DOPAMINE D_2 AND 5-HT_{2A} RECEPTOR GENE EXPRESSION: THEIR FUNCTIONAL ROLE ON ALDEHYDE DEHYDROGENASE REGULATION IN ETHANOL TREATED RATS

THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BIOTECHNOLOGY

UNDER THE FACULTY OF SCIENCE

OF

COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

BY

AKASH K. GEORGE

DEPARTMENT OF BIOTECHNOLOGY
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN - 682 022, KERALA, INDIA.

SEPTEMBER 2006

CERTIFICATE

This is to certify that the thesis entitled **"DOPAMINE D_2 AND 5-HT_{2A} RECEPTOR GENE EXPRESSION: THEIR FUNCTIONAL ROLE ON ALDEHYDE DEHYDROGENASE REGULATION IN ETHANOL TREATED RATS"** is a bonafide record of the research work carried out by **Mr. AKASH K. GEORGE** under my guidance and supervision in the Department of Biotechnology, Cochin University of Science and Technology and that no part thereof has been presented for the award of any other degree.

Cochin - 682 022
September 15, 2006

Dr. C. S. Paulose
READER & HEAD
Department of Biotechnology
Cochin University of Science and Technology

Dr. C.S. PAULOSE M.Sc., Ph.D. FIMSA, FGSI
DIRECTOR, CENTRE FOR NEUROSCIENCE
HEAD, DEPT. OF BIOTECHNOLOGY
Cochin University of Science & Technology
Cochin-682 022, Kerala, India

DECLARATION

I hereby declare that this thesis entitled **"DOPAMINE D_2 AND 5-HT_{2A} RECEPTOR GENE EXPRESSION: THEIR FUNCTIONAL ROLE ON ALDEHYDE DEHYDROGENASE REGULATION IN ETHANOL TREATED RATS"** is based on the original research work carried out by me at the Department of Biotechnology, Cochin University of Science and Technology, under the guidance of **Dr. C. S. Paulose,** Reader and Head, Department of Biotechnology and no part thereof has been presented for the award of any other degree, diploma, associateship or other similar titles or recognition.

Cochin - 682 022
September 15, 2006

Akash K. George
Reg. No. 2857
Department of Biotechnology
Cochin University of Science and Technology

ACKNOWLEDGEMENT

It is my pleasure to express my sincere heartfelt gratitude to my supervising guide, Dr. C. S. Paulose for his sustained interest, motivation and exemplary guidance throughout the course of this work. His encouragement and timely advice will always be remembered with a deep sense of gratitude. I wish to express my sincere gratitude to him as the Head of the Department of Biotechnology for providing me necessary facilities to complete this work.

I offer my profound gratitude to Prof. Babu Philip, Department of Marine Biology, Microbiology and Biochemistry for his critical comments and creative suggestions.

I use this opportunity to express my gratitude to all teaching staff of this department for their help and encouragements.

I sincerely acknowledge my senior colleagues for their timely help and unfailing support.

My friends and colleagues had always been there with a helping hand through out the period of my work. I thank them for all their affection, friendship and help which I shall always cherish. I would also like to thank my juniors for their timely help and encouragement.

I thank all the non teaching office staff of department-present and past- for their help and support. I also thank all other research scholars and all M. Sc. students of the department for their friendship, help and co-operation. I wish to record my gratitude to and for their kind support.

I would like to extend my heartfelt gratitude to Dr. M. Chandrasekharan, Nair's Hospital Cochin and Dr. Kuruvilla Thomas, Lissy Hospital, Cochin for their suggestions and help.

My friends, are always been a source of inspiration, encouragement and support in all my endeavors. I thank them for all their affection, counsel and caution which I shall always cherish. I wish to record my heartfelt gratitude to my room mate Mr. Bibin Babu for his constant support. I wish to thank all my M. Sc. classmates especially Mr. Jaison Louis for his encouragement.

I would like to extend my sincere thanks to Prof. V. Unnikrishnan Nair, Dean, Faculty of Science, Cochin University of Science and Technology. I specially thank research scholars of Department of Physics, Photonics and Chemistry for their help and friendship.

Special thanks is due to the staff of Department of Instrumentation for their expertise and help in fabrication of Instruments and the authorities of Animal Breeding Centre, Mannuthy, KAU and Amrita Institute of Medical Science, Cochin for readily providing animals for this work.

My special thanks are due to the authorities and staff of Cochin University of Science and Technology for their help and co-operation.

I thank all the teachers of my school days, graduation and post-graduation for their blessings and encouragements. I would like to extend my sincere thanks to Indian Council of Medical Research for supporting this work with fellowships.

I owe a lot to my relatives, and my cousins.

Last, but not least I would like to express my heartfelt gratitude to my beloved ones- my father Mr. K. J. George and mother Late Mrs. Gracyamma George for

their blessings and prayers and my brother Mr. Adarsh K. George for his constant encouragement, affection and support, without which this work would not have been complete. There are so many others whom I may have inadvertently left out and I sincerely thank all of them for their help.

