International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Neuroprotective Effect of Chlorpromazine on Hippocampus of Ketamine-Induced Psychotic Rat Model

Elizabeth Finbarrs-Bello[1,2], Ignatius Ikemefuna Ozor[1], Vivian Onyinye Atuadu[1], Epete Michael Arinze[2]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology, Parklane Enugu State. Nigeria

[2] Department of Anatomy, Faculty of Basic Medical Sciences, Ebonyi State University. Abakaliki. Ebonyi State. Nigeria

Abstract: ***Ketamine has been known to produce psychotic symptoms like delusion and hallucination in rodents and humans. Chlorpromazine (CPZ) a dopamine receptor antagonist of the typical anti-psychotic drug is used to treat schizophrenia. This study investigated the neuroprotective effect of chlorpromazine on hippocampus of ketamine-induced psychotic rat model. Twenty (20) rats were divided intoexperimental and control groups. The control (n=5) received 0.1ml saline (p.o) while psychosis was induced in experimental rats (n=15) using 25mg/kg of ketamine (i.p) for 7days. On the eight (8th) day the psychotic model rats (n=15) were subdivided into three groups 1, 2and3of five ketamine induced rats each.The control and Group 1 were sacrificed; then groups 2 and 3 were treated with 25mg/kg of chlorpromazine (p.o) and 0.1ml of saline (p.o) respectively for 21days. On the 22nd daythe rats were anaesthetized by 50mg/kg of thiopental sodium, aortic perfusion was performed and the brain was dissected. Deparaffinized tissue sections were processed and stained. The H & E stained section of the control group showed normal neurons, untreated model shows features of cytoplasmic vacuolation, and chlorpromazine treated model showed relatively normal neuronal cells, while the saline treated model group showed shrunken cytoplasm.TheGolgi silver stained hippocampal sections revealed numerous granular cells with accumulation of silver deposit in the untreated model, chlorpromazine treated model had few granular cells with silver depositsand Saline treated model shows cell with moderate granular cells with silver deposits. The Nissl bodies stained by Cresyl fast violet method decreased in the untreated and saline treated models, while itincreased in the model treated with chlorpromazine. This result put together we conclude that chlorpromazine an antipsychotic drug has neuroprotective effect on the hippocampus in ketamine-induced psychosis in rat.***

Keywords: Ketamine, Psychosis, Chlorpromazine, Hippocampus**,** Nissl Bodies, Silver Stain

1. Introduction

1.1 Background of the Study

Psychosis is a mental state often involving loss of contact with reality. The commonest form of psychosis is schizophrenia [1]. Sufferers of psychosis exhibit personality cognitive and thought disorders and so described as being psychotic [2]. The assessments of psychosis in a client or patient require extensive physical, laboratory and sometimes neuro imaging evaluations [3]. This is because the causes are difficult to elucidate, beside certain genetic predispositions as in the case of schizophrenia. The most prevalent etiological factor is linked to societal indulgence in substance abuse also termed toxic or substance-induced psychosis [4]. The substances include agents such as cocaine, cannabis, amphetamine and ketamine used by the young and old in different parts of the world as recreational drugs [3].These agents are capable of inducing the negative and positive symptoms of the disorder in rodents and humans [1], [5].

Ketamine is anN- methyl-D-aspartic acid(NMDA) receptor antagonist but also acts on numerous other receptor sites including, opoid receptor and monoamine transporters. It is a classified anaesthetic and dissociative agent [4], [5]. Its subanesthetic doses have been known to induce the symptoms of schizophrenia in healthy volunteers and increase susceptibility in patients [6, 7]. Schizophrenia and ketamine-induced psychotic symptoms are the same, their neurobiological mechanisms and pharmacological therapies employed in their treatment are also the same apart from hospitalisation [7]. The major therapy is the use of antipsychotic medications such as clozapine and chlorpromazine [8].

Chlorpromazine is a first generation antipsychotic [7, 9]. It is an antagonist of the D2 dopamine receptor. In addition to its antipsychotic action on this receptor, Chlorpromazine also possesses anti-adrenergic, anti-serotonergic, anti-cholinergic and anti-histaminergic properties and used to treat schizophrenia [10]. This agent treats the physiological and cognitive defects seen in the disorder which majorly affects function of specific areas of the limbic system like the amygdala and hippocampus [11].

