NEURODEGENERATION AND BEHAVIOURAL EFFECT IN KETAMINE AND METHAMPHETAMINE IN RATS

BY

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ABSTRACT

Ketamine and methamphetamine (METH) are increasingly becoming a popular choice of drugs among drug abusers. The abuse has reached epidemic proportion worldwide. Prolong exposure to these drugs is thought to cause neurodegeneration resulting in loss of hippocampal neurons and functions. This study examined the neurodegenerative effect of ketamine and METH on adult rats' hippocampus, its relation to exploratory behaviour in different doses and duration of exposure and correlation between neuron CA1 and CA3. Fifty-five Sprague-Dawley rats (male, 4 weeks old, and 150-200g) were divided into acute (1 day) and chronic (5 days) drug treatment groups. The acute treatment groups were treated with different doses of ketamine (10 mg/kg, 20 mg/kg and 50 mg/kg, n=5 per subgroup) and methamphetamine (5 mg/kg and 10 mg/kg, n=5 per subgroup) for 4 injections at 2-hour intervals. The same protocol was repeated for 5 consecutive days in the chronic regimen. Behavioural test was performed using hole-board maze (16 holes, 40 cm x 40 cm, flexiglass) under well controlled condition for 5 minutes at well-define time points. The rats were sacrificed 12 hours after the last exposure to the drugs. Viable neuronal cell count was performed in CA1 and CA3 regions of the hippocampus on cresyl violet stained sections (Image J Software; 0.48 mm^2 areas). Correlation between the number of CA1 neurons and CA3 neurons was analyzed using Pearson correlation coefficient test. Linear regression was used to analyze the causality effect of CA3 to CA1 neurons. The result showed that the acute and chronic ketamine did not show significant neuronal reduction in CA1 region. Acute METH treatment groups, 5mg/kg and 10 mg/kg with mean value 80 cells ± 5.10 per 0.48 mm^2 and 78 cells ± 6.20 per 0.48 mm^2 respectively caused significant CA1 region neuronal loss. Similar reduction was observed in chronic METH regimen, with mean value for 5mg/kg and 10mg/kg were 66 cells ± 6.18 per 0.48 mm^2 and 61 cells ± 5.63 per 0.48 mm^2 respectively. CA3 region neuronal count was significantly reduced in acute ketamine (50 mg/kg; 153 cells ± 5.59 per 0.48 mm^2) and acute METH (5 mg/kg; 120 cells ± 7.18 per 0.48 mm^2, 10 mg/kg; 107 ± 7.79 per 0.48 mm^2) treatment groups. All treatment groups for the chronic regimen demonstrated significant CA3 region neuronal count reduction. For exploratory behaviour, head dipping data was considered as unreliable. Statistical analysis was done with ANOVA with post-hoc analysis and the differences are significant at $p < 0.05$. A positive correlation was observed between CA1 and CA3 region neuron counts in chronic Ketamine ($F=8.341$, $p=0.010$), acute ($F=95.076$, $p=0.000$) and chronic METH ($F=288.434$, $p=0.000$) regimen. These data demonstrated that only high dose of acute 50 mg/kg and chronic doses of ketamine caused neurodegeneration in CA3 region with no significant results for both regimens in CA1 region while both METH regimens exhibited significant neuronal degeneration in CA1 and CA3 regions of hippocampus.