Over and above all I am immensely thankful to ***The Almighty God****, for his blessings, which I need for ever.*

AKASH K. GEORGE

Dedicated to St. George of Aruvithura

ABBREVIATIONS USED IN THE TEXT

5-HIAA	5-Hydroxyindole acetic acid
5-HIAL	5-Hydroxy phenyl acetaldehyde
5-HT	5-Hydroxy tryptamine
5-HTP	5-Hydroxy tryptophan
ACh	Acetylcholine
ADP	Adenosine diphosphate
ALDH	Aldehyde dehydrogenase
ATP	Adenosine triphosphate
B_{max}	Maximal binding
BS	Brainstem
cAMP	cyclic Adenosine mono phosphate
CC	Cerebral cortex
cDNA	Complimentary DNA
CNS	Central nervous system
CS	Corpus striatum
CSF	Cerebro spinal fluid
Ct	Crossing threshold
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
DEPC	Di ethyl pyro carbonate
dGTP	Deoxyguanosine triphosphate
dNTP	Deoxyribonucleotide triphosphate
DOPAC	Dihydroxy phenyl acetic acid
DA	Dopamine
EPI	Epinephrine

EtOH	Ethyl alcohol
GABA	Gamma aminobutyric acid
GTP	Guanosine triphosphate
HPLC	High performance liquid chromatography
HVA	Homovanillic acid
HYPO	Hypothalamus
HPA	Hypothalamic–pituitary–adrenal
K_d	Dissociation constant
K_m	Michaelis Menten constant
mRNA	messenger Ribonucleic acid
Mu MLV	Murine moloney leukemia virus reverse transcriptase
NAD^+	Nicotinamide adenine dinucleotide (oxidised)
NADH	Nicotinamide adenine dinucleotide (reduced)
NADP	Nicotinamide adenine dinucleotide phosphate (oxidised)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NE	Norepinephrine
p	Level of significance
PBS	phosphate buffered saline
RT-PCR	Reverse-transcription-polymerase chain reaction
S.E.M.	Standard Error of Mean
V_{max}	Maximal velocity
VTA	Ventral tegmental area

CONTENTS

INTRODUCTION	1
Objectives of the present study	7
LITERATURE REVIEW	8
Central Nervous system and ethanol	8
Dopamine	12
DA receptors	13
DA receptor classification	14
DA D_1-like family	15
DA D_1 receptor	16
DA D_5 receptors	16
DA D_2-like family	17
DA D_2 receptors	18
DA D_3 receptors	19
DA D_4 receptors	20
Effect of ethanol on brain DA receptors	21
DA receptor gene expression and ethanol	22
Serotonin	23
5-HT receptor classification	25
5-HT_1 receptor	26
5-HT_2 receptor	27
5-HT_3 receptor	28
5-HT_4 receptor	29
5-HT_5 receptor	29
5-HT_6 receptor	29
5-HT_7 receptor	30
Effect of ethanol on brain 5-HT receptors	30
5-HT receptor gene expression and ethanol	31
Brain neurotransmitters and ethanol	32
DA and 5-HT	32
Acetylcholine	34
Epinephrine and norepinephrine	35
Gamma aminobutyric acid and glutamate	37
Liver and ethanol	38

Aldehyde dehydrogenase and ethanol 38
Nervous system and hepatic functions 41
Nervous system and ALDH 44
Ethanol perfusion and ALDH 45
Ethanol mediated electrophysiological changes 46

MATERIALS AND METHODS 49

Biochemicals and their sources 49
Important chemicals used for the present study 49
Biochemicals 49
Radiochemicals 49
Molecular biology chemicals 50
Animals 50
Ethanol treatment to animals 50
Sacrifice and tissue preparation 50
Kinetic studies of aldehyde dehydrogenase 51
HPLC quantification of 5-HT and dopamine in liver and various brain regions 51
Dopamine D2 receptor binding studies using [^{3}H] YM-09151-2 in cerebral cortex and brain stem 52
Dopamine D_2 receptor binding studies using [^{3}H]YM-09151-2 in corpus striatum, cerebellum and hypothalamus 53
5-HT_{2A} Receptor binding studies using [^{3}H]MDL 100907 radioligand in brain regions 54
5-HT_{2A} Receptor binding studies using [^{3}H]MDL 100907 radioligand in liver 55
Protein estimation 56
Analysis of the receptor binding data 56
Receptor binding parameters analysis 56
Displacement curve analysis 57
Expression studies of dopamine D_2, 5-HT_{2A} receptors and ALDH in different brain regions 57
RT-PCR (Reverse Transcription Polymerase Chain Reaction) 57
Preparation of RNA 57
Isolation of RNA 58
Primers 58
RT-PCR of dopamine D_2, 5-HT_{2A} receptors, ALDH and β-actin 59

Thermocycling profile used for Real -Time PCR 60
Thermocycling profile used for dopamine D_2 receptor 60
Thermocycling profile used for 5-HT_{2A} receptor 61
Thermocycling profile used for ALDH 61
Thermocycling profile used for β-actin 61
Analysis of Real - Time PCR product 62
Liver perfusion with PBS 62
Electroencephalogram 62
Statistics 63

RESULTS 64

Body weight and ethanol consumption 64
Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in cerebral cortex 64
Real - Time PCR analysis of ALDH 64
Kinetic parameters, V_{max} and k_m, of aldehyde dehydrogenase in brain stem 64
Kinetic parameters, V_{max} and k_m, of aldehyde dehydrogenase in cerebellum 65
Kinetic parameters, V_{max} and k_m, of aldehyde dehydrogenase in plasma 65
Kinetic parameters, V_{max} and k_m, of aldehyde dehydrogenase in liver 65
Real - Time PCR analysis of ALDH 65
Quantification of DA, 5-HT and their metabolites by HPLC in the cerebral cortex 66
Quantification of DA, 5-HT and their metabolites by HPLC in the brainstem 66
Quantification of DA, 5-HT and their metabolites by HPLC in the hypothalamus 66
Quantification of DA, 5-HT and their metabolites by HPLC in the corpus striatum 67
Quantification of DA, 5-HT and their metabolites by HPLC in the liver 67
ALTERED BRAIN DOPAMINE D_2 RECEPTOR BINDING PARAMETERS 67
Cerebral cortex 67
Displacement analysis of [^{3}H]YM-09151-2 68