The hippocampus is a major component of the human brain and that of other vertebrates, principally responsible for the consolidation of information from short term memory to long term memory and spatial navigation[12]. Structural modifications in neurons, glia and neurochemicals in this area underlie the specific functional defects observed in schizophrenia or ketamine- induced psychotic symptoms in human and rodent models of the disease [13]. Likewise, the therapeutic responsiveness of the specific and/ or collective areas of the limbic system. For example, the effects of conventional antipsychotics on the hippocampus and amygdala in patients and animal models of psychosis. The neuropharmacological mechanisms involved in psychosis including schizophrenia and the specific

Licensed Under Creative Commons Attribution CC BY

pharmacotherapeutic agents (antipsychotic) have been the focus of previous studies [14].This present study is a histological perspective on the therapeutic effect of an antipsychotic therapy (chlorpromazine) on the hippocampus in induced psychosis. Consequently, it provides insight on the target areas for preclinical psychiatric drug research or discovery.

2. Materials and Method

2.1 Drugs and Chemicals

2.1.1 Drugs

Chlorpromazine hydrochloride tablets were used as a standard reference antipsychotic drug while, Ketamine hydrochloride injection (Rotex Medical Germany) was used to induce psychosis in the rat's model. The drugs were purchased from a standard and registered pharmacy in Enugu. Nigeria. The doses of drugs were selected based on data from literature [6] and drug information leaflets. All reagents used in the study are of standard analytical grades.

2.2 Animal Procurement

Twenty (20) adult wistar rats of both sexes, average weight of 180g were purchased from the animal house of the Department of Physiology, College of Medicine University of Nigeria. Enugu Campus and housed at the animal facility of Anatomy Department, Ebonyi State University. The animals were housed in netted iron cages in group of five, fed with grower's mash and given water ad libitum. The rats were maintained under laboratory conditions (temperature 24±2+oc, with relative humidity 60-70% and a 12-hour light-dark cycle). The animals were also acclimatized for two weeks before the experiment.

2.3 Ethical Statement

The experiment procedures and techniques used in the study were in accordance with accepted principles for laboratory animal use and care by NIH, 1985 and EU directive of 1989:86/609/EEC. All protocols used were approved by the Research Ethic Committee of Faculty of Medicine Ebonyi State University with certificate number EBSU/FREC/MPC14/10.

2.4 Treatment of Animals

Twenty (20) rats were divided intocontrol and experimental groups.Psychosis was induced in the experimental group(n=15) using25mg.kg,-1 ketamine hydrochloride per body weight intraperitoneally (I.p), for 7days. The control (n=5) received 0.1ml saline (I.p) for 7days. The symptoms observed were side to side head rocking and continuous staggering locomotion.

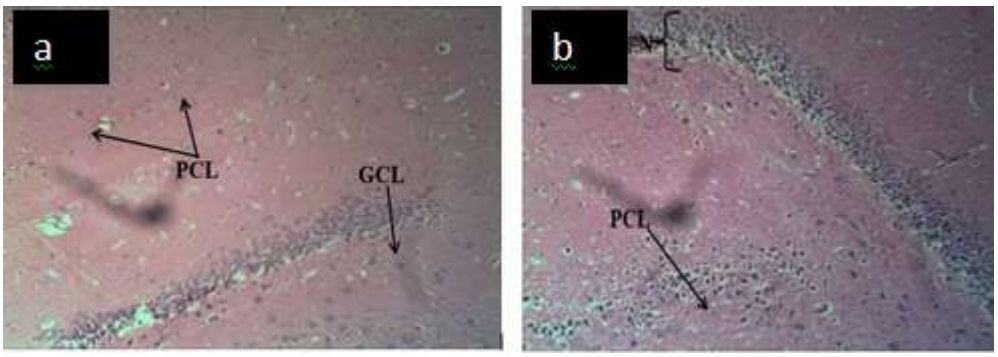
On the eight (8^{th}) day the experimental group (n=15) were further divided into three groups 1, 2 and3 (n=5 each).Group 1 and the control were sacrificed on day eight of the study. Whilegroups 2and3 were treated with 25mg/kg of chlorpromazine and 0.1ml of saline orally respectively for 21days. On twenty-ninth day (i.e. day 22 post induction of psychosis) of the study rats in groups 2 and 3 were anaesthetized with 50mg/kg/ body weight of thiopental sodium injection, aortic perfusion-fixation using 4%paraformaldehyde via the heart was performed on the rats. The brain was dissected out and further fixed in 10% paraformaldehyde overnight for histological studies.

2.5 Histological Studies

Fixed specimens were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serials sections of 10um thick were obtained using a rotatory microtome. The sections were deparaffinized and stained using haematoxylin and eosin and Golgi silver method to stain neuronal architecture while Nissl bodies was stained using the Cresyl fast violet method both stain purple-blue and deep-brown or yellow respectively. Photomicrograph was captured by Amiscope research microscope model 3 at the Biotechnology Centre Ebonyi State University

3. Results

3.1 Histological Findings



Licensed Under Creative Commons Attribution CC BY

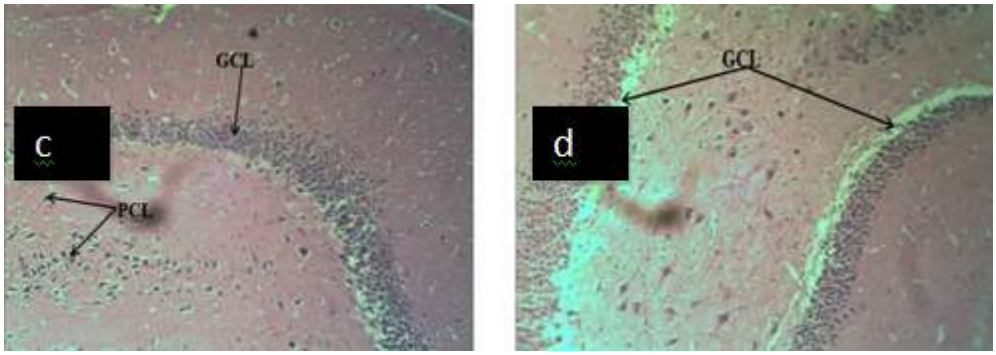


Figure 1: Photomicrographs of hippocampus of rat (**a**) control, molecular layer shows normal neuronal cells with cytoplasm and centric nuclei in the pyramidal cells (PCL) and granular cell layers (GCL). **(b)**Ketamine untreated model shows cytoplasmic vacoulations and eccentric nucleic (Arrows) inpyramidal cells (PCL).**(c)**Chlorpromazine treated model shows normal neurons in the granule layers (GCL) and Pyramidal Cells (PCL). (**d**) Saline treated model shows few neurons with shrunken cytoplasm and eccentric nuclei in granular cell layer (GCL). **H&E.x200**.

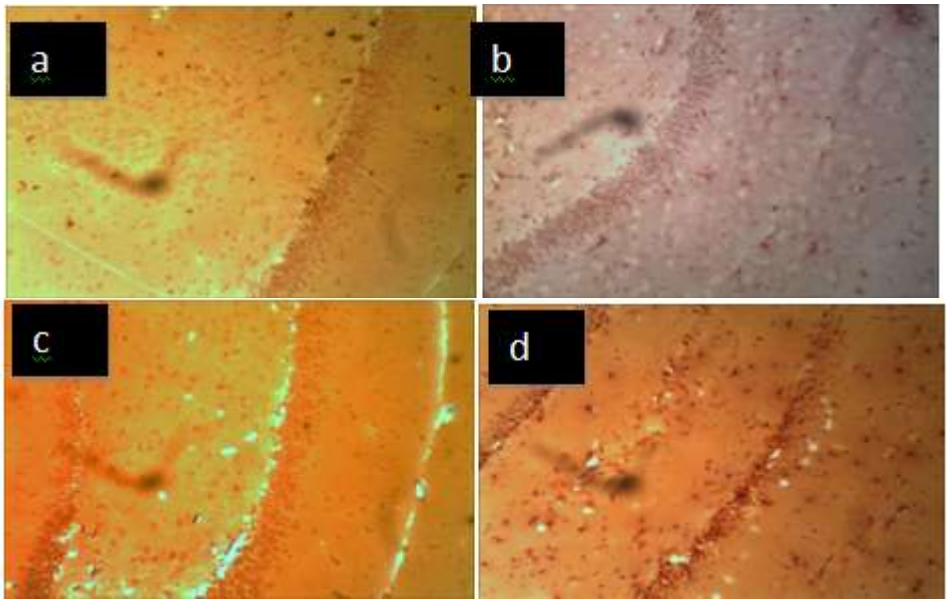


Figure 2: Photomicrographs of the Hippocampus of rat **(a)**control group showed positive cells and granular cell silver deposit **(b)**Ketamin**e** untreated model showed positive cells with numerous granular cell accumulation of silver deposit **(c)**Chlorpromazine treated model shows positive cell with few granular cell accumulation of silver deposit (**d)**Saline treated model shows positive cell with moderate granular cells accumulation of silver deposit. **Golgi SilverMethod.x200**.