خلاصة البحث

الكيتامين والميثامفيتامين يتزايد خيارا شعبيا للمخدرات بين متعاطي المخدرات. وكثيرا ما يرتبط الحصين في الذاكرة والسلوك. سعت الدراسة الحالية لدراسة تأثير الاعصاب الكيتامين والميثامفيتامين في قرن آمون الفئران البالغين وعلاقتها استكشافية السلوك. لمجموعة الحادة ، وكانت تدار الكيتامين والميثامفيتامين intraperitoneally في سبراغ داولي الفئران ، (4 أسابيع من العمر ، 150 - 200 (Gعلى جرعات مختلفة (10 mg/kg ، 20 mg/kg و50 mg/kg لالكيتامين: 5 mg/kg و10 mg/kg لالميثامفيتامين) أربع مرات يوميا مع فاصل 2 ساعة. وتكرر نفس البروتوكول بعد 5 أيام في فوج المزمنة. تم حقن مجموعة المراقبة مع المالحة. الفئران ثم تعرض لثقب متن الاختبار (40 (cmx40cmلمدة 5 دقائق استكشافية لمراقبة السلوك. سجلت اثنين من المحققين المستقلين إلى أن عددا من العد غمس الرأس. وقد ضحى مزيد من الفئران بعد 12 ساعة عقب التعرض الماضي. وتم التحقيق في تنكس عصبي الحصين عن طريق حساب عدد الخلايا العصبية CA1 وقابلة للحياة CA3 باستخدام صورة البرنامج .J وقد استخدم في اتجاه واحد أنوفا مع مرحلة ما بعد التحليل مخصص لتحليل البيانات. وجرى تحليل العلاقة بين الخلايا العصبيةCA1 ، CA3الخلايا العصبية ورئيس غمس باستخدام اختبار معامل ارتباط بيرسون. واستخدمت انحدارات خطية متعددة لتحليل تأثير سببية CA1 و CA3الخلايا العصبية لرئيس السلوك غمس. وأظهرت النتيجة أن الكيتامين الحاد (10 80 mg/kg خلايا لكل 0.48 (15.50 ± mm2 والميثامفيتامين 10 mg/kg الحادة (74 في الخلايا 0.48 (15.95 ± mm2 تسبب خسارة كبيرة CA1 العصبية. لمجموعة المزمنة ، 5 mg/kg الميثامفيتامين والميثامفيتامين 10 mg/kg أظهرت انخفاضا كبيرا مع قيمة الخلايا العصبية 67 CA1 خلايا لكل 0.48 9.05 ± mm2 و 73 في الخلايا 0.48 7.40 ± mm2 على التوالي. وكان لسيطرة مجموعة CA1 عدد الخلايا العصبية في 0.48 .15.69 ± 141 mm2 وكان عدد الخلايا العصبية CA3 خسارة كبيرة في 50 mg/kg الكيتامين الحادة (92 في الخلايا 0.48 ± mm2 (7.54والميثامفيتامين 10 mg/kg الحادة (78 في الخلايا 0.48 .(4.28 ± mm2 أظهر جميع الفئات العلاج للحد من فوج كبير العصبية المزمنة CA3. 10 mg/kg الميتامفيتامين مزمن له القيمة من 68 خلايا لكل 0.48 6.60 ± mm2 ، تليها 10 mg/kg الكيتامين المزمن (78 في الخلايا 0.48 (7.32 ± mm2 ، والمزمنة الكيتامين 20 mg/kg (82 خلايا لكل 0.48 (8.30 ± mm2 ، والمزمنة الميثامفيتامين 5 mg/kg (82خلايا لكل 0.48 (4.43 ± mm2 والكيتامين المزمنة 50 (94 خلايا لكل 0.48 mm2 .(6.38 ±لمجموعة المراقبة ، وكان عدد الخلايا العصبية 145 CA3 خلية لكل 0.48 .17.05 ± mm2 استكشافية للسلوك ، تم تخفيض رأس غمس فقط في 5 mg/kg الميثامفيتامين الحاد (1 مرة في 5 دقائق ± 0.4) بينما في نظام المزمنة ، لوحظ انخفاض في 10 mg/kg الميثامفيتامين المزمن 0 مرة في 5 دقائق ± 3.18. جميع البيانات الواردة أعلاه مهم مع ف القيمة أقل من 0.05. أظهرت علاقة ارتباط إيجابية بين) CA1 ص - 0.656 ، (P = 0.008،) CA3ص - 0.849 ، (P = 0.000بحساب عدد الخلايا العصبية مع غمس الرأس في نظام الميثامفيتامين المزمنة. نتائج الانحدار المتعدد أثبتت أن جرعة الميتامفيتامين في نظام المزمن كان مؤشرا كبيرا لسلوك رئيس غمس(F = 16.741 ، P = 0.000 ، .(R2 = 0.736