against sulpiride in cerebral cortex
Real -Time-PCR analysis of DA D_2 receptor 68
Brainstem 68
Displacement analysis of [^{3}H]YM-09151-2 against sulpiride in brainstem 68
Hypothalamus 69
Displacement analysis of [^{3}H]YM-09151-2 against sulpiride in hypothalamus 69
Real - Time PCR analysis of DA D_2 receptor 69
Corpus striatum 70
Displacement analysis of [^{3}H]YM-09151-2 against sulpiride in corpus striatum 70
Real - Time PCR analysis of DA D_2 receptor 70
Cerebellum 70
Displacement analysis of [^{3}H]YM-09151-2 against sulpiride in cerebellum 71
Real - Time PCR analysis of D_2 receptor 71
ALTERED BRAIN 5-HT_{2A} RECEPTOR BINDING PARAMETERS 71
Cerebral cortex 71
Displacement analysis of [^{3}H]MDL 100907 against ketanserin in cerebral cortex 71
Real -Time PCR analysis of 5-HT_{2A} receptor 72
Brainstem 72
Displacement analysis of [^{3}H]MDL 100907 against ketanserin in brainstem 72
Hypothalamus 72
Displacement analysis of [^{3}H]MDL 100907 against ketanserin in hypothalamus 73
Real - Time PCR analysis of 5-HT_{2A} receptor 73
Corpus striatum 73
Displacement analysis of [^{3}H]MDL 100907 against ketanserin in corpus striatum. 74
Real - Time PCR analysis of 5-HT_{2A} receptor 74
Cerebellum 74
Displacement analysis of [^{3}H]MDL 100907 against ketanserin in cerebellum. 74
Real -Time PCR analysis of 5-HT_{2A} receptor 75
Liver 75

Displacement analysis of [^{3}H]MDL 100907 against ketanserin in liver 75
Real - Time PCR analysis of $5HT_{2A}$ receptor 76
Kinetic parameters of aldehyde dehydrogenase in perfused liver 76
Electroencephalogram analysis 77

DISCUSSION 78

Ethanol induced aldehyde dehydrogenase activity disparity in liver, plasma and brain regions 79
Brain DA and HVA changes during ethanol treatment 80
Brain 5-HT and 5-HIAA changes during ethanol treatment 83
Liver DA, 5-HT and their metabolite changes during ethanol treatment 85
DA D_2 receptor alteration in brain regions 86
5-HT_{2A} receptor alteration in brain regions 87
Hepatic 5-HT_{2A} receptor alterations 88
Ethanol induced ALDH, DA D_2 and 5-HT_{2A} receptor gene expression changes 89
Central, Periperal DA, 5-HT and Liver ALDH activity 90
Dopaminergic and serotonergic regulation on kinetic parameters of aldehyde dehydrogenase 92
Ethanol mediated electrophysiological changes 93

CONCLUSION 96

SUMMARY 99

REFERENCES 101

LIST OF PAPERS PRESENTED / PUBLISHED 140

INTRODUCTION

INTRODUCTION

Neuroscience research is essential for understanding the biological basis of ethanol-related brain alterations and for identifying the molecular targets for therapeutic compounds that can alter ethanol's actions in the brain and body. Many different biological systems in the brain are influenced by ethanol and result in brain adaptations that form the underpinnings of ethanol addiction. Brain is the major target for the actions of ethanol and it can affect the brain and behaviour in a variety of ways, multiple factors can influence these effects. The neurotoxicity of ethanol determines, modulates or modifies the brain functions during the course of ethanol treatment. Ethanol stimulates the release of β-endorphins, responsible for euphoria and anesthesia, accounting for some of the intoxicating effects of ethanol. Ethanol can cause physical addiction directly through its effects on many receptor sites in the postsynaptic membranes of neurons. Changes in the brain include depletion and interference in neurons and chemical messengers involved in signalling that result in a dependence on ethanol. Brain neurotransmitters through their receptors or hormonal pathway play an important role in governing the cellular activities.

Consumption of ethanol interferes differentially with transmission processes in the central nervous system (CNS), affecting many of the neurotransmitter systems. Ethanol acts at many sites - including the reticular formation, spinal cord, cerebellum, cerebral cortex and on many neurotransmitter systems. The effects of ethanol on the brain result mainly from its action on the postsynaptic receptor sites for various neurotransmitters. Brain serotonin and

dopamine along with other neurotransmitters play an important role in the brain process underlying ethanol addiction. Development of addiction appears to be with abnormal neurotransmitter systems.

Serotonin (5-HT) and dopamine (DA) are the two major neurotransmitters involved in ethanol addiction. Ethanol alters neuronal cell membranes as well as their ion channels, enzymes and receptors. Ethanol also binds directly to the receptors causing the prolonged stimulating or inhibiting impulse, depending on which area of the brain it is present. Ethanol not only affects the neurotransmitters individually, but also influences the interactions of these neurotransmitters, opening of the chloride ion channels and the greater uptake of chloride ions by the post-synaptic cell. Ethanol addiction leads to morphological and functional degeneration of rat peripheral sympathetic nervous system. 5-HT does not act alone within the brain. Instead, serotonergic neurons are parts of larger circuits of interconnected neurons that transmit information within and among brain regions. Many neurons within these circuits release neurotransmitters other than serotonin. The exact effect of ethanol on these neurotransmitters is still under study. Some of the 5-HT mediated neuronal responses to ethanol may arise from interactions between serotonin and other neurotransmitters. Serotonin can alter dopaminergic neuronal activity through 5-HT_2 receptors by its interaction with the dopaminergic system. Systemic administration of ethanol increases the firing rate of mesolimbic dopamine neurons. Ethanol appears to facilitate dopamine release by increasing opioidergic activity and dopaminergic neurons by inhibition of GABAergic neurotransmission *via* opioid receptors in the ventral tegmental area (VTA).

Mesolimbic dopamine release induced by ethanol consumption indicates that ethanol-related stimuli are important.