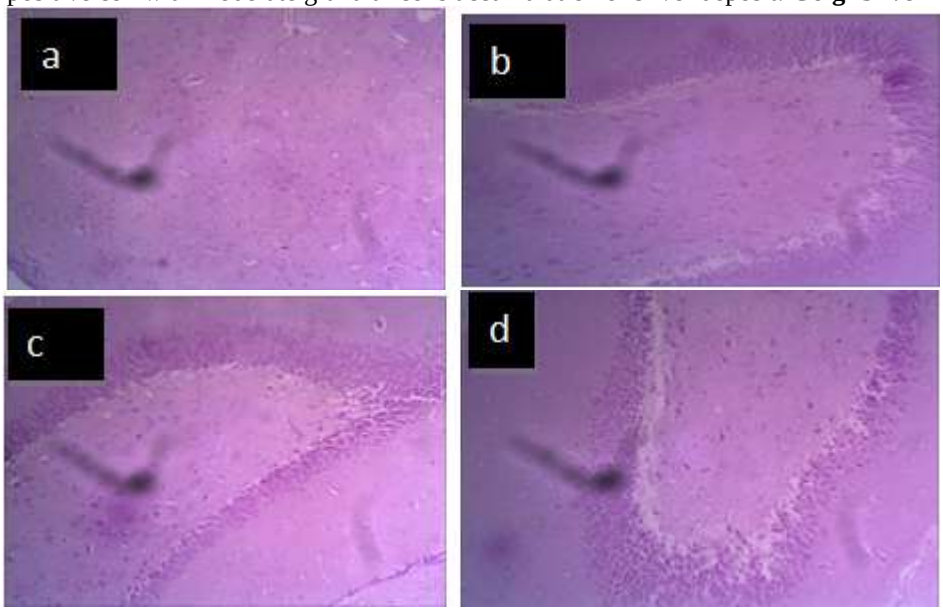


Figure 3: Photomicrographs of the Hippocampus of rat (**a**) Control shows normal Nissl bodies'.**(b)**Ketamine untreated modelshows decrease stain affinity in hippocampal neuron. **(c)**Chlorpromazine treated model shows increased staining in granular and pyramidal layers.**(d)** Saline treated model shows decreased stain for Nissl bodies. **Cresyl Fast Violet. x200.**

Licensed Under Creative Commons Attribution CC BY

4. Discussion

Basically, normal neurons have cytoplasms with centrally located nuclei as found in any other cells. Structural dis-arrangement, disorganization or displacement of cell components signifies some sort of cell damage. In the present study, hippocampal section of the control revealed normal neurons with centrally placed nuclei within their cytoplasm (figure 1a) but, untreated model induced with 25mg/kg of ketamine showed cytoplasmic vacoulations and eccentric nuclei (figure1b). While the treated group which was induced with 25mg/kg ketamine and treated with 25mg/kg of chlorpromazine showed relatively normal neuronal cells compared to control and ketamine induced groups. Likewise the group induced with ketamine and treated with 0.1ml saline showed cytoplasmic vacoulations and eccentric nuclei compared to the chlorpromazine groups.

Inconsistent with previous studies, cytoplasmic vacoulations and pyknosis entail signs of neurotoxicity or neurodegeneration[6], [11]. The ketamine induction causes neuronal degeneration in the hippocampus. This was restored by chlorpromazine treatment, but the saline treatment, produces less pronounced reversal effect on the hippocampus. Its treatment outcome was attributed to ketamine withdrawal effect and not the saline treatment [12]. By contrast, chlorpromazine had reversed the neurotoxic and degenerative effect of ketamine.

In addition, the control group showed negative silver stain with no granular cells accumulation of silver deposit (figure 2a). On the contrary, untreated models showed silver positive cells with numerous granular cells rich of silver deposits (figure 2b). The models treated with chlorpromazine shows silver positive cell with scanty cells containing silver deposit (figure 2c).However, saline treatment fails to provide reasonable effect (figure2d).Silver stain is known to detect protein, but many factors influence the colour intensity, and every protein has its own characteristics which is not limited to purity of reagents and water [15]. In addition, histologically, silver impregnation is one of the techniques that can detect degeneration in neurons [16]. Within the present study, chlorpromazine treatment provided a curative or protective effect than saline going by their differential staining effect and intensity. These indicate that degeneration of neurons by ketamine can be reversed by chlorpromazine. This finding also agrees with the result observed in the H&E stained sections of the corresponding groups. The chlorpromazine actually elicited some kind of treatment effect in reversing the ketamine effect.

The study also investigated the effects on the distribution and staining intensity of Nissl bodies. Normally, Nissl bodies are evenly distributed in the soma and proximal dendrite of neurons. They are seen as basophilic material in pyramidal cells and dispersed in powdered form at other sites. However, their visibility, size, form and distribution vary greatly in neurons. These variations are important in neuropathology [17]. The decrease Nissl stain in the ketamine untreated model (figure 3b) confirmed the earlier neurotoxic effect of ketamine reported in the H and E stain (figure 1b). This is a fact when the cell bodies and dendrites are severed thus, Nissl bodies disappear (chromatolysis). Then deeply basophilic material becomes diffused in the cytoplasm as it attempts to repair the lost neurons [15].The decreases in the number of nissl bodies in neurons indicate neurodegenerative process, as occur in inflammatory diseases, oxygen deprivation or trauma [6].But, increased Nissl stain in the chlorpromazine treated group (figure 3c) depicts the ketamine reversal effects observed in the Hand E and silver stains. Notably, Nissl bodies are synonymous to the rough endoplasmic reticulum, as sites for production and release of chemical substance such as proteins and peptides. These form building blocks for neurotransmitters and neuromodulators [18],[19]. Hence, Chlorpromazine might have stimulated neuronal secretion of neurotransmitters such as glutamate and/or GABA.

Withdrawal of ketamine by saline treatment didn't resolve or reversed the neuronal damage induced by ketamine (figure 3d). Ketamine inductions of the disease (psychosis) must have decreased the level of neuronal synthetic activity such as neurotransmitter such as glutamate. But, upon administration of the treatment chlorpromazine increased synthetic activity, culminating into enhanced release of neurotransmitter like GABA in the respective neural pathway(s). This shows reciprocal modulation of neurotransmitter synthesis by the Nissl bodies (endoplasmic reticulum) in the soma of the neurons in order to correct or balance transmitter defect in the ketamine-induced psychosis.

5. Conclusion

The findings in this study showed that induction of psychosis using ketamine caused neuronal degeneration which was restored by chlorpromazine treatment and not saline in the hippocampus. These will affect neurons response in the synthesis of neurotransmitters, learning and memory in/of the hippocampus but chlorpromazine provided neuroprotective effect.

References

[1] P. Tanveer, A. Udyawar,. N. Azhar, C. Pantelis, D. Velakoulis, PD. McGorry, SJ. Wood, SJ. J. Suckling,LJ. Phillips, AR. Yung, ET. Bullmore,W. Brewer, B. Soulsby, P. Desmond, PK. McGuire. "Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison", Lancet, (25), pp. 281–8, 2003.

[2] T. R. E. Barnes, M. J. Drake, C. Paton, "Nocturnal enuresis with antipsychotic medication", The British journal of psychiatry, (1), pp. 87-89, 2010.

[3] A.U Freudenreich, Oliver. "Differential diagnosis of psychotic symptom: medical mimics, Psychiatric times UBM Media.

[4] SA. Bergman, Ketamine: 'Review of its pharmacology and its use in pediatric'' The American journal of pediatric psychiatry, pp. 124-132, 1999.

[5] H.A Adams. "Ketamine. Circulatory interactions during total intravenous anaesthesia and analgesia-sedation", Der Anaesthetist, 46(12), pp. 1081–7, 1997.

[6] FB.Elizabeth, E.N.Obikili, E. A E Esom, G.E Anyanwu. "Curative effect of aqueous leaf extract of crinumgiganteum on NMDA-Receptor Antagonist-

Licensed Under Creative Commons Attribution CC BY

induced Schizophrenia Wistar Rat Model". Macedonian journal of medical sciences.4(3);337-341.2016
[7] Bertram G.K, Serzan B. M, Anthony T.T., "Basic and Clinical pharmacology".11th International ed, McGraw Hill Companies inc. 371-509. 2009
[8] R.Y Cho, Gilbert, A. Lewis, DA. "Ch 22 the neurobiology of schizophrenia". In Charney DS, Nestler EJ. *Neurobiology of Mental Illness*. Oxford University Press US., 2005.
[9] Y. Daisy. "Throughout History. Defining Schizophrenia Remains A Challenge", Scientific America Mind, 2013.
[10] H, David. "The creation of psychopharmacology" Cambridge Harvard University press, 2004.
[11] P. Boyer, J.L., Phillips, F.L Rousseau. "Hippocampal abnormalities and memory deficits: new evidence of a strong pathophysiological link in schizophrenia", Brain Research Rev, 54 (1), pp. 192–112, 2007.
[12] M.Chevallet, S. Luche, T. Rabilloud."Silver staining of protein in polyacrylamide gels". Nat. Protoc 1(4),pp 1852-1858.2006.
[13] H .T. Richard. "The brain: A Neuroscience Primer," Macmillan, pp 35. 2013
[14] EC. Brown, C. Tas, M. Brune, "Potential therapeutic avenues to tackle social cognition problems inschizophrenia", Expert Rev Neurother, 12 (1), pp. 71-81, 2012.
[15] S. J. Baloyannis. "Staining neurons with Golgi techniques in degenerative diseases of the brain". Neural. Regen. Res. 10(5). pp 693-695.2015.
[16] K. R. Ibrahim, Z.E. M Hala, "Histological changes of the adult Albino rat's entrohinal cortex under the effect of tramadol administration: Histological and Morphometric study". Alexandria journal of medicine 53(2), pp123-133. .2017
[17] B. L., McNaughton, L. L., Chen, E. J. Markus. "Dead reckoning," landmark learning, and the sense of direction", A neurophysiological and computational hypothesis. J. Cogn.Neurosci, 3, pp. 190-202, 1991.
[18] R. Kohrs, and M.E., Durieux. "Ketamine: Teaching an old drug new tricks", Anesthesia & Analgesia, 87 (5), pp. 1186–93, 1998.
[19] L. Brunton, B. Chabner, B. Knollman, Goodman and Gilman's. "The Pharmacological Basis of Therapeutics", 2010.

Author Profile

ElizabethFinbarrs-Bello obtained B.Sc (2002), M.Sc (2008), Ph.D (Neuroanatomy, 2016) from the Universities of Maiduguri, Calabar and Nigeria. She is a lecturer and researcher at the Department of Anatomy, Faculty of Basic Medical Sciences. College of Medicine, Enugu State University of Science and Technology. She has lectured also at Ebonyi State University, Abakaliki**.** Her core areas of pre-clinical and clinical research interests include neuroscience, cellular and molecular histology, neurohistochemisty and plant targets for drug discovery**.** This has reflected in her publications.

Ignatius Ikemefuna Ozor (MBBS, M.Sc, FWACS (Neurosurgery). He is a lecturer in both Anatomy and Surgery departments of theCollege of Medicine. He is also a doctorate student of the Anatomy department. Enugu State University of Science and Technology. A consultant neurosurgeon at the Enugu State University Teaching Hospital. He is strongly involved in preclinical and clinical researches on Central nervous system disorders.

Vivian Onyinye Atuadu(B.Sc, M.Sc) is a young Assistant lecturer in the Department of Anatomy, Faculty of Basic Medical Sciences. College of Medicine.Enugu State University of Science and Technology. She has been teaching since the year 2015 till date. Her special interest is in Developmental Anatomy and Neuroanatomy,which has reflected in her research publications.

Epete Michael Arinze(B.Sc, M.Sc Ebonyi) is a young Research Assistant in the Department of Anatomy, Faculty of Basic Medical Sciences. College of Medicine. Ebonyi State University. He has been involved in undergraduate, postgraduate and departmental researches. He is specially interested Neuroanatomy and has research publications in this area.

Licensed Under Creative Commons Attribution CC BY