ABSTRAK

Ketamin dan methamphetamin (METH) semakin menjadi pilihan popular dadah di kalangan penagih dadah. Penyalahgunaan telah mencapai tahap epidemik di seluruh dunia. Pendedahan berpanjangan kepada ubat-ubatan ini yang dianggap penyebab kepada neurodegenerasi menyebabkan kehilangan neuron dan fungsi hippokampus. Kajian ini mengkaji kesan neurodegeneratif ketamin dan METH kepada hippokampus tikus dewasa kaitannya kepada tingkah laku penerokaan dalam dos dan tempoh yang berlainan serta korrelasi antara neuron CA1 dan CA3. Lima puluh lima ekor tikus Sprague-Dawley (jantan, berusia 4 minggu, dan 150-200g) telah dibahagikan kepada kumpulan-kumpulan dadah akut (1 hari) dan kronik (5 hari). Kumpulan rawatan akut dirawat dengan dos yang berbeza ketamin (10 mg / kg, 20 mg / kg dan 50 mg / kg, n = 5) dan methamphetamine (5 mg / kg dan 10 mg / kg, n = 5) untuk 4 suntikan pada selang 2-jam. Protokol yang sama diulang selama 5 hari berturut-turut dalam regimen yang kronik. Ujian Tingkah Laku dilakukan menggunakan lubang maze (16 lubang, 40 cm x 40 cm, flexiglass) di bawah keadaan yang dikawal dengan baik selama 5 minit pada masa yang ditentukan. Tikus-tikus dikorbankan 12 jam selepas pendedahan terakhir kepada dadah. Kiraan sel neuron hidup dilakukan di kawasan hippocampus CA1 dan CA3 yang diwarnakan dengan cresyl violet (Imej J Perisian; 0,48 mm2 kawasan). Korelasi antara bilangan CA1 neuron dan CA3 neuron dianalisis menggunakan ujian pekali korelasi Pearson. Berbilang terurus linear telah digunakan untuk menganalisis kesan sebab dan akibat CA1 dan CA3 neuron untuk mengetuai tingkah laku mencelup. Hasilnya menunjukkan ketamin akut dan kronik tidak memberi kesan kepada jumlah neuron CA1. Manakala kumpulan akut METH, 5mg/kg (80 sel ± 5.10 setiap 0.48 mm^2) dan 10mg/kg (78 sel ± 6.20 setiap 0.48 mm^2) masing-masing menunjukkan kehilangan neuron yang ketara di kawasan CA1. Begitu juga dengan kumpulan kronik METH, pengurangan ketara dikesan (5 mg/kg; 66 sel ± 6.18 setiap 0.48 mm^2, 10 mg/kg; 61 sel ± 5.63 setiap 0.48 mm^2). Bagi kawasan CA3, dalam kumpulan ketamin akut (50 mg/kg; 153 sel ± 5.59 setiap 0.48 mm^2) dan METH akut (5 mg/kg; 120 sel ± 7.18 setiap 0.48 mm^2, 10 mg/kg; 107 sel ± 7.79 setiap 0.48 mm^2). Semua kumpulan rawatan untuk regimen kronik menunjukkan pengurangan ketara neuron CA3. Untuk tingkah laku penerokaan, perbandingan tidak dilakukan kerana data tidak relevan. Analisis statistik telah dilakukan dengan ANOVA dengan analisis post-hoc dan perbezaan dianggap signifikan pada $p < 0.05$. Korelasi positif ditunjukkan antara neuron kawasan CA3 dan CA1 dalam kumpulan dalam ketamine kronik, akut dan kronik METH. Keputusan regresi linear menunjukkan bahawa kiraan neuron CA3 adalah peramal signifikan dalam regimen ketamine kronik ($F=8.341$, $p=0.010$),, akut ($F=95.076$, $p=0.000$) dan kronik METH ($F=288.434$, $p=0.000$). Data-data ini menunjukkan bahawa hanya dos yang tinggi akut 50 mg / kg dan kronik ketamin menyebabkan neurodegenerasi dalam kawasan CA3 serta tiada kesan ditunjukkan dalam kedua-dua rejimen di rantau CA1 manakala kedua-dua rejimen METH mempamerkan degenerasi neuron yang ketara dalam CA1 dan CA3 hippocampus.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Medical Sciences.

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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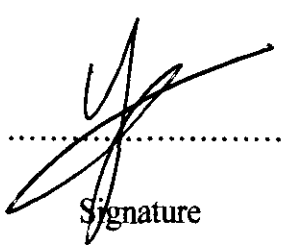

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Date

I would like to dedicate this research to my family and all Muslim ummah especially

in Palestine, Iraq, Kashmir, and Egypt

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LIST OF ABBREVIATIONS

Ak10	Acute ketamine 10 mg/kg
Ak20	Acute ketamine 20 mg/kg
Ak50	Acute ketamine 50 mg/kg
Am5	Acute methamphetamine 5 mg/kg
Am10	Acute methamphetamine 10 mg/kg
Ck10	Chronic ketamine 10 mg/kg
Ck20	Chronic ketamine 20 mg/kg
Ck50	Chronic ketamine 50 mg/kg
Cm5	Chronic methamphetamine 5 mg/kg
Cm10	Chronic methamphetamine 10 mg/kg
CV	Coefficient of variance
kg	kilogram
METH	Methamphetamine
mg	milligram
µl	microliter
mg	milligram
NMDA	N-methyl-D-aspartate
C^{o}	Degree Celsius
Ca^{2+}	Calcium
v/v	volume per volume
±	Plus minus
=	Equal
>	More than
<	Less than

CHAPTER ONE

1. INTRODUCTION

1.1 BACKGROUND AND JUSTIFICATION OF STUDY

Neurodegeneration is a slow process of cell death which includes the alteration of the structure and function of neuron (Carole, Kate, Adrian, & Judy, 2011). It is believed to be the underlying pathophysiological process of neurodegenerative diseases. Devastating and expensive cost exceeding several hundred billion dollars are required in order to seek the treatment for these diseases as the current treatments modalities are inadequate. Several factors contributed to neurodegeneration process, these include the illicit use of drugs namely ketamine and METH.

Ketamine is an anesthetic agent commonly used since the early 1970s. It is a noncompetitive antagonist of N-methyl-D-aspartate receptor (NMDA) (Bhutta, Venkatesan, Rovnaghi, & Anand, 2007). It has been used as short-term dissociative anesthesia for adult and pediatric patients. Frequently, it is used in infants for elective surgeries as well as for emergency room procedures (Bergman, 1999). Besides its usefulness in the clinical settings, ketamine abuse has however become a major problem in many countries including East and Southeast Asia. Many reports stated that illicit use of ketamine has surpassed many club drugs. This trend can be seen in Hong Kong as early 2000s and other Asian countries (Li, Vicknasingam, & Cheung)

The trend is not unique to ketamine alone but also to a highly addictive psychostimulant drug called methamphetamine (METH). METH illicit use causes major problems around the world in terms of economic, social and public health burden (Yuan, Quiocho, Kim, Wee, & Mandyam, 2011). It is estimated that 15-16

million people are subjected to METH abuse worldwide making it the most widely abused drug after cannabis exceeding the number of heroin abusers (Krasnova & Cadet, 2009). Despite the strict regulation by the authorities on METH, the emergence of clandestine drug laboratories has resulted in over production and the surge in the abuse (Barr et al., 2006)

Current investigations indicate that repeated exposures to ketamine and METH cause neurotoxicity effects on human and rodents. Ketamine is shown to cause dose-dependent widespread apoptotic neurodegeneration in the immature rat brain (Ikonomidou et al., 1999; V Jevtovic-Todorovic et al., 2003; Scallet et al., 2004). METH administration on the other hand causes terminal degeneration and neuronal apoptosis induced by oxidative stress, hyperthermia, neuroinflammatory response, mitochondria dysfunction and excitotoxicity (Krasnova & Cadet, 2009). Ketamine and METH neurotoxicity effects have resulted in the damage of several regions of the brain including striatum, cerebral cortex and hippocampus. The duration of exposure both acute and chronic also crucially influence the level of neurotoxicity (P. M. Thompson et al., 2004; Venâncio, Magalhães, Antunes, & Summavielle, 2011).

Hippocampus is vital in memory, learning and behavioural functions. (Goto & Grace, 2008). Neurodegeneration of neurons in the hippocampal region will significantly alter these functions. In terms of memory function, hippocampus is important in developing cognitive map whereby it binds together information gain from neocortex and represents it as a whole memory (Manns & Eichenbaum, 2009). However, relative contribution of CA1 and CA3 regions of hippocampus in memory processes are not well studied (Vazdarjanova & Guzowski, 2004). Although adjacently located, CA1 and CA3 regions exhibited differences in the number and type of receptors, genes expression, neuronal circuitry and contribution to memory

functions (Aida, Fujiwara, & Shimoji, 1994; Ginsberg & Che, 2005; I. Lee, Yoganarasimha, Rao, & Knierim, 2004; Monyer, Burnashev, Laurie, Sakmann, & Seeburg, 1994; Stanika, Winters, Pivovarova, & Andrews, 2010). These differences lead to different susceptibility towards neurodegeneration (Coultrap, Nixon, Alvestad, Fernando Valenzuela, & Browning, 2005).

In relation to behavioural functions, hippocampus is largely related to exploratory behaviour. Exploratory behaviours assist in memory performance (Voss, Gonsalves, Federmeier, Tranel, & Cohen, 2010). It is one of the vital aspects of behaviour and is an important part of learning process. Several studies in human and animal experimental reported that application of ketamine and METH resulted in modification of behaviour by disrupting the serotonergic, dopaminergic and noradrenergic systems (Duncan, Miyamoto, Leipzig, & Lieberman, 1999).

The present study is therefore designed to demonstrate the effect of different doses and duration of ketamine and METH administration on neurodegeneration in adult rat hippocampus. The study involved two subregions of hippocampus, CA1 and CA3 which known to have significant differences in vulnerability towards neurodegeneration as well as different functions on human behaviour. The study also examines the outcome of exploratory behaviour in relation to the administration of different doses and duration of ketamine and METH. In addition, the study sought to establish the correlation of hippocampus neurodegeneration on CA1 and CA3 neuron. The design of this study differs from the previous studies in that it can provide more input on ketamine and METH abuse in terms of dose and duration of exposures in association with neurodegeneration and behaviour.

1.2 RESEARCH HYPOTHESIS

Different doses and duration of exposure of ketamine and METH administration will cause neurodegeneration of hippocampus, behavioural changes and correlation of regions in hippocampus (CA1 and CA3) can be established.

1.3 RESEARCH OBJECTIVES

1.3.1 General objective

Investigation of potential neurodegeneration of hippocampus and behavioural changes in the adult Sprague-Dawley rats treated with ketamine and METH.

1.3.2 Specific objectives

1. To compare the number of viable neurons in CA1 and CA3 regions at different doses and exposures of ketamine and METH administration.
2. To observe the behavioural change of adult rats exposed to different doses and exposures of ketamine and METH administration.
3. To study the correlation between the number of viable neurons in CA1 and CA3 regions in different doses and duration of exposure of ketamine and METH administration

CHAPTER TWO

2. LITERATURE REVIEW

2.1 NEURODEGENERATION

Neurodegeneration is defined as loss of structure and function of neurons. It is a gradual and progressive process that leads to cell death (Robillard, Federico, Tairyan, Ivinson, & Illes, 2011). It is involved in many central nervous system (CNS) diseases such as Parkinson's, Alzheimer's, and Huntington's disease (Thompson, 2008). The prevalence of neurodegeneration diseases has increased significantly over the years. According to World Health Organization (WHO), 15 million people worldwide are suffering from Alzheimer's disease (AD) alone. By 2050, the number of affected individuals in Europe and United States is estimated to triple to 13.2 million and 16.2 million respectively. There is generally a great urge for new discovery of effective treatment and preventive intervention for better management of neurodegenerative diseases (Forman, Trojanowski, & Lee, 2004).

The mechanisms of neurodegeneration in neurodegenerative diseases are complex and associated with many processes. Protein aggregation, mitochondrial mutation and oxidative stress are some of the important processes in neurodegeneration (Cherra Iii, Dagda, & Chu, 2010). Among the contributing factors suggested include gender, poor education, endocrine conditions, inflammation, stroke, hypertension, diabetes, smoking, head trauma, depression, infection, tumour, vitamin deficiencies, immune and metabolic conditions as well as chemical exposure (Brown, Lockwood, & Sonawane, 2005).

2.2 HIPPOCAMPUS

2.2.1 Anatomy

Hippocampus is a cortical grey matter formation in the central nervous system. It is formed by an enfolding of the inferomedial cortical portion of the temporal lobe into the inferior horn of the lateral ventricle of the brain (Fig. 2.1).

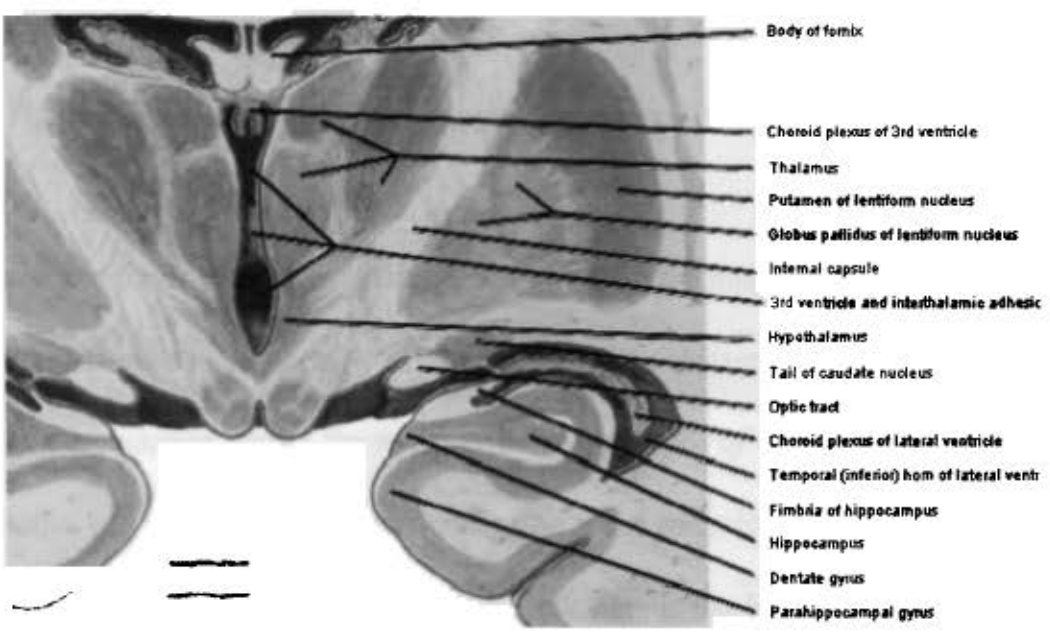

Figure 2.1: Posterior view of brain coronal section (Monkhouse, 1996)

The hippocampus formation consists of three parts: the dentate gyrus, the parahippocampal gyrus and the hippocampus proper. The hippocampal sulcus lies just above the anterior part of the parahippocampal gyrus, where the hippocampus projects into the floor of the inferior horn of the lateral ventricle. On its ventricular surface is a thin film of white matter, which thickens medially to form the fimbria (Fig. 2.2). This breaks free from the hippocampus as the crus (posterior pillar) of the fornix. The dentate gyrus is a small part of the hippocampus, as seen from the medial side, lies between the fimbria and the parahippocampal gyrus.

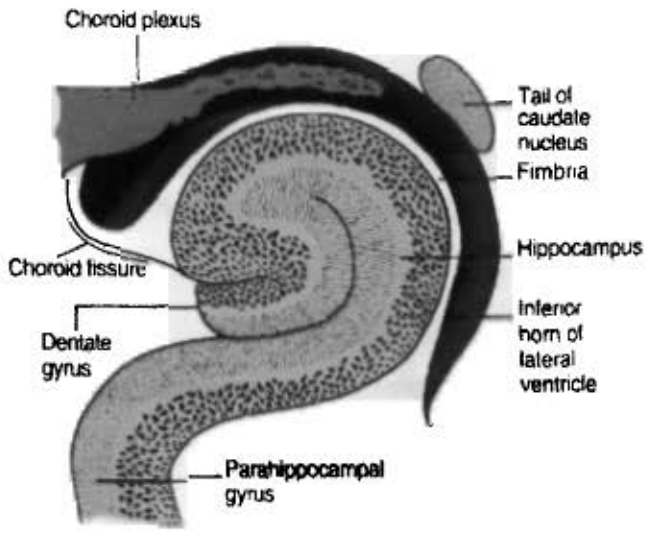

Figure 2.2: Transverse section through hippocampus (Crossman & Neary, 1995)

The fornix is the great efferent pathway from the hippocampus. As a flat band continuous with the fimbria, it curves up behind the thalamus to join its fellow in a partial decussation across the midline, the *commissure of the fornix*. It is really a chiasma, and is an association tract rather than a true commissure. The conjoined mass of white matter, lying beneath the corpus callosum, is the *body of the fornix* (Figure 2.3).

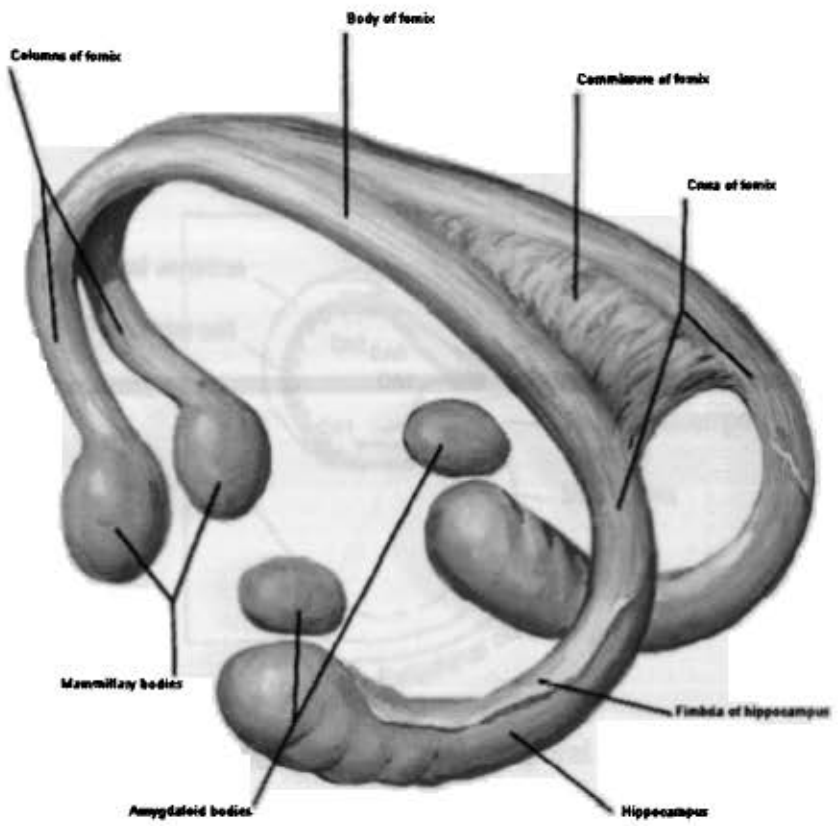

Figure 2.3: Hippocampus and fornix (Hansen, 2001)

From it, the conjoined anterior columns arch down in front to the anterior poles of the thalami, forming the anterior margins of the interventricular foramina. The columns of the fornix pass both anterior and posterior to the anterior commissure. The anterior fibers pass mainly to the septal nuclei near the lamina terminalis. The posterior fibers pass directly to the thalamus or into the mamillary body. From the mamillary body, the fibers pass in the lateral wall of the third ventricle as the mamillothalamic tract to the anterior pole of the thalamus. Here they relay and the thalamic neurons send their fibers through the internal capsule to the cingulated gyrus.

Hippocampus proper consists of longitudinal oriented regions referred as CA1, CA2, CA3 and CA4 (Figure 2.4). CA stands for *Cornu Ammonis* or Ammon's Horn which is an earlier name of hippocampus. CA1 lies ventrolaterally and CA4 lies medially near the origin of the fornix. The pyramidal cell is the most distinctive cell of