Both short and long-term ethanol exposure also affect the serotonin receptors that convert the chemical signal produced by serotonin into functional changes in the signal-receiving cell. Neuronal dopamine receptors are widely distributed in the central and the peripheral nervous system at different levels. Serotonin seems to be involved in ethanol's acute reinforcing effects. The exact mechanisms that may be involved still need to be clarified. Depending on the dose, ethanol stimulates locomotor activity and produces an increase in dopamine levels in the nucleus accumbens. Brain peptide corticotropin releasing factor (CRF) with ethanol appears to influence neurotransmission in the amygdala, by increasing the transmission of gamma amino butyric acid (GABA). Ethanol not only affects the neurotransmitters individually, but also influences the interactions of these neurotransmitters when working together as 5-HT may interact with neurons that secrete GABA. If ethanol is present, the ethanol influenced 5-HT may affect the actions of GABA neurons in areas involving behavioural output such as the hippocampal formation, where cognitive decisions are made. Similarly, ethanol influenced 5-HT stimulates dopamine production and thus more extreme behavioural outputs.

Postsynaptic receptor sites for various neurotransmitters are affected by the acute effects of ethanol. They exert their function through receptors present in both neuronal and non neuronal cell surface that trigger second messenger signalling pathways. Chronic ethanol consumption has been associated with an increased dopamine turnover rate and decreased dopamine uptake. Genetic

variability in the 5-HT_{2A} receptor is involved in the development of ethanol dependence. Another series of studies suggest that ethanol-induced reward is independent of the activation of DA D_2 receptors mediated through 5-HT_{1B} and 5-HT_2 receptors. Ethanol increases the amount of dopamine acting on receptors and enhances the normal feeling of pleasure associated with the dopaminergic system. Chronic ethanol treatment may decrease serotonergic neurotransmission in selective brain regions. Ethanol has several actions on the central nervous system believed to be mediated by non-specific physicochemical effects on the membrane or by actions through specific receptors. Ethanol has a variety of effects on neuroendocrine function and there is a great deal of interest in investigating the effects of ethanol on the hypothalamic–pituitary–adrenal (HPA) axis. Ethanol administration activates the HPA axis. Acetaldehyde formed during ethanol metabolism in brain is able to activate the HPA axis at a central level.

Brain plays an important regulatory role in hepatic functions. The liver is richly innervated and signalling occurs between the liver and brain (Kerfoot *et al.*, 2006). Liver dysfunction is associated with more extensive brain dysfunction in liver cirrhosis patients (Tarter *et al.*, 1993). Brain monoamines and aldehyde dehydrogenase (ALDH) level together plays a decisive role in the ethanol addiction. The liver plays a primary role in body homeostasis. It regulates levels of circulating nutrients, excretes waste products into the bile, reduces circulating ammonia through production of urea, produces important serum proteins and produces bile acids required in digestion of lipids and acts as the primary site of metabolic defense. The ethanol induced neurotransmitters mediate changes in intracellular communications not only within the central nervous system but also in the peripheral tissues. The ethanol metabolism in the rat liver is functionally controlled directly by sympathetic nerves. With long-term use, adolescent rats

have shown massive neuronal loss in their cerebellum, basal forebrain and neocortex. Strong ethanol preferences are associated with reduced serotonergic functions either directly or indirectly by increasing dopamine neurotransmission particularly in the ventral striatum. Serotonergic system appears to be involved in ethanol consumption and reinforcement by activating dopaminergic reward system. Acetaldehyde produced from ethanol is metabolized quickly to acetate by liver ALDH. Biogenic aldehydes, the metabolic intermediate of ethanol, interfere in some way with the oxidative metabolism of the brain. Chronic ethanol exposure has been shown to cause degenerative changes in several areas of the brain, including cerebral cortex, hippocampus, cerebellum, brainstem and also in the peripheral nervous system. Acute ethanol intoxication may cause changes in hepatic enzymes (Hegyi *et al.*, 2003).

Most of the acetaldehyde produced from ethanol is metabolized quickly to acetate by liver ALDH, the principal enzyme involved in serotonin and dopamine metabolism. The 5-hydroxyindole-3-acetaldehyde (5-HIAL), 3, 4-dihydroxyphenylacetaldehyde (DOPAL) are produced by the first step of metabolism of serotonin and dopamine respectively. Both DOPAL and 5-HIAL are excellent substrates for ALDH. Differences in acetaldehyde elimination may contribute to ethanol preference. Accumulation of acetaldehyde in blood following ethanol ingestion, due to a lower activity of ALDH, is believed to play a preventive role against ethanol addiction.

This study focuses on the effect of ethanol treatment and its functional correlation with dopaminergic and serotonergic system with regard to its suitability as a model of human ethanol consumption. The work that is presented

here is an attempt to understand the role of dopamine, serotonin acting through DA D_2 and 5-HT_{2A} receptors in the functional regulation of ALDH in ethanol treated rats. Neurobiological mechanisms that are responsible for ethanol addiction and the role of ALDH have been given special emphasis with dopamine and serotonin receptor subtype specificity. Also, the brain activity is studied using electroencephalogram confirming the neurotransmitters functional regulation and ethanol treatment.

OBJECTIVES OF THE PRESENT STUDY

1. To create the animal model for ethanol consumption by ethanol treatment and study the rate of ethanol consumption.
2. To study the kinetic parameters of aldehyde dehydrogenase in brain regions of control and ethanol treated rats.
3. To study the kinetic parameters of aldehyde dehydrogenase in liver and plasma of control and ethanol treated rats.
4. To study the changes in DA, 5-HT, HVA and 5-HIAA content in liver and various rat brain regions – corpus striatum (CS), cerebral cortex (CC), brainstem (BS) and hypothalamus (HYPO) of control and ethanol treated rats using High Performance Liquid Chromatography.
5. To study the DA D_2 and 5-HT_{2A} receptor alterations in liver and different brain regions like cerebral cortex, brainstem, hypothalamus, corpus striatum and cerebellum of control and ethanol treated rats.
6. To study the gene expression of DA D_2 and 5-HT_{2A} receptors, their functional role on ALDH regulation in control and ethanol treated rats.
7. To study the impact of dopaminergic and serotonergic system in functional regulation on the kinetic parameters of aldehyde dehydrogenase.
8. To perform neurophysiologic analysis of the electrical activity of the brain using electroencephalogram (EEG) in ethanol treated rats.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Drug and ethanol seeking behaviour has become a great global problem affecting millions of inhabitants with a cost to society in the billions. The etiology of ethanol dependence is a complex interaction of psychosocial and biologic factors (Konishi *et al.*, 2004). The central nervous system (CNS) plays an important role in the peripheral regulation. Neurotransmitters mediate rapid intracellular communications not only within the central nervous system but also in the peripheral tissues. They exert their function through receptors present in both neuronal and non neuronal cell surface that trigger second messenger signalling pathways (Julius *et al.*, 1989). Central nervous system believed to be mediated by non-specific physicochemical effects on the membrane or by actions through specific receptors (Deitrich *et al.*, 1989; Eckardt *et al.*, 1998). Determining the specific neurotransmitters and receptor subtypes that may be involved in the development and effects of ethanol abuse is the first step in developing medications to treat ethanol addiction (Hunt, 1993; Deitrich & Erwin, 1996).

Central Nervous System and Ethanol

The etiology of ethanol dependence is a complex interaction of psychosocial and biologic factors (Konishi *et al.*, 2004). The effects of ethanol on the brain result mainly from its action on the postsynaptic receptor sites for various neurotransmitters. Heavy ethanol consumption has both immediate and long-term detrimental effects on the brain and neuropsychological functioning (Delin & Lee, 1992; Evert & Oscar-Berman, 1995). Ethanol interferes with communication between nerve cells and all other cells, suppressing the activities

of excitatory nerve pathways and increasing the activities of inhibitory nerve pathways. Central nervous system has a crucial role in ethanol addiction, several actions believed to be mediated by non-specific physicochemical effects on the membrane or by actions through specific receptors (Deitrich *et al.,* 1989; Eckardt *et al.,* 1998). Chronic and excessive consumption of ethanol in humans and animals has been shown to cause cellular damages in many body organs, including neurons and glial cells in the central nervous system (Miller, 1992; Hunt, 1993; Luo & Miller, 1998). Biogenic amines have been implicated in the regulation of aggression (Kravitz, 2000) and memory (Hasselmo, 1995). Brain serotonin (5-HT) modulates the neural and behavioural effects of ethanol in a manner that remains poorly understood (Daws *et al.,* 2006). Ethanol-induced changes in thyroid function may contribute to the development of mood disorders (Liappas *et al.,* 2006). Ethanol ingestion for short as well as long time has been shown to induce significant changes in neurotransmitter systems (Imperato & Di Chiara, 1986; Samson & Harris, 1992), among these DA and 5-HT have received special attention because of their putative role in the motivational effects of ethanol (Cloninger, 1987; Sellers *et al.,* 1992; Wallis *et al.,* 1993). Changes in central DA neurotransmission are implicated in processes as diverse as muscle rigidity, hormonal regulation, thought disorder and cocaine addiction. Peripheral DA mediate changes in blood flow, glomerular filtration rate, sodium excretion and catecholamine release. In the adolescent brain, drinking cessation can partially ameliorate the ethanol-induced morphological changes on neurons and astrocytes but cannot fully return it to the basal state (Evrard *et al.,* 2006). DA itself has a regulatory effect on the synthesis of post-synaptic receptors. Schizophrenia causes an increased DA D_2 receptor synthesis due to dopaminergic blockade by neuroleptics. In Parkinson's disease DA deficiency causes an

increase in DA D_2 receptors. The nicotinic acetylcholine receptor (nAChR) is the prototype for a superfamily of ligand gated ion channels (Corringer *et al.*, 2000) that includes inhibitory [glycine (Gly), $GABA_A$, and GABA receptors] as well as excitatory receptors (nAChRs and 5-HT_3 receptors). These receptors have a pentameric structure, whereby the five subunits are arranged in a quasisymmetric distribution around a central pore (Unwin *et al.*, 1988). Each subunit presents a large extracellular amino-terminal domain, folsess binding sites for ethanol (Crews *et al.*, 1996). GABA, the major inhibitory neurotransmitter of the CNS is affected by even short-term exposure to ethanol and increases GABAergic function. Long-term ethanol exposure is associated with reduced GABA-benzodiazepine receptor (GBzR) levels and function (Lingford-Hughes *et al.*, 2002). Ethanol enhances the activity of GABA, but reduces the excitatory effects of glutamate. These actions are the main reason that ethanol is often thought of as a depressant. $GABA_A$ receptor is involved in ethanol's acute and chronic effects (Mehta & Ticku, 1999; Buck & Finn, 2000; Cagett *et al.*, 2003). Baclofen, agonist of GABA activates another type of GABA receptor ($GABA_B$), has recently been shown in a preliminary study to be effective in inducing abstinence from ethanol and reducing ethanol craving and consumption (Addolorato *et al.*, 2002). Serotonin and dopamine are the major neurotransmitters involved in ethanol addiction *in vivo* (Tank, 1981). Serotonin produced and released from neurons that originate within discrete regions, or nuclei, in the brain (Cooper & Bloom, 1991). Along with other neurotransmitters, serotonin plays an important role in the brain process underlying ethanol abuse (David, 1999). Alterations in monoamines are observed in the striatum after chronic ethanol administration (Vasconcelos *et al.*, 2004). DA is a neurotransmitter that has been implicated in various central neuronal degenerative disorders like Parkinson's disease and

behavioural diseases like Schizophrenia. DA is synthesised from tyrosine, stored in vesicles in axon terminals and released when the neuron is depolarised. DA interacts with specific membrane receptors to produce its effects. These effects are terminated by re-uptake of DA into the presynaptic neuron by a DA transporter or by metabolic inactivation by monoamine oxidase B (MAO-B) or catechol-*O*-methyltransferase (COMT). DA plays an important role both centrally and peripherally. Nonetheless, the mesolimbic DA system has been shown to play a role in the rewarding effects of ethanol. The recent identification of five DA receptor subtypes provides a basis for understanding DA's central and peripheral actions. Stimulation of the DA D_1 receptor gives rise to increased production of cAMP. DA D_2 receptors inhibit cAMP production, but activate the inositol phosphate second messenger system. Impairment of central DA neurotransmission causes muscle rigidity, hormonal regulation, thought disorder and cocaine addiction. Ethanol enhanced 5-HT_{3A} receptor function, but had no effect on mouse 5-$HT_{3A/B}$ receptor mediated currents (Hayrapetyan *et al.*, 2005). Ethanol administration activates the HPA axis (Ellis, 1966, Rivier *et al.*, 1984, Rivier & Vale, 1988; Thiagarajan *et al.*, 1989; Rivier, 1996; Rivier & Lee, 1996; Ogilvie *et al.*, 1997). Acetaldehyde formed in brain is able to activate the HPA axis at a central level (Hiroshi *et al.*, 2001). Aldehyde dehydrogenase, the primary enzyme responsible for acetaldehyde metabolism, is highly correlated with voluntary ethanol consumption in several strains of rats and mice (Amir, 1977). Brain ALDH plays an important role in the biosynthesis of biogenic amines (Tipton *et al.*, 1977), which may be one of the important factors in modifying ethanol-induced behaviour (Roberta *et al.*, 2001). Ethanol is found to cause several biochemical changes in the NA, such as increased levels of tyrosine

hydroxylase, NMDA R1 and Glutamate R1 receptor subunits and decreased levels of subunit α1 of the $GABA_A$ receptor complex (Ortiz *et al.*, 1995).

Dopamine

Dopamine (DA) exerts its functions mediated through various receptors and these actions are terminated to prevent continuous stimulation of the receptors. This inactivation is brought about by reuptake mechanisms and metabolism of DA. Reuptake of DA is accomplished by a high affinity carrier present in the membrane, the DA transporter (DAT). DA containing neurons arise mainly from DA cell bodies in the substantia nigra and ventral tegmental area in mid-brain region (Carlsson, 1993; Lookingland *et al.*, 1995; Creese *et al.*, 1997; Tarazi *et al.*, 1996, 2001). Dopaminergic system is organized into four major subsystems (i) the nigrostriatal system involving neurons projecting from the substantia nigra - the major DA system in the brain as it accounts for about 70% of the total DA in the brain, and its degeneration makes a major contribution to the pathophysiology of Parkinson's disease; (ii) the mesolimbic system that originates in the midbrain tegmentum and projects to the nucleus accumbens septi and lateral septal nuclei of the basal forebrain as well as the amygdala, hippocampus and the entorhinal cortex, all of which are considered components of the limbic system and so are of particular interest for the patho-physiology of idiopathic psychiatric disorders; (iii) mesocortical pathway arising from the arcuate and other nuclei of the hypothalamus the mesocortical system, which also arises from neuronal cell bodies in the tegmentum which project their axons to the cerebral cortex, particularly the medial prefrontal regions; (iv) the tuberinfundibular pathway, which is a neuroendocrinological and ending in the median eminence of the inferior hypothalamus. DA released in this system

exerts regulatory effects in the anterior pituitary and inhibits the release of prolactin. DA is involved in the control of both motor and emotional behaviour. Despite the large number of crucial functions it performs, this chemical messenger is found in a relatively small number of brain cells. In fact, while there are a total of 10 billion cells in the cerebral cortex alone, there are only one million dopaminergic cells in the entire brain. The DA transporter recycles extracellular DA by actively pumping it back into the nerve terminal. The DA content which is about 70 to 80% in the striatal synaptic cleft is inactivated by this process. Drugs, such as cocaine, are able to block the action of the DA transporter, thereby sustaining the presence of DA in the synaptic cleft and its action on DA receptors. Part of the DA is inactivated by conversion to inactive compounds by metabolic enzymes, which are present both intra- and extraneuronally. Monoamine oxidase (MAO), aldehyde dehydrogenase and COMT are responsible for the metabolism of DA. DA after reuptake may intraneuronally be deaminated by MAO to give 3, 4-dihydroxyphenyl acetaldehyde (DOPAL), which subsequently is converted to 3, 4-dihydroxyphenylacetic acid (DOPAC) by ALDH. DOPAC is then methylated by COMT to give homovanillic acid (HVA).

DA receptors

DA mediates its actions *via* membrane receptor proteins. DA receptors are found on postsynaptic neurons in brain regions that are DA-enriched. In addition, they reside presynaptically on DA neuronal cell bodies and dendrites in the midbrain as well as on their terminals in the forebrain. DA receptors belong to a family of large peptides that are coupled to G-proteins which are modified by attached carbohydrate, lipid-ester or phosphate groups. The topologies of the

five DA receptors are predicted to be the same as all the other G-protein-coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions. The third intracytoplasmic loop is functionally critical and interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects (Carlsson, 1993; Tarazi *et al.*, 1996; Creese *et. al.*, 1997). In their putative transmembrane domains, the DA D_1 and D_5 receptors are 79% identical to each other, while they are only 40–45% identical to the DA D_2, D_3, and D_4 receptors. Conversely, the DA D_2, D_3, and D_4 receptors are between 75% and 51% identical to each other. They contain seven putative membrane-spanning helices which would form a narrow dihedral hydrophobic cleft surrounded by three extracellular and three intracellular loops. The receptor polypeptides are probably further anchored to the membranes through palmitoylation of a conserved Cys residue found in their carboxy tails, 347 in DA D_1, the C-terminus in DA D_2 like receptors. The DA receptors are glycosylated in their N-terminal domains. DA D_1 like subtypes has potential glycosylation sites in their first extra cytoplasmic loop.

DA receptor classification

DA receptors are divided into two families on the presence or absence of ability of DA to stimulate adenylyl cyclase and produce the second-messenger molecule cyclic-AMP (cAMP) (Calne, 1979; Schwartz *et al.*, 1992; Civelli *et al.*, 1993; Jackson *et al.*, 1994; Ogawa, 1995; Strange, 1996). This classification is based on similarities in structure, pharmacology, function and distribution. DA D_1 like receptors are characterized initially as mediating the stimulation of cAMP

production. DA D_2 like receptors inhibit the production of cAMP. This pharmacological characterization is based on the ability of some DA agents to block adenylyl cyclase activity to inhibit the release of prolactin *in vivo* and *in vitro* in a cAMP-independent fashion (Seeman, 1980). Applications of recent technical advances in molecular genetics have greatly facilitated the isolation and characterization of novel DA receptors, DA D_3, D_4 and D_5, with different anatomical localization from traditional DA D_1 or DA D_2 receptors. Based upon their pharmacological profiles, including their effects on different signal transduction cascades, these receptors are currently divided into two families: the DA D_1-like family which includes DA D_1 and D_5 receptors. The DA D_2-like family includes DA D_2, D_3 and D_4 receptors (Shen *et al.*, 1993).

DA D_1-like family

The DA D_1-like receptors are characterized by a short third loop as in many receptors coupled to Gs protein (Civelli *et al.*, 1993). They are classified into DA D_1 and D_5. The DA D_1-like receptors have short third intracellular loops and long carboxy terminal tails. The DA D_1 receptor is the most abundant DA receptor in the central nervous system. In the DA D_1 and DA D_5 receptors third intracellular loop and the carboxy terminus are similar in size but divergent in their sequence. The small cytoplasmic loops 1 and 2 are highly conserved so that any difference in the biology of these receptors can be probably related to the third cytoplasmic loop and the carboxy terminal tail (Gingrich *et al.*, 1993; Dowd, 1993). The external loop between transmembrane domain (TM) TM4 and TM5 is considerably different in the two receptor subtypes, being shorter

(27 amino acids) in the D_1 receptor than in the D_5 receptor (41 amino acids). The amino acid sequence of this loop is divergent in the DA D_5 receptor (Marc *et al.*, 1998).

DA D_1 receptor

DA D_1 receptors are found at high levels in the typical DA regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercles. DA D_1 receptor seems to mediate important actions of DA to control movement, cognitive function and cardiovascular function. The DA D_1 receptors show characteristic ability to stimulate adenylyl cyclase and generate inositol 1, 4, 5-trisphosphate (IP_3) and diacylglycerol *via* the activation of phospholipase C (Sibley *et al.*, 1990; Monsma *et al.*, 1990). DA D_1 receptors are highly expressed in basal ganglia followed by cerebral cortex, hypothalamus and thalamus. DA D_1 receptors mRNA is colocalized in striatal neurons of the basal ganglia with mRNA for DA receptor phospho protein (DARPP-32; KD) which is a DA and cyclic-AMP-regulated phosphoprotein. DA receptor phosphoprotein contributes to the actions of D_1 receptor (Hemmings & Greengard, 1986; Greengard *et al.*, 1987).

DA D_5 receptors

The gene encoding the human DA D_5 protein is located at the short arm of chromosome 4, the same region where the Huntington disease gene has been located (Gusella, 1989). The DA D_5 receptor gene is intronless and encodes a protein that extends for 477 amino acids (George *et al.*, 1991). This protein has an overall 50% homology with DA D_1 receptor and 80% if only the seven transmembrane segments are considered. Two DA D_5 receptor pseudogenes

having 154 amino acids have been identified with 90% homology (Gusella, 1989). These pseudogenes, however, contain stop codons in their coding regions that prevent them from expressing functional receptors. The functions of these pseudogenes, which appear so far to be specific to humans, are not yet known (Allen *et al.*, 1991). DA D_5 receptors, like DA D_1 receptors, appear to interact with G-proteins and can stimulate adenylyl cyclase, with relatively high affinity for DA and DA D_1-selective agonists (George *et al.*, 1991). DA D_5 receptor mRNA expression is unique and limited to the hippocampus and parafascicular nucleus of the thalamus (Civelli *et al.*, 1992). It is involved in the thalamic processing of painful stimuli (Basbaum *et al.*, 1979).

DA D_2-like family

The dopamine D_2 receptor is one of at least five physiologically distinct dopamine receptors (D_1, D_2, D_3, D_4 and D_5) found on the synaptic membranes of neurons in the brain (Sibley & Monsma, 1990). DA D_2-like receptors are characterized by a long extracellular amino terminus which has several glycosylation sites and a shorter carboxy terminal tail with putative phosphorylation sites. DA D_2-like receptors belong to the G-protein coupled receptors and have 400 amino acid residues. The function of sugar moieties is unclear (Marc *et al.*, 1998; Sibley, 1999). The unique feature of DA D_2-like receptors family is that they posses a bigger third cytoplasmic (intracellular) loop in common, which is thought to be the site where the G-protein couples (Marc *et al.*, 1998). It is generally believed that the membrane enclosed part of the amino-acid chain of G-protein coupled receptors is folded into seven α-helices. The transmembrane helices consist primarily of hydrophobic amino-acid residues. Between the different DA receptors, the third loop also displays the

greatest variability in amino-acid sequence. This may have consequences for their respective second messenger systems. The DA D_2-like receptors are coupled to Gi-protein and inhibit the formation of cyclic AMP. The DA D_2 receptors tertiary structure is stabilized by two cysteine disulphide bridges.

DA D_2 receptors

The DA D_2 receptor gene encodes a protein that extends for 415 amino acids. Similar to other G-protein coupled receptors, the DA D_2 receptor has seven transmembrane segments, but in contrast to DA D_1-like receptors, the third cytoplasmic domain is long and the carboxy terminus is short. The gene encoding this DA D_2 receptor was found to reside on q22-q23 of human chromosome 11 (Makam *et al.*, 1989). The DA D_2 receptor was the first receptor to be cloned (Chrisre *et al.*, 1988). The DA D_2 receptors are involved in several signal transduction cascades, including inhibition of cAMP production (Vallar & Meldolesi, 1989), inhibition of phosphoinositide turnover (Epelbaum *et al.*, 1986) activation of potassium channels and potentiation of arachidonic acid release (Axelrod *et al.*, 1991). The DA D_2 receptors are highly expressed in basal ganglia, nucleus accumbens septi and ventral tegmental area (Schwartz *et al.*, 1991). The DA D_2 receptor exists as two alternatively spliced isoforms differing in the insertion of a stretch of 29 amino acids in the third intracellular loop and are designated as DA D_{2S} and DA D_{2L} (Seeburg *et al.*, 1989; Marc *et al.*, 1998). DA D_2 receptor isoforms (DA D_{2L} and DA D_{2S}) vary within each species by the presence or absence of a 29-amino acid sequence in the third cytoplasmic domain of the DA D_2 receptor peptide chain. Both variants share the same distribution pattern; with the shorter form less abundantly transcribed in addition they appear to differ in their mode of regulation (Marc *et al.*, 1998). Because this loop seems

to play a central role in receptor coupling, the existence of a splicing mechanism at this level could imply functional diversity. However, in spite of the efforts of several groups, no obvious differences have emerged so far between the two DA D_2 receptor isoforms. The two isoforms derived from the same gene by alternative RNA splicing which occurs during the maturation of the DA D_2 receptor pre-mRNA (Schwartz *et al.*, 1989). Pharmacologically, both isoforms exhibit nearly similar profiles in terms of their affinities to different DA D_2-selective agents, and inhibit adenylyl cyclase activity. However, these isoforms display an opposite regulatory effect (Sibley *et al.*, 1994). These isoforms have the same pharmacological profile, even though a marginal difference in the affinity of some substituted response to DA treatment is reported: DA induces the up-regulation of DA D_{2L} isoform of DA D_2 receptors (Castro & Strange, 1993). When expressed in host cell lines, both isoforms inhibited adenylyl cyclase (Marc *et al.*, 1998; Sibley, 1999). However, the DA D_{2S} receptor isoform displayed higher affinity than the DA D_{2L} in this effect (Seeburg *et al.*, 1989; Marc *et al.*, 1998). The isoforms of DA D_2 mediate a phosphatidylinositol-linked mobilization of intracellular calcium in mouse Ltk [-] fibroblasts. Protein kinase C (PKC), however, differentially modulates DA D_{2S} and DA D_{2L}-activated transmembrane signalling in this system with a selective inhibitory effect on the DA D_{2S}-mediated response.

DA D_3 receptors

The gene encoding this receptor resides on chromosome 3 (Giros *et al.*, 1990). DA D_3 mRNA occurs in longer and shorter spliced forms generated from the same gene (Schwartz *et al.*, 1991). DA D_3 receptor gene contains five introns and encodes a 446 amino acid protein (Schwartz *et al.*, 1990). The DA D_3

receptors bear close structural and pharmacological similarities to the DA D_2 receptors. Distribution of DA D_3 receptor mRNA are distributed and expressed mainly in subcortical limbic regions including islands of Calleja, nucleus accumbens septi and olfactory tubercle, with low levels of expression in the basal ganglia (Marc *et al.*, 1998). D_3 receptor mRNA has also been found in neurons of the cerebellum, which may regulate eye-movements (Levesque *et al.*, 1992). The structural similarity with DA D_2 receptor raises the possibility that DA D_3 receptor may also inhibit adenylyl cyclase activity in its normal cellular setting. More recent studies reported that DA D_3 receptors might mediate positive regulatory influences of DA on production of the peptide neurotensin (Levesque *et al.*, 1995; Marc *et al.*, 1998). The status of the DA D_3 molecular entity as a functional receptor remains uncertain since it neither couples to G-proteins nor consistently transduces an effector mechanism (Schwartz *et al.*, 1990; Sokoloff *et al.*, 1992; Marc *et al.*, 1998).

DA D_4 receptors

The gene encoding the human DA D_4 protein is located at the tip of the short arm of chromosome 11 (Civelli *et al.*, 1992; Marc *et al.*, 1998). DA D_4 receptor gene has been localized in brain regions like hippocampus and frontal cortex using specific histoprobes (Civelli *et al.*, 1994). DA D_4 receptor gene contains four introns and encodes a 387 amino acid protein (Van *et al.*, 1991). The overall homology of the DA D_4 receptor to the DA D_2 and D_3 receptors is about 41% and 39% respectively, but this homology increases to 56% for both receptors when only the transmembrane spanning segments are considered. In humans, DA D_4 receptor occurs in several genomic polymorphic variants that contain two to eleven repeats of a 48 base pair segment that is expressed in the

third cytoplasmic domain (Marc *et al.*, 1998). These are called the DA D_4 alleles, which are represented as DA $D_{4.2}$, $D_{4.4}$ and $D_{4.7}$. These may contribute to the pathophysiology of certain neuropsychiatric disorders (Jackson & Westlind, 1994). The stimulation of DA D_4 receptor inhibits adenylyl cyclase activity and release arachidonic acid in brain neurons (Huff *et al.*, 1994; Marc *et al.*, 1998).

Effect of ethanol on brain DA receptors

Since the first report by Blum *et al.*, (1990) suggesting an association of DA D_2 receptor gene and ethanol addiction, the possible role of DA D_2 receptor locus in the etiology of ethanol addiction has been the focus of considerable attention (Noble, 2000). The brain of ethanol addicts seems to contain abnormalities that reduce the effectiveness of the dopaminergic system. Chronