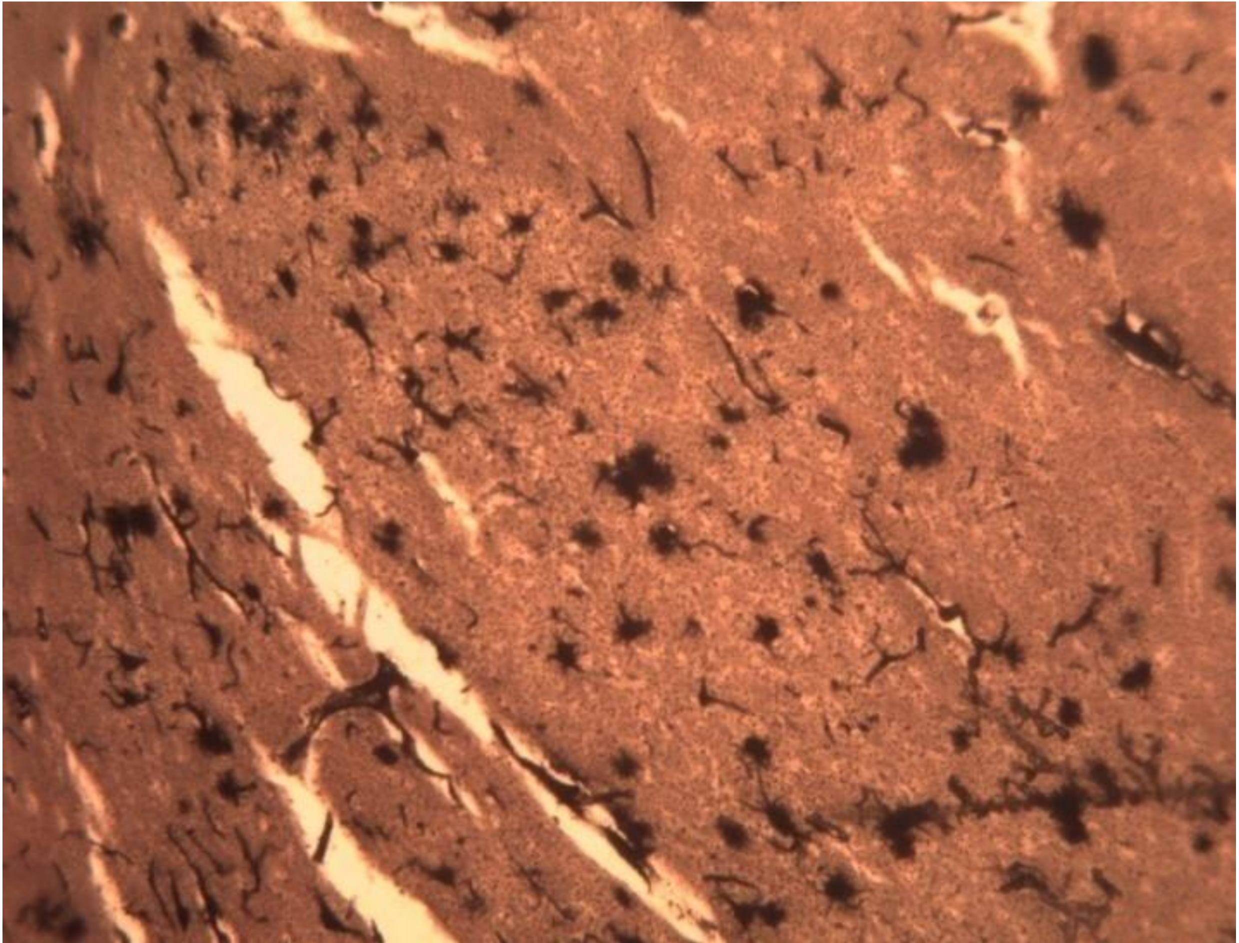


Plate XXV. Photomicrograph showing cells of the basolateral complex of sheep (Golgi silver, X40). Basolateral cells of sheep amygdala are larger than those of goat but more than in dog.. Their dendritic aborizations were not as elaborate as those of dog but more than goat.

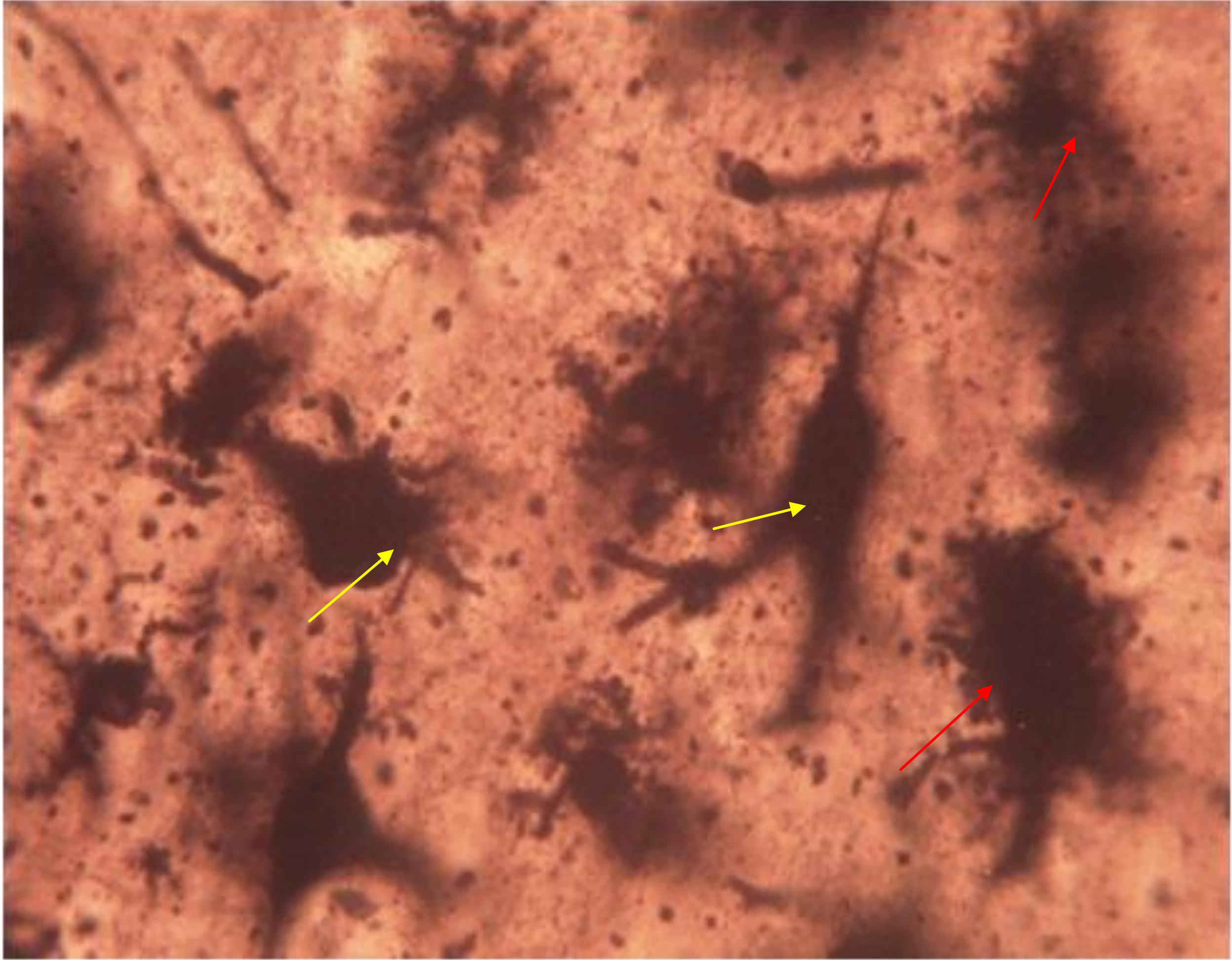


Plate XXVI. Photomicrograph showing cells of the basolateral complex of dog (Golgi silver, X250). Neurons of the basolateral complex of dog are the largest (yellow arrow). Neurons here are pyramidal (yellow arrow) or stellate in shape (red arrow). Stellate neurons have complex aborization and their staining intensity is deep such that the cell bodies are almost completely obscured. Spines are not obvious on the dendrites of these astrocytes.

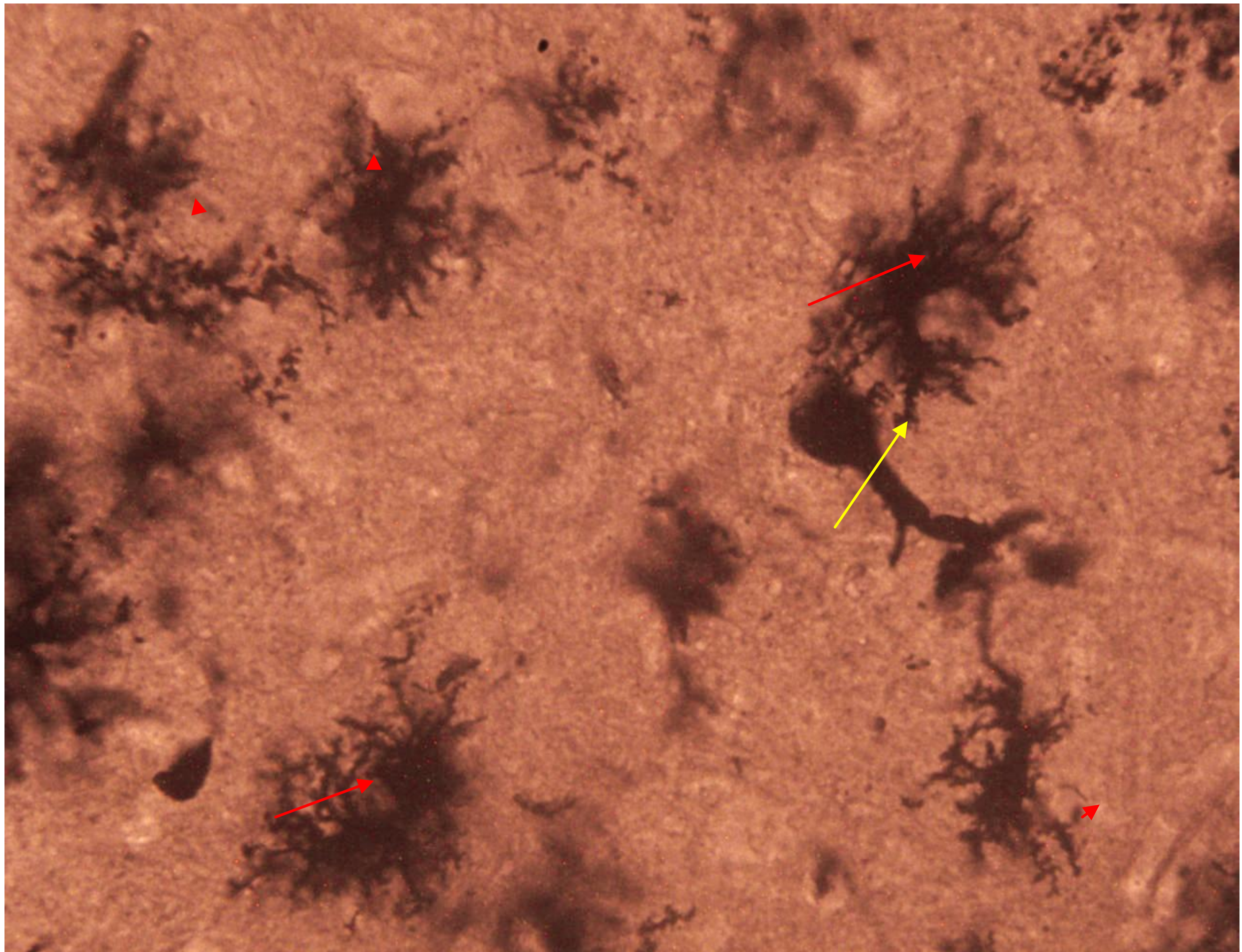


Plate XXVII: Photomicrograph showing cells of the basolateral complex of goat (Golgi silver, X250). Neurons of the basolateral complex of sheep are not as large as those of dog (yellow arrow). More numerous stellate neurons were interspersed among the pyramidal-like neurons (red arrow) which have well ramified dendritic aborization. Their staining intensity is high but not as much as in dog. Stellate neurons in goat are spinous (red arrow heads).

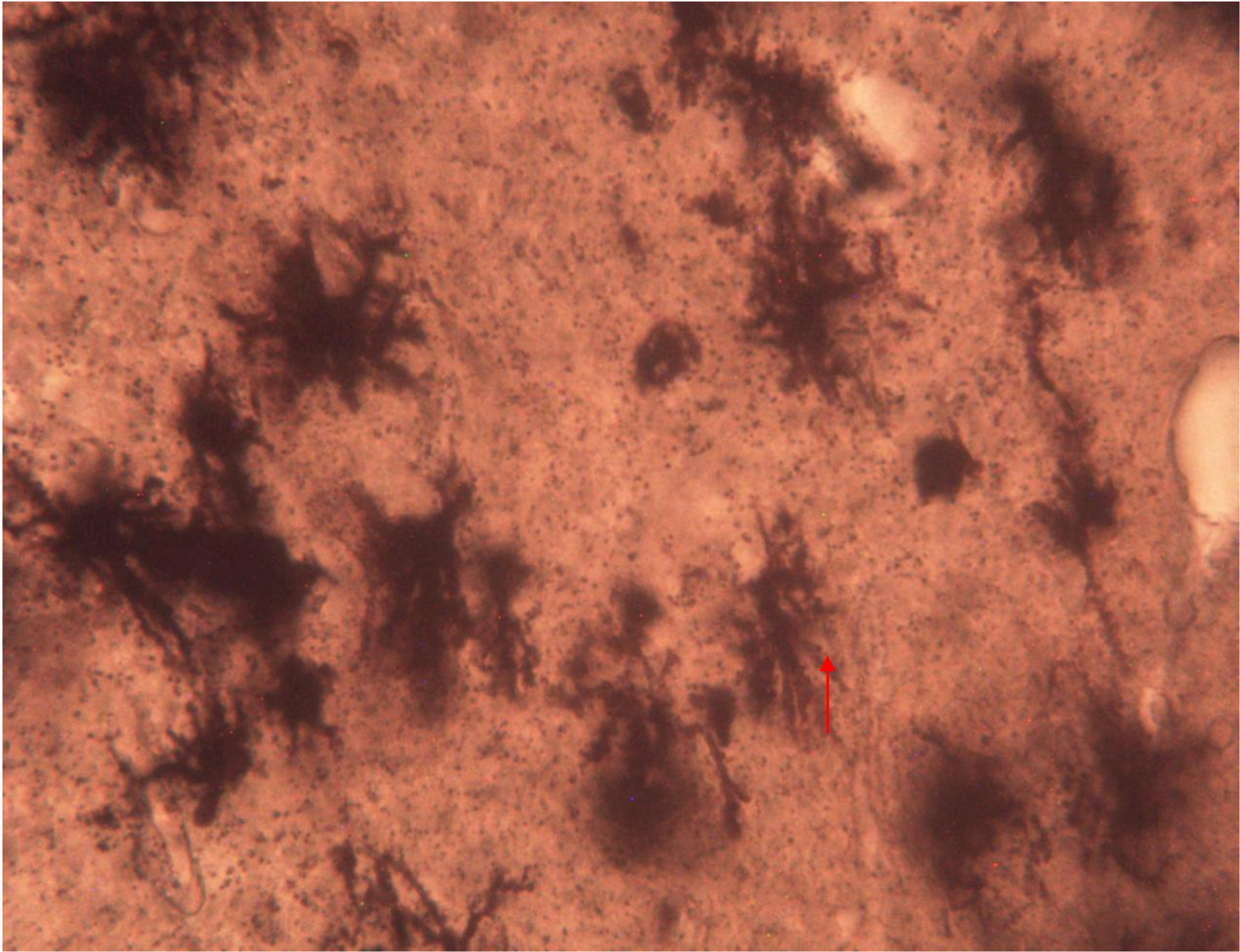


Plate XXVIII. Photomicrograph showing cells of the basolateral complex of sheep (Golgi silver, X250). Neurons of the basolateral complex of sheep are also large but fewer than in dog (yellow arrow). More numerous stellate shaped neurons were found here (red arrow). They are well ramified with complex aborization that is not as in dog but more than in goat. Their staining intensity is deep such that the cell bodies are almost completely obscured as in dog. Dendrites are sparsely spinous.

Table 4.4 Histomorphometry of basolateral complex cells of dog, goat, and sheep

Stain	Variable	DG	GT	SH
	Area(µm2)	1619.16±726.65*	582.97±187.49*	1175.90±409.54*
Toludine	**Perimeter(µm)**	76.10924±28.90*	51.78±11.06*	65.27±18.21*
Blue	**Diameter (µm)**	27.92816±11.10*	19.92±4.70*	24.06±6.89*
	Cell count	193*	173*	272*
	Area ($µm^2$	10588.8±5328.86*	554.38±3282.67*	7921.13±554.38*
Golgi	**Perimeter(µm)**	282.382±128.07*	39.20±63.35*	187.75±9.20 *
	Diameter (µm)	88.06442±38.15*	10.12±22.09*	65.42±10.12*
	Cells measured	62	81	87

Data are expressed as mean ±standard deviation (Mean±SD).The first row shows the mean soma diameters of lateral cells of dog, goat, and sheep. The dog lateral nucleus cells are the largest in both soma size and dendritic aborization, while sheep had the most cells in its lateral nucleus. Values in first row were obtained from analysis of lateral cells of toludine blue stained photomicrographs. Values in the second row were obtained by analysis of photomicrographs prepared using golgi silver techniques. *= Statistically significant. p≤0.05

4.3.7 Purkinje-like neurons in goat's amygdala

There was observed in goat amygdala, purkinje-like neurons. This was similar to purkinje cells found in the gray matter of cerebellum. It appeared as a huge flask-shaped cell which sent two or more primary thick dendrites that in turn branch into secondary dendrites (plate 4.35, 4.30). This cell morphology was unique in goat amygdala.

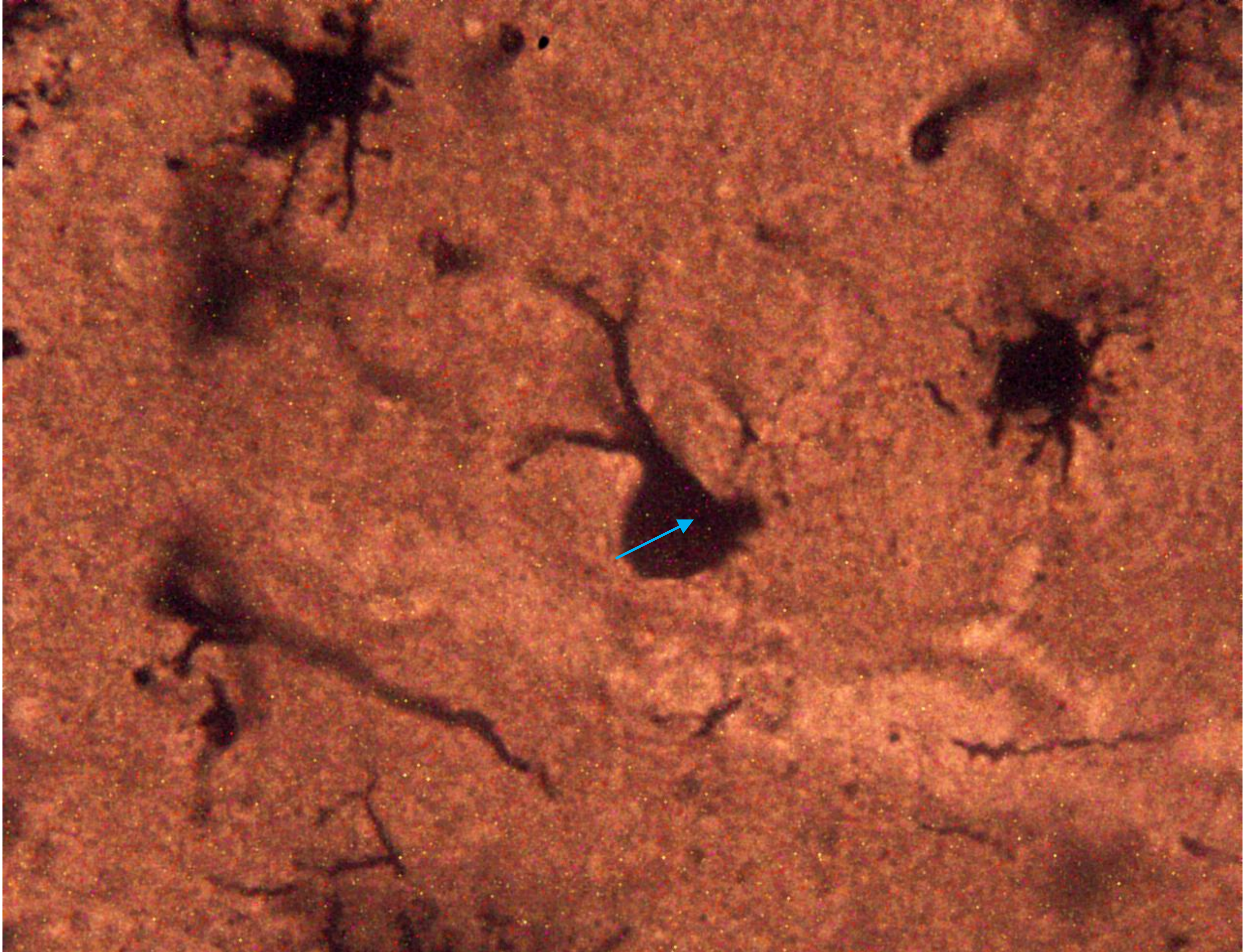


Plate XXVIX: Purkinje-like neurons in goat amygdala
Blue arrow indicates purkinje-like neuron. It is flask-shaped in appearance (Golgi stain, magX400). It is similar to purkinje cells found in the cerebellum

4.3.8. Histomorphology and Histomorphometry of Protoplasmic astrocytes surrounding the cortical region of the amygdala

Numerous protoplasmic astrocytes surrounded the surfaces of the gray matter in cortical region of the amygdala. Protoplasmic astrocytes were found in the gray matter and differ from fibrous astrocytes by having thicker and symmetrical dendritic processes (Singh, 2006). They were found attached either in relation to blood vessels or the surfaces of the brain. Dendrites of goat astrocytes (plate XXIX) around the cortical region of the amygdala appeared thicker than those of sheep(XXXI) and dog(XXX) and the dendrites ramified greatly in dog, thereby resulting in a complex dendritic aborization (plates XXIX-XXXI). There were more protoplasmic astrocytes in the cortical region of goat's amygdala (table 4.5) and it was significant in goat. This was followed by dog, then sheep. The staining intensity of the soma of goat astrocytes was more than those of dog and sheep..

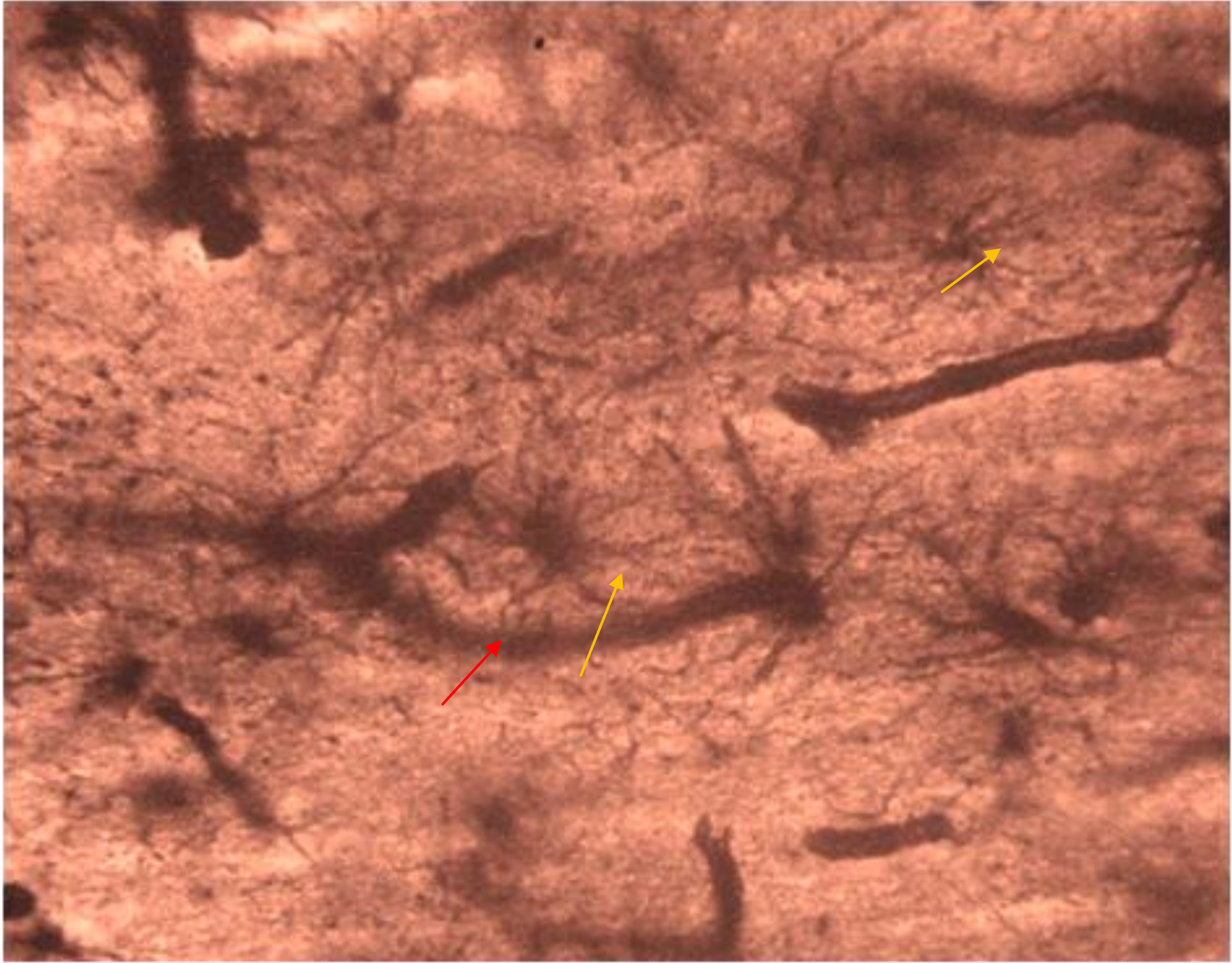


Plate XXIX: Protoplasmic astrocytes surrounding the cortical region of dog amygdala (Golgi silver, X250). Red arrow shows blood vessel, while yellow arrows indicate protoplasmic astrocytes. There were more protoplasmic astrocytes in the cortical region of dog amygdala than in sheep, but less than in goat. The staining intensity of the cell body of dog astrocytes was not as much as in goat but more than in sheep.

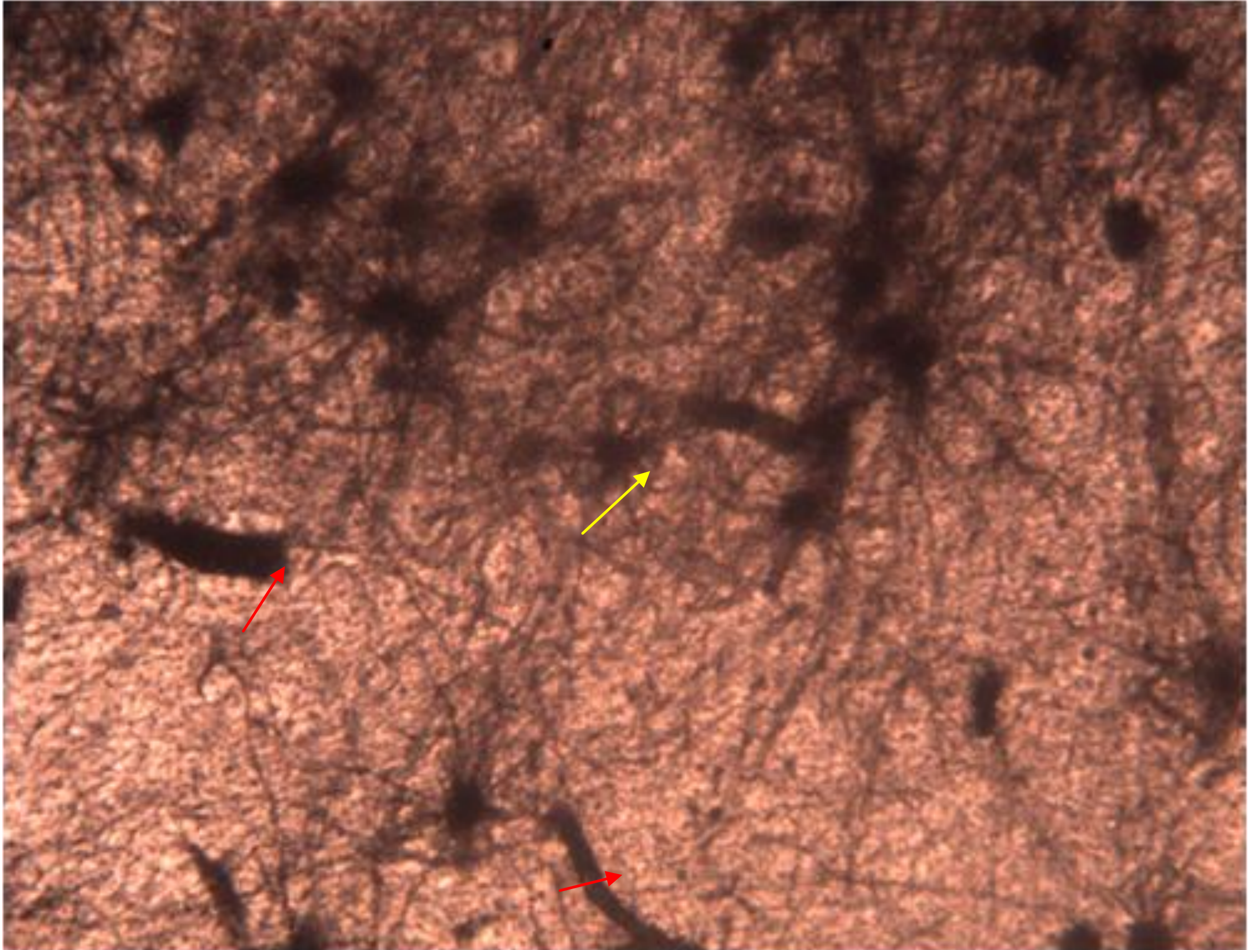


Plate XXX. Protoplasmic astrocytes surrounding the cortical region of goat amygdala (Golgi silver, X250). Red arrow shows blood vessel, while yellow arrow indicate protoplasmic astrocytes. There were more protoplasmic astrocytes in the cortical region of goat's amygdala. The staining intensity of the cell body of goat astrocytes was more than that of dog and sheep. The dendritic aborization of goat astrocytes in the cortical region of the amygdala is greatly ramified when compared with dog and sheep.

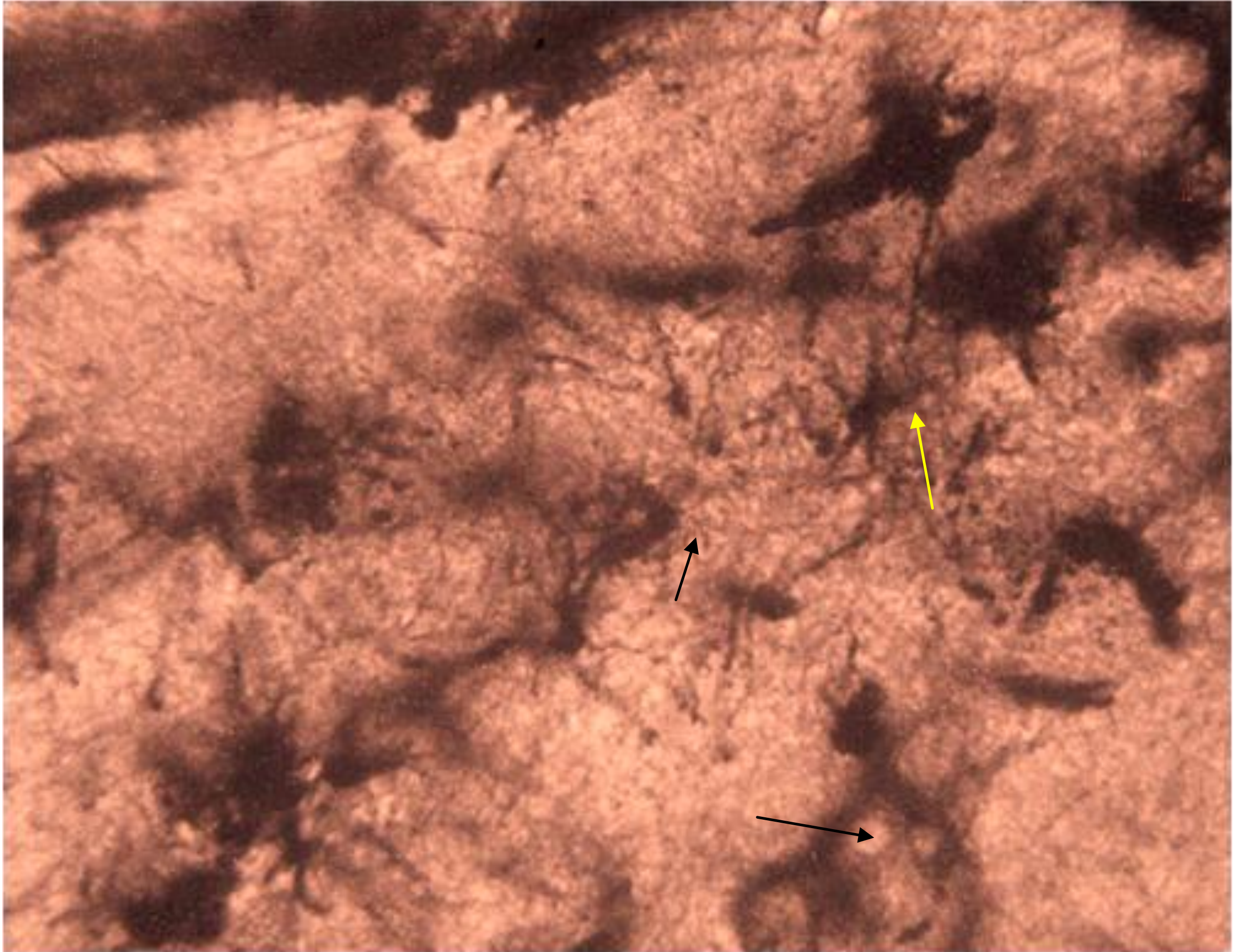


Plate XXXI. Protoplasmic astrocytes surrounding the cortical region of sheep amygdala (Golgi silver, X250). Red arrow shows blood vessel, while yellow arrow indicate protoplasmic astrocytes. There were more protoplasmic astrocytes in the cortical region of sheep amygdala. The staining intensity of the cell body was not as much as in goat.

Table 4.5. Cell count of Protoplasmic astrocytes surrounding the cortical region of the amygdala

Variable	DG	GT	SH
Number of protoplasmic Astrocytes			
	210± 0.19	252±0.06*	73±0.13

Data are expressed as mean ±standard Error of Mean (Mean±S.E.M.). Dog (DG), goat (GT), and sheep (SH). Goat had the highest number of protoplasmic astrocytes, followed by dog, then sheep. P≤0.05. *= statistically significant.

4.3.9. Histomorphology of neurons in the central nucleus of the amygdala of sheep, goat, and dog

H and E histological slides showed dog as having more ovoid shaped cells (plate XXXII). Same was observed with goat (XXXIII). Sheep central cells were more fusiform in shape than goat and dog (XXXIV). Cells were fewer in sheep. Toludine blue sections revealed more about the morphology of the soma of central cells in sheep, goat, and dog. Dog central cells appeared fusiform in shape and more in density (Plate XXXV). The cells stained deeply that most nucleoli were not observed. Goat cells were fewer and ovoid in shape. The staining intensity of cells was not as much as dog. Nucleolus could be clearly seen (plate XXXVI). Sheep cells were deeply stained as in dog but appeared fewer in number (plate XXXVII). Cell count revealed that goat had the most cells. This was followed by goat, then sheep. The soma diameter of dog was the largest, followed by sheep (table 4.6).

Golgi silver techniques revealed more about the dendritc aborization of cells in the central nucleus region of the amygdala. The dendritic aborization of goat was the most elaborate, followed by dog. Sheep had the least dendritic aborization of cells in its central nucleus (plates XXXVII-XXXXIX, table 4.6). The dendrites branched greatly in goat, giving rise to long dendritc shafts (Plate XXXVIII). Also, the morphology of the soma of neurons was clearly outlined by golgi stain. The neurons were ovoid of fusiform in shape. The somata of the neurons appeared more ovoid in shape with more than two primary dendrites arising from it. Numerous networks of dendrites were found in the central nucleus of the amygdala.

4.3.10. Histomorphometry of the central nucleus of dog, goat, sheep

Cell count showed that dog had the most number of cells in the central nucleus of the amygdala. This was followed by goat, then sheep (table 4.6). The soma sizes of toludine blue stained sections of dog cells were the largest in size (26.56±17.23µm). This was followed by sheep (21.80±6.74 µm), then goat (16.70±5.50 µm). The dendritic aborization of the neurons was most in goat (79.66±42.94 µm), with very long primary dendrites arising from the soma (table 4.6).

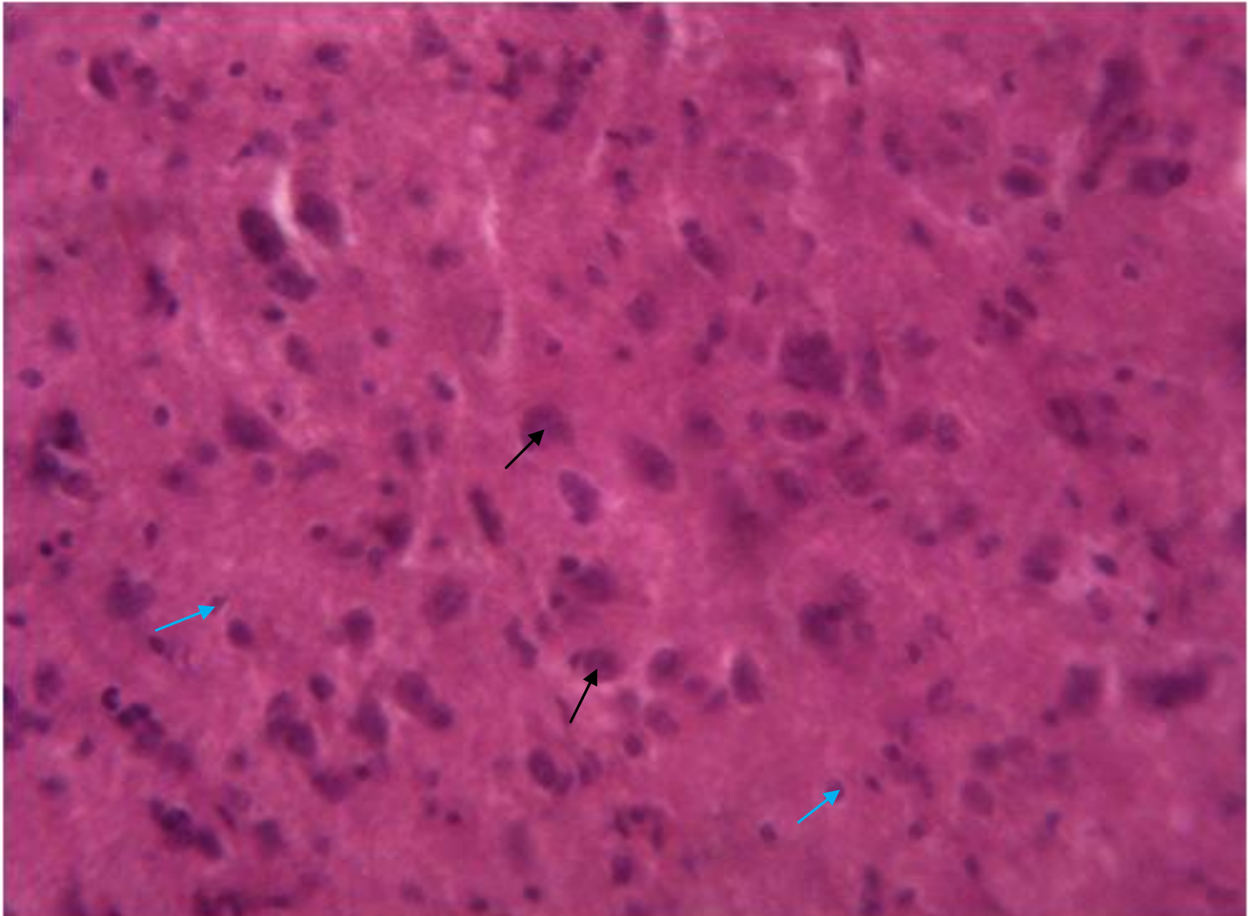


Plate XXXII: Oval or round shaped cells of dog amygdala (Haematoxylin and Eosin stain , X250). Numerous glia cells were interspersed among the cells

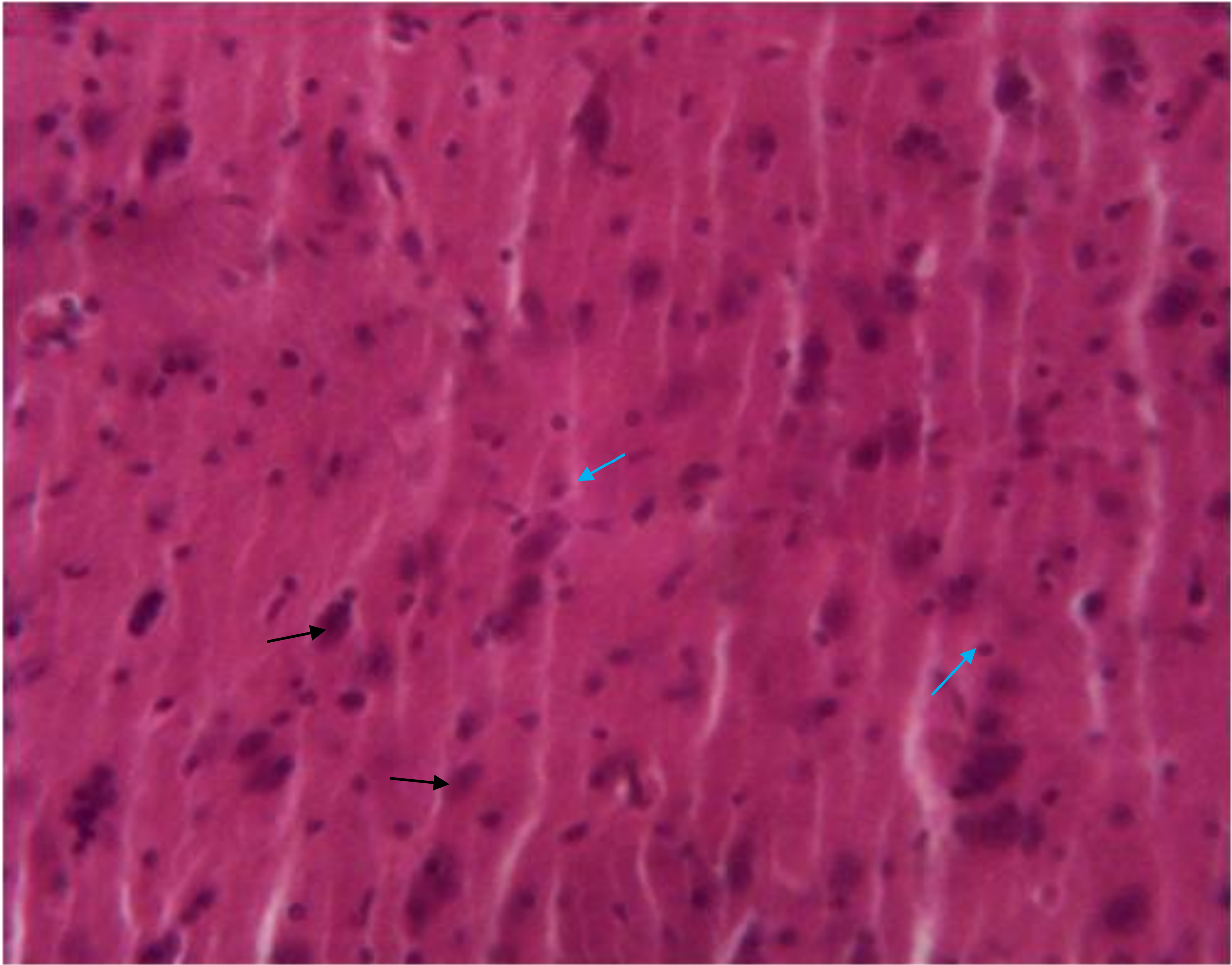


Plate XXXIII: Oval or fusiform shaped cells (black arrow) of goat amygdala (Haematoxylin and Eosin stain , X250). Numerous glia cells (blue arrow) were interspersed among the cells

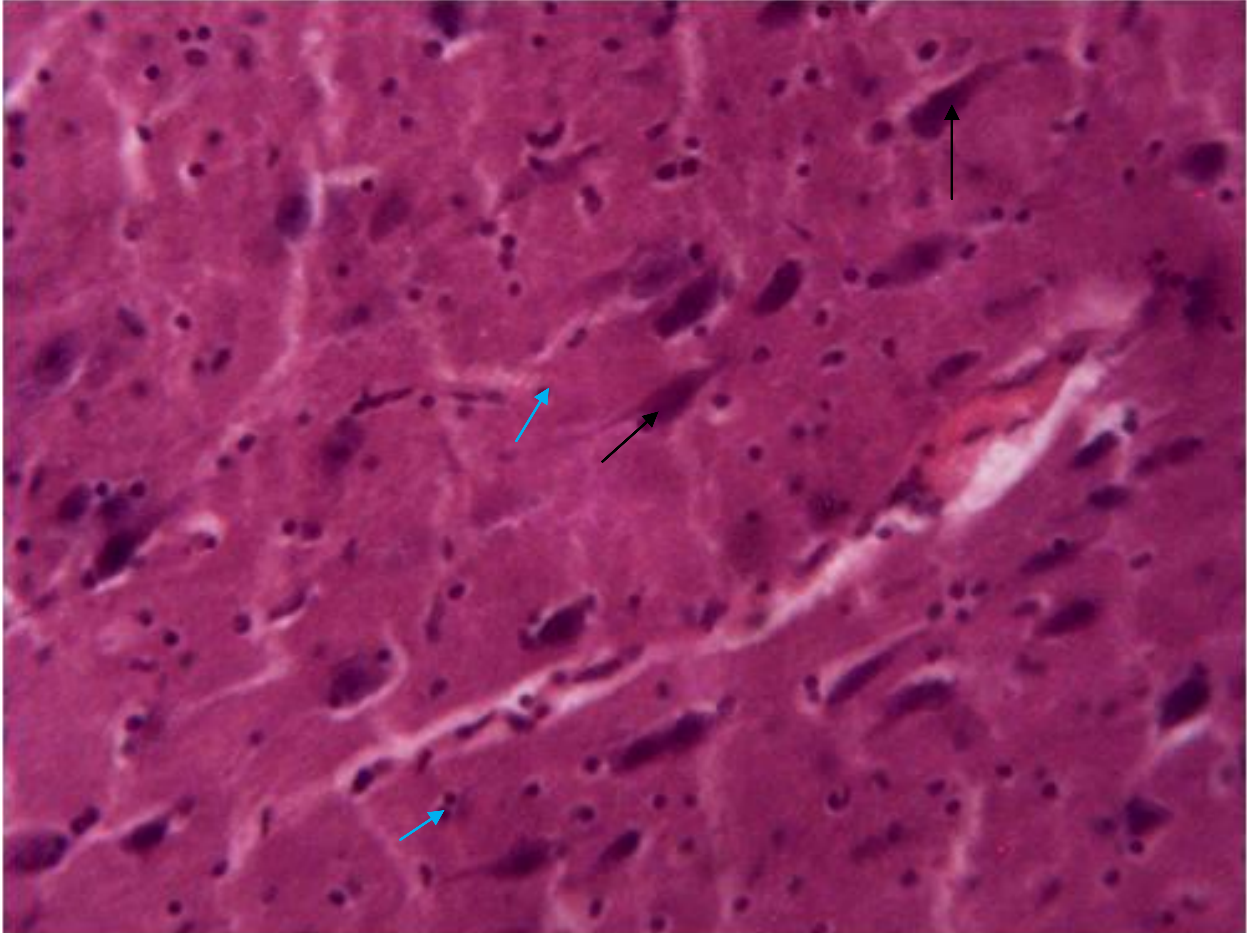


Plate XXXIV. Fusiform shaped cells of sheep amygdala (Haematoxylin and Eosin stain , X250). Cells are more fusiform and enlongated in shape with fewer glia cells interspersed among the cells when compared with dog and goat.

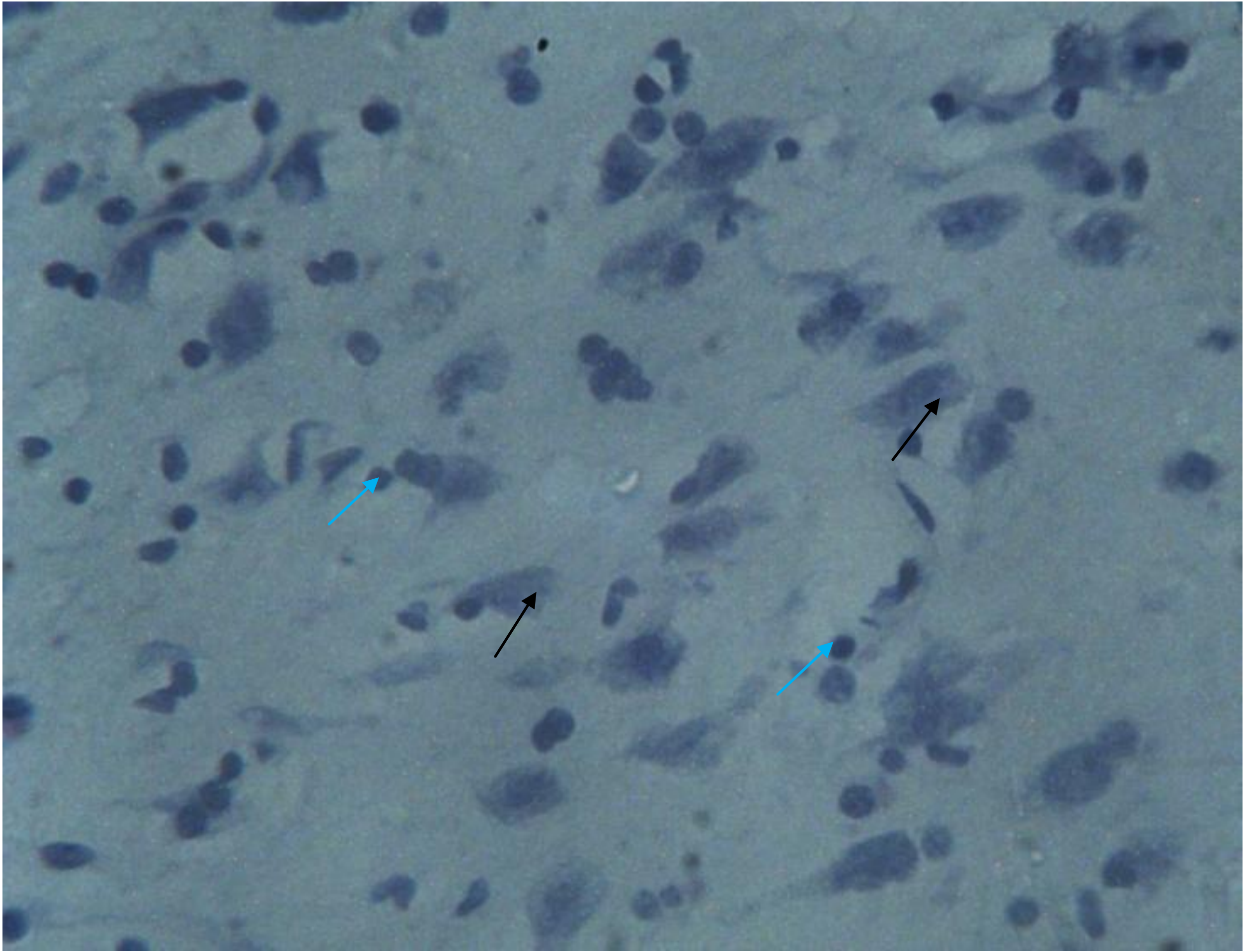


Plate XXXV: Fusiform shaped cells (black arrow) of dog amygdala (Toludine blue stain, X250). Numerous glia cells (blue arrow) were interspersed among the cells

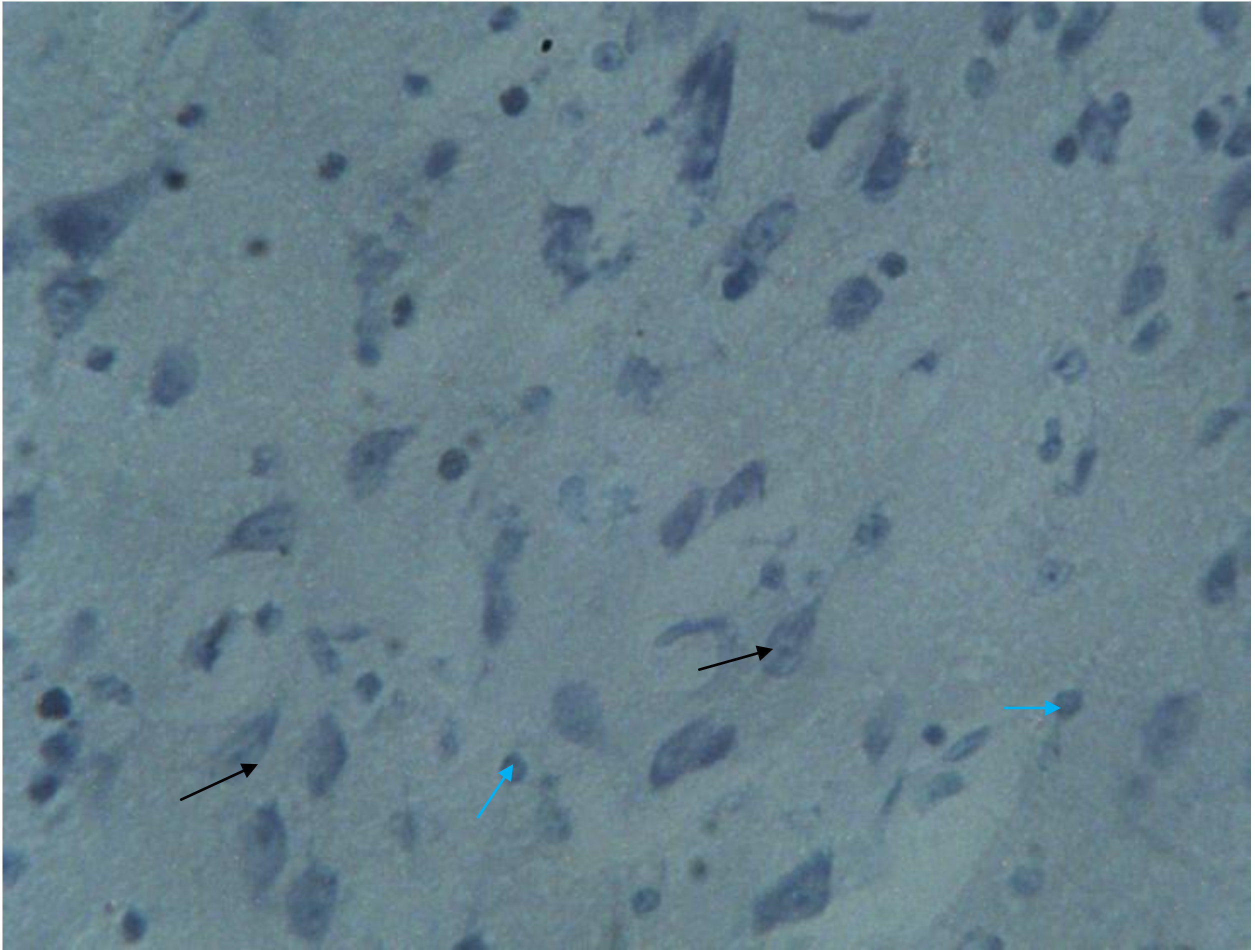


Plate XXXVI: Fusiform shaped cells (black arrow) of goat amygdala (Toludine blue stain, X250). Numerous glia cells (blue arrow) were interspersed among the cells

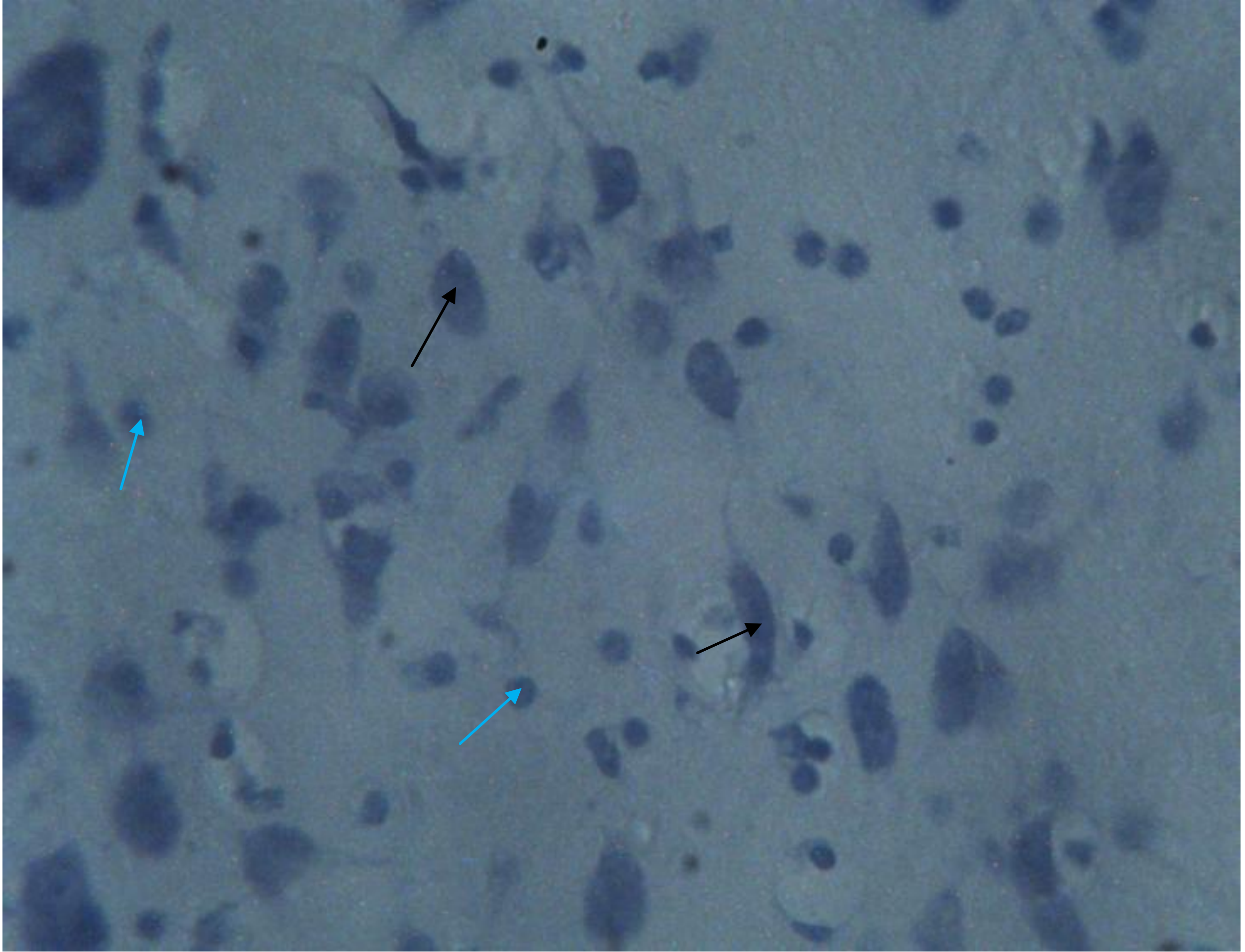


Plate XXXVI. Neurons ranged from fusiform to ovoid shape (black arrow) in sheep amygdala (Toludine blue stain, X250). Numerous glia cells (blue arrow) were interspersed among the cells

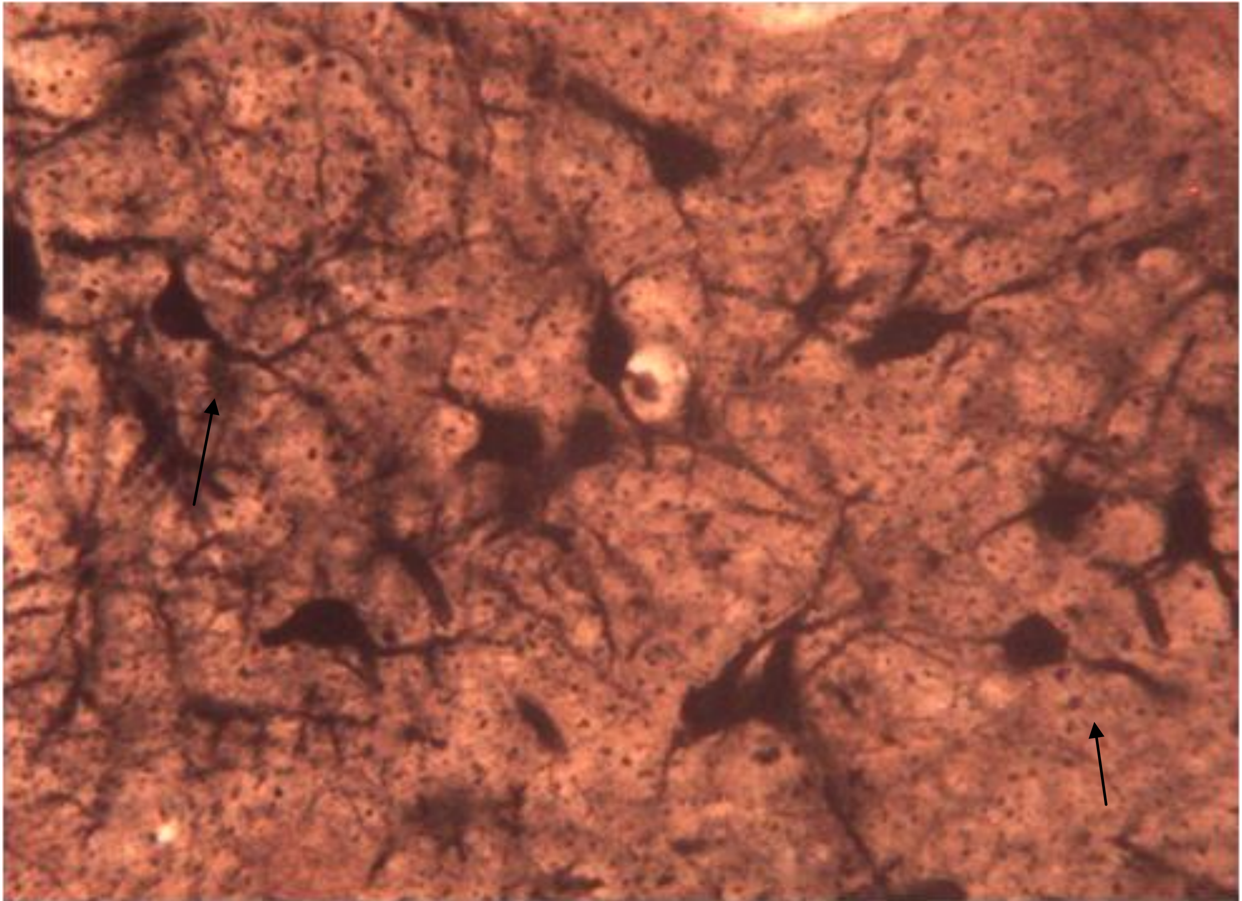


Plate XXXVII. Ovoid shaped neurons in the central nucleus of dog amygdala(Golgi Stain X250). The soma of the neurons appear more ovoid in shape with more than two primary dendrites arising from the soma, which futher gives rise to secondary dendrites. Numerous network of dendrites were found in the central nucleus of the amygdala.

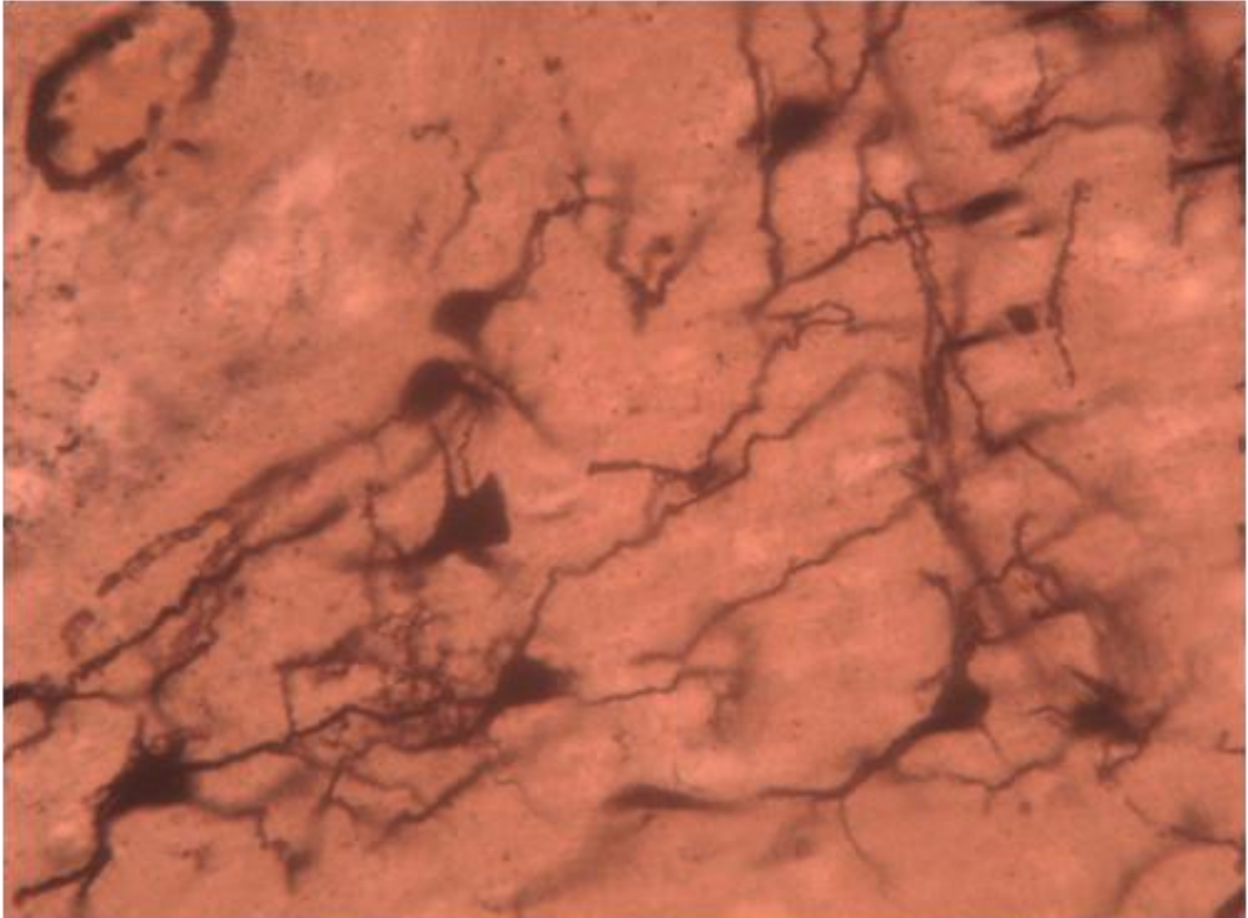


PLATE XXXVIII: Ovoid shaped neurons (black arrow) in the central nucleus of goat amygdala(Golgi Stain, X250). The dendrites branched greatly in goat, giving rise to long dendritc shafts. The soma of the neurons appear more ovoid in shape with more than two primary dendrites arising from the soma, which futher gives rise to secondary dendrites. Numerous network of dendrites were found in the central nucleus of rhe amygdala.

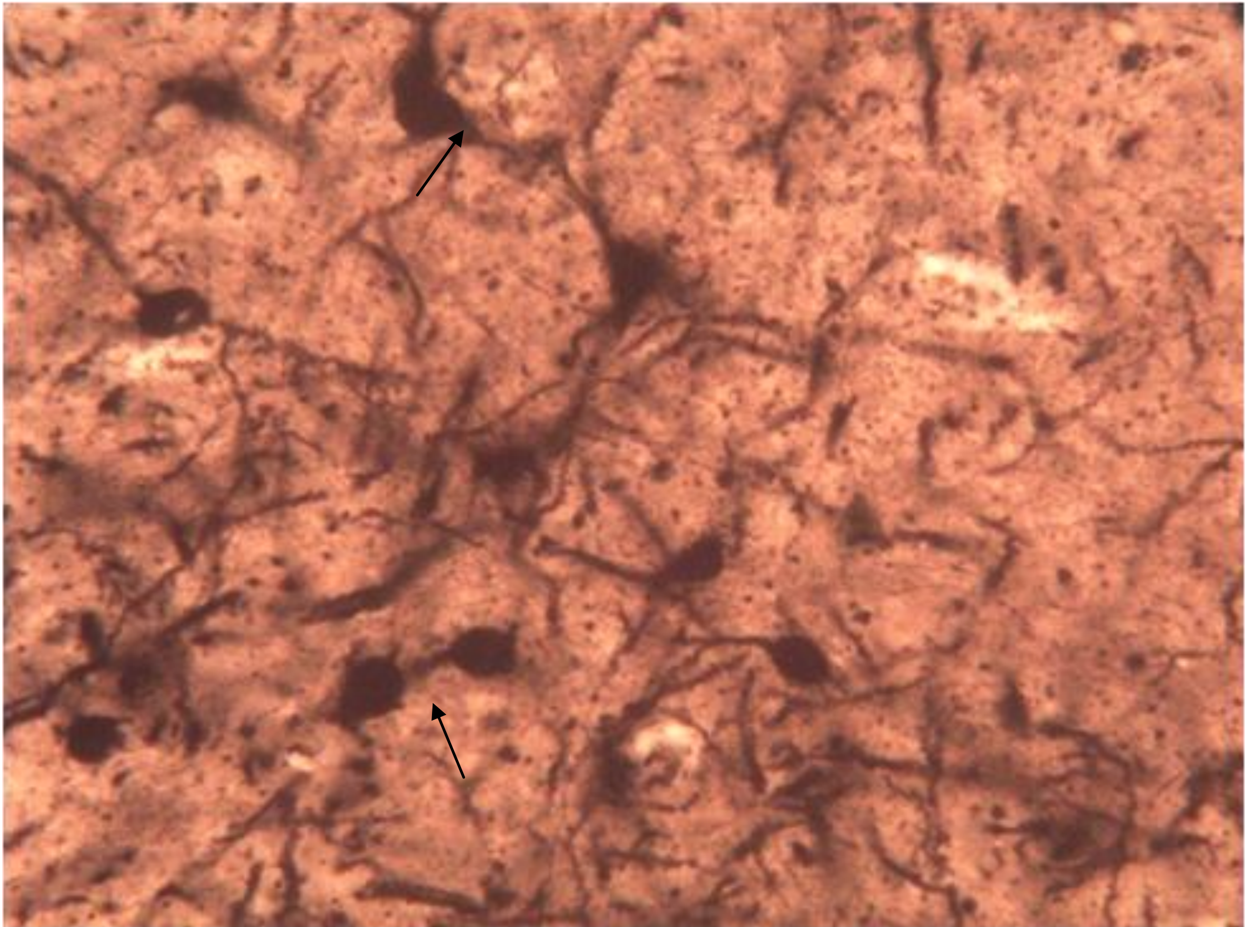


PLATE XXXIX. Ovoid shaped neurons in the central nucleus of sheep amygdala(Golgi Stain x250mg). The soma of the neurons appear more ovoid in shape with more than two primary dendrites arising from the soma, which further gives rise to secondary dendrites (black arrow). Numerous network of dendrites were found in the central nucleus of the amygdala.

Table 4.6 Histomorphometry of the central nucleus of dog, goat, sheep

Stain	Variable	DG	GT	SH
	Area(μm^2)	864.03±733.85*	3540.83±163.42*	616.25±212.65*
Toludine	**Perimeter(µm)**	66.70±34.58*	40.73±13.00*	54.46±15.37*
Blue	**Diameter (µm)**	26.56±17.23*	16.70±5.50*	21.80±6.74*
	cell count	306*	271*	230*
	Area(μm^2)	2261.36±1212.70*	2332.76±1312.24*	1487.92±624.23*
Golgi	**Perimeter(µm)**	172.89±103.19*	250.78±133.69*	114.22±46.68*
	Diameter (µm)	57.01±24.19*	79.66±42.94*	43.43±14.35*
	Cells measured	84	76	71

Data are expressed as mean ±standard deviation (Mean±SD). The first row shows the mean soma diameters of central cells of dog, goat, and sheep. Goat had the highest number of central cells in its hippocampus The dog central nucleus cells are the largest in both soma size and dendritic aborization. Sheep has the least number of cells, soma size, and dendritic aborization, Values in first row were obtained from analysis of central cells of toludine blue stained photomicrographs. Values in the second row were obtained by analysis of photomicrographs prepared using golgi silver techniques. *= $p \leq 0.05$

CHAPTER FIVE

5.0 DISCUSSION

5.1 Gross morphological and morphometric studies

Parameters of specific parts of brain show differences that reflect functional requirements between and within species (Kruska, 1988; Kaas &Collins, 2001). Changes in parameters of certain brain parts have been attributed either to an increase in the number of neurons, the size of such neurons and/ or their connectivity (Kaas, 2000). Although the brain size and volume were largest in sheep, followed by goat, and then dog, sheep brain had the most intricate folding of the gyri and sulci on the cerebrum. This was followed by goat. Dog had the least intricate folding of the cerebrum, thus indicative of larger brain surface area on the cerebrum of sheep and goat, than dog. Interestingly, there was a highly significant difference in the number of gyri and sulci between sheep and goat (Table 4.2). This result suggested that the degree of gyrification was essentially determined by the size of the brain. As the brain size increased, the gyrification, as well as the number of gyri and sulci, increased. This is in agreement with works done by Wosinski *et al.,* (2006) and Zilles *et al.,* (2013).

Olopade and Owuka (2002) determined the brain weight to body weight ratio of the Red Sokoto goat to be 1: 165 while data obtained from Igado (2011) showed the brain weight to body ratio of the Mongrel breed (*Canis lupus familiaris*) of dog in Nigeria 1:294. This showed the brain of dog in relation to body size to be far smaller in weight in comparison with goat. Similar results were observed in table 4.1 in which the weight and volume of goat brains were more than those of dog.

Although dog brain was smaller, it had larger caudolateral expansions than sheep and goat. This was also observed in the dog having the most cerebral width (table 4.2). This caudolateral expansion of the cerebrum of dog, together with its pointed frontal lobe of cerebrum, makes it similar to rodents phylogenetically. On the other hand, the rostocaudolateral expansion of the cerebrum of sheep makes it similar to humans phylogenetically. Therefore dog, goat, and sheep could be said to have similar ontogeny, but different phylogeny, and dogs could be older phylogenetically than sheep and goat on the evolutionary tree as it is similar to rodents in the morphology of their cerebrum. The difference between rodent brain and dog brain was in the gyrencephaly of dog brain while those of rodents were lissencephalic with little or no gyrus at all on their cerebrum.

Overlapping of the cerebellum by the cerebrum is a feature seen in the human brain. This was also observed in dog. Therefore, dog could be said to serve as a “bridge” between rodents and humans on the evolutionary tree on the one hand. Sheep and goat had little or no overlap of their cerebellum by cerebrum. This is a feature seen in rodents. Hence, goat and sheep could also be said to serve as a “bridge” between humans and rodents on the evolutionary tree on the other hand. Although dog brain had the least size and volume, its cerebral length and width were the most. This could be attributed either to an increase in the number of neurons, the size of such neurons and/ or their connectivity in the cerebrum(Kaas, 2000). In this present study, dog had the most number of cells in its prefrontal cortex of the cerebrum (Plates I, IV, VI), although the interconnectivity (dendritc arborization) and sizes of neurons in goat were the most (plates II, V, VII). Increased density of neurons in dog could be the reason for the longer length and width of its cerebrum since Kaas (2000) stated that changes in parameters of certain brain parts could be attributed to this. This could be responsible for the higher cognitive

function of dog and their ability to sense and respond faster to threats and danger than sheep and goat.

5.2 Histomorphological and histomorphometric studies of the prefrontal cortex, hippocampus, and the amygdala

It has been argued that differences in the neocortex could be mainly a factor of differences in neuron number (Herculano-Houzel, 2007) in animals with different sized brains and varying degrees of behavioral complexity. Other alterations such as changes in the types of neurons and glia present, connectivity, and the addition of new cortical fields are postulated to play a role as well (Krubitzer, 2009). In this study, these were examined and deductions were made based on observed facts.

5.2.1 Prefrontal cortex

The basic components of fear circuitry were well preserved across species and exert similar functions in humans (Lisa and Isreal, 2010). The Prefrontal cortex (PFC) is involved in higher-level cognitive processes (Baddeley, 1992; Fuster, 2000; Jurado and Rosselli, 2007). It has six layers: I-VI. Layer V included large pyramidal cells whose sizes were larger than those of the pyramidal cells of layer III (Semendeferi *et al.,* 2001). The major source of sensory information to the amygdala is the cerebral cortex (Mc Donald, 1998). These projections are glutamatergic, predominantly arising from layer V pyramidal neurons (Amaral and Insausti, 1992; Ottersen *et al.,* 1986). For this reason, together with the fact that large pyramidal neurons are found here, layer V cells of the prefrontal cortex was investigated in this study.

In studying layer V cells of the prefrontal cortex specifically, dog had the largest soma size with a diameter of 57.60±9.65µm (table 4.2, plates VIII) based on results from cell count of toludine blue stained photomicrographs. However, the density of neurons was the most in dog. Since the prefrontal cortex is responsible for higher cognitive function (Baddeley, 1992; Fuster, 2000; Jurado and Rosselli, 2007), increase in the density of neurons in dog prefrontal cortex explains why dogs are keen, swift, specialized in alertness, and respond faster to threats and danger than goat and sheep. Kaas, in 2000, listed increase in the density of neurons as one of the factors relating to changes in certain brain parameters. Therefore, increase in the cerebral length and width of dog than of sheep and goat could account for increased density of neurons in the prefrontal cortex of dog's cerebrum, even though dog brain was the smallest in size.

5.2.2 Hippocampus

CA1 and CA2 of the hippocampus form zones of small pyramidal cells while CA3 and CA4 formed of zone of large pyramidal cells (Amin *et al.,* 2013). The CA3 cells were differentiated from CA2 by their larger pyramidal cells (Amin *et al.,* 2013). Dentate gyrus granule cells receive excitatory neuron input from the entorhinal cortex and send excitatory output to the hippocampal CA3 region via its mossy fibers. Pyramidal cells of CA3 send their axons to CA1. Pyramidal cells of CA1 send their axons to the subiculum and deep layers of the enthorinal cortex. Subicular neurons send their axons mainly to the EC (Anderson *et al.,* 1971). However, in the present study, the density of neurons in CA3 region of the hippocampus of dog was the most. This is revealed by neuronal cell count of both toludine blue stained and H & E histological sections. Sheep had the least number of cells and the

largest soma diameter (41.61±11.46μm). Although the pyramidal cells of sheep CA3 cells were the largest in soma diameter, the density of neurons here were the least. Therefore, fewer cells in the CA3 of sheep receive signals from the dentate gyrus which are then projected to other parts of the hippocampus. Since CA3 cells of the hippocampus is responsible for long term memory storage and learning ((Baddeley, 1992; Fuster, 2000; Jurado and Rosselli, 2007) and mediating of larger scale spatial information processing (Hunsaker *et al.,* 2008), it could be said that fewer sensory input gets to sheep than goat and dog. Hence, the sheep responds slowly to threats and danger than goat and dog. It could also be said that sheep has a poor memory of fearful or threatening situations since fewer cells of its CA3 cells play the role of projecting sensory input relating to memory. It "forgets easily" a threatening situation, while goat and dog had greater density of neurons in this region of the hippocampus, hence their prompt and quick response to threats and danger.

5.2.3 Dentate gyrus

The dentate gyrus (DG), is a tightly packed layer of small granular cells wrapped around the end of the hippocampus proper, forming a pointed wedge in some cross-sections, a semicircle in others (Amaral and Lavenex, 2006). Dentate gyrus (DG) was seen surrounding CA4 by its upper & lower limbs (Amin *et al.,* 2013). Granular cells of dentate gyrus send their axons (called "mossy fibers") to CA3 cells during flow of signals through the hippocampus (Anderson *et al.,* 1971). In some coronal sections of the dog hippocampus, however, the dentate gyrus was observed to form a cluster of diffused region of densely packed cluster of granular cells which tapers gradually towards the upper and the lower limb of the dentate gyrus (plate XVI). This is different from the conventional pointed wedged appearance in some

cross-sections, or a semicircular in others reported by Amaral and Lavenex in 2006. The cluster of diffused granular cells was seen at the region of the loop of the dentate gyrus. The upper and lower limbs of the ends of the dentate gyrus were of approximately equal length at this coronal section (plates XVI). This was not observed in the sheep and goat at any coronal section through the hippocampus. This is important because it has been suggested that the dentate gyrus (DG) and CA3 cooperate to efficiently process spatial information. The DG has been proposed to be important for fine spatial discrimination, and the CA3 has been proposed to mediate larger scale spatial information processing (Hunsaker *et al.,* 2008). Therefore, the presence of densely packed cluster of cells at the loop of the dentate gyrus suggests that the transmission of information from the dentate gyrus to the CA3 cells of dog hippocampus is quicker than in sheep and goat as there are more granular cells in dog dentate gyrus. This could account for dog's quick response to threats and danger than the sheep and the goat. It also explains why dogs "easily remember" a threatening situation and react promptly to it. Throughout the brain, the dentate gyrus is unique, because adult neurogenesis takes place in this region. Thus, new neurons are generated and functionally integrated throughout life (Lledo *et al.,* 2006). And region of clusters of cells with increased density of neurons in dog's dentate gyrus suggest more neurogenesis in dog when compared to sheep and goat.

5.2.4 Amygdala

The internal organization of the amygdala has been shown to vary across species both qualitatively (Pitkeanen and Kemppainen, 2002) and quantitatively (Stephan *et al.,* 1987; Barger *et al.,* 2007). The amygdala plays a role in the acquisition of fear conditioned response,

a function that is conserved across a wide variety of species (LeDoux, 2000; Antoniadis *et al.,* 2007)

5.2.4.1 Basolateral complex cells

In the basolateral complex, basal cells are somewhat larger cells when compared with lateral and central nucleus cells (McDonald, 1982b; Millhouse and DeOlmos, 1983). 70% of the cells are pyramidal-like or projection neuron. For neurons within the basolateral complex, cells described as pyramidal-like comprise a morphological continum from pyramidal-like to semi-pyramidal to stellate shape (Faber *et al.,* 2001; McDonald, 1992; Pare *et al.,* 1995), In the rat lateral amygdala, the pyramidal-like or projection neurons had an average soma diameter of 10-15µm (McDonald, 1982b; Millhouse and DeOlmos, 1983) . In the present study, however, dog lateral cells had the largest soma diameter (27.93±11.10µm) and their dendritic trees were the most elaborate (88.0±38.15µm). This is reasonable when compared with the soma diameter of rat lateral cells (10-15 µm) since goat, dog, and sheep brains are larger than rat brain. Goat had the least dendritic aborization of the basal cells (table 4.5). Yet goat responds faster to threats and danger than sheep.

A significance of the basolateral complex of the amygdala is that sensory inputs to the amygdala (fig 2.3) terminate mainly in the lateral nucleus (LA) of the basolateral complex (Amaral *et al.,* 1992; LeDoux *et al.,* 1990a; Mascagni *et al.,* 1993; McDonald, 1998; Romanski and LeDoux, 1993; Turner and Herkenham, 1991). When an unconditioned sensory input from the sensory cortex is sent, it is projected to the amygdala directly, passing through the lateral nucleus, and then to the central nucleus, the brain region that generates fear responses (shin, 2012; Knobloch *et al.,* 2012).Therefore, increased density of neurons and dendritic arborization of neurons in the lateral nucleus of dog basolateral complex explains

why dogs are generally faster than sheep and goat when it comes to responding to threatening situations. Hence, cells in the basolateral complex serve as an important link in this pathway of fear. Moreover, even though there are more cells in sheep lateral nucleus, there are more elaborate dendritic aborization of dog lateral cells that in turn project the sensory inputs to the central nucleus or the basal nucleus, depending on the pathway of fear and type of sensory stimuli (conditioned or unconditioned stimuli), so as to bring about a quicker fear response in dog than sheep. Goat, on the other hand, had the least of cell density and dendritic aborization of cells. Yet, it responds faster to threats and danger than sheep. This shows that other factors are involved in goat which contributed to their quicker response to threats and danger than sheep.

Spines were observed on the stellate neurons of goat basolateral complex. The presence of spines increased synaptic connections between neurons. Most synapses impinging on neurons are located in dendritic spines (Luiz and Jose, 2005). Dendritic spines are the first processing locale for synaptic signals arriving on a neuron. They also participate in the plasticity changes that underlie adaptation, learning, and memory. They are dynamic structures with a morphological plasticity based on the cytoskeletal protein, actin, which is related to the development of synapse and their functional adaptation (Luiz and Jose, 2005). This could be the reason for goat's faster response to threats and danger than sheep even though sheep had more stellate neurons and greater dendritic arborizations of these neurons than goat. Dog's greatly ramified dendritic arborization accounts for its fastest response to threats and danger than goat and sheep

5.2.4.2 Protoplasmic astrocytes in the cortical region of the amygdala

Astrocytes have processes that frequently end in expansions in relation to blood vessels or in relation to the surface of the brain. Small swellings called gliosomes are present on the processes of astrocytes (Singh, 2006). In the present study, numerous astrocytes were observed in the basolateral complex and around the cortical region of the brain in relation to blood vessel. Processes of astrocytes are united to those of other astrocytes through gap junctions (Singh, 2006). Astrocytes regulate the stability, dynamics, and maturation of dendritic spines (Haber *et al.,* 2006; Nishida and Okabe, 2007).

5.2.4.3 Central nucleus

Goat, sheep, and dog central amygdala neurons were aspiny in this study. Martina *et al.,*(1999), McDonald (1982a), and Schiess *et al.* (1999) described some neurons of the central nucleus as having an ovoid or fusiform soma and three to five non-spiny primary dendrites from which moderately spiny, sparsely branching secondary and tertiary dendrites arise. A second type of neuron has also been described that has a somewhat larger soma and a thick primary aspiny dendrite that tapers into sparsely spiny secondary dendrites (Cassell and Gray, 1989; McDonald, 1982a; Schiess *et al.,* 1999). In addition, a small number of aspiny neurons have also been described (Cassell and Gray, 1989). These three cell types are said to be distributed homogeneously throughout the central amygdala. However, in the present study, the central neurons of sheep, goat, and dog were aspiny.

Neuronal cell count using toludine blue stained photomicrographs revealed dog to have the largest number of cells in its central nucleus. The soma size of neurons was largest in dog and

least in goat. However, the goat had the most elaborate dendritic aborization, followed by dog. Sheep had the least of cell count and dendritic aborization. The central nucleus of the amygdala (CE) is the interface with motor systems. It receives inputs from the basolateral complex (Pitkanen *et al.,* 1997). Damage to CE interferes with the expression of fear responses (Gentile *et al.,* 1986; Hitchcock and Davis, 1986; Iwata *et al.,* 1986; Kapp *et al.,* 1979; Van de Kar *et al.,* 1991). Since sheep had the least of cell count and dendritic aborization in its central nucleus, this could be responsible for its slow response to threats and danger when compared with the dog. Also, dog had the largest number of cells in its central nucleus and responds faster to threats and danger.

CHAPTER SIX

6.0 Summary, Conclusion, and Recommendation

6.1 Summary

Sheep had the largest brain size, greatest brain volume, and the most number of gyri and sulci on its cerebrum. Yet, dog responds faster to threats and danger than sheep. Although goat had the largest soma size and greatly ramified dendritic aborization of layer V cells of the prefrontal cortex which is involved in higher cognitive functions, dog is still quicker than the goat when it comes to responding to threatening situations. This means that other factors could be responsible for higher cognitive function in dogs than in goats. Also, considering the longer cerebral length of dog, this explains why the density of neurons in the prefrontal cortex were most in dog since Kaas (2000) listed increase in the density of neurons as one of the factors relating to changes in certain brain parameters.

Dog CA3 cells had the most density of neurons. Studies have shown the CA3 cells to be responsible for long term memory and learning. Hence, more neurons in the CA3 cells recieve sensory inputs from the prefrontal cortex of dog. Moreover, the dentate gyrus of dog at some coronal sections had clusters of cells with mossy fibres around its loop which project numerous inputs to the CA3 cells. That meant that more projections would be sent to dog’s CA3 cells than the sheep and goat from the dentate gyrus. Since the CA3 cells are responsible for memory and learning, this could explain why a dog “remembers” a threatening situation and responds faster than the sheep and the goat.

All sensory inputs to the amygdala terminate mainly in the basolateral complex of the amygdala. When a conditioned sensory input reaches the thalamus, it could be projected to the amygdala directly (short route of fear), or first to the sensory cortex, then the hippocampus (which is responsible for memory and learning) which in turn projects to the basolateral complex of the amygdala, which then projects to the central nucleus of the amygdala (CA) which is the brain region that generates fear responses (shin, 2012). Hence, the basolateral complex serves as an important link in the pathway of fear. The lateral nucleus of the basolateral complex project sensory inputs to the central nucleus or the basal nucleus, depending on the pathway of fear (long route or short route) and the type of sensory stimuli (conditioned or unconditioned stimul), so as to bring about a quicker fear response (shin, 2012; Trepeta, 2008). There are more cells and greater dendritic arborization of the basolateral complex of dog. This could be responsible for dog's quick reception of sensory stimuli relating to the emotion of fear and prompt response to a threatening situation than goat and sheep. Hence, sheep could be described as "stupid" since it does not quickly evade a threatening situation. The central nucleus brings about fear response (Gentile *et al.,* 1986; Hitchcock and Davis, 1986; Iwata *et al.,* 1986; Kapp *et al.,* 1979; Van de Kar *et al.,* 1991). In the central nucleus of dog, the soma diameter was the largest and the density of neurons was the greatest. Goat had the most elaborate and greatly ramified dendritic arborization. Sheep had the least of dendritic aborization and cell count in its central nucleus. This could be the reason for the slower response of sheep to threats and danger than dog. Also, although goat had the most number of cells in its central nucleus, dog still responds faster to threats and danger, followed by goat, and then sheep. This shows that other factors, such as better olfaction and vision,

could be involved in dog considering the smaller brain size and volume, and less intricate gyrification of the cerebrum of dog in comparison with the sheep and goat.

6.2. Conclusion

Structural differences in the structures of the fear circuitry of sheep, goat, and dog exist and could be related to their behavior or functional differences. Dog, although the smallest in brain size and volume, it had the greatest cerebral length and the most density of neurons in its prefrontal cortex (which is involved in higher cognitive function), hippocampus (which is responsible for higher cognitive function), and central nucleus of the amygdala (which plays a role in the expression of fear response). This could be the reason for the dog's quick response to threats and dangers. Goat had the most dendritic arborization of its layer V cells of prefrontal cortex and central amygdala neurons, and spinous stellate neurons in its basolateral complex. This explains why the goat is faster than sheep when it comes to responding to threats and danger but slower than dog. Sheep had the largest brain size and volume and more intricate gyrification, yet there was decreased density of neurons in its hippocampus and relatively fewer cells in its prefrontal cortex, and the least number of central nucleus cells. This could be the reason for the slow response of sheep to threats and danger.

6.3 Recommendations

i. Cytoarchitectural and chemoarchitectural studies including neuronal tracing on prefrontal cortex, hippocampus, and amygdala of dog, goat, and sheep should be carried out for a better understanding of these structures of fear circuitry.

ii. Other structures of the fear circuitry such as the thalamus, septum, and brainstem should be compared in dog, goat, and sheep.

iii. The depth of gyri and sulci in the cerebrum of sheep, goat, and dog should be studied

iv. Other structures of the fear circuitry such as the thalamus, septum, and brainstem should be compared in dog, goat, and sheep.

v. Studies should include other breeds of domestic animals.

vi. The immunoreactivities of the prefrontal cortex, hippocampus, and amygdala of sheep, goat, and dog with Calbidin D28k should be carried out

vii. The olfaction and vision of sheep, goat, and dog should be studied and compared as this could play a role in their behavioural differences when it comes to responding to threats and danger.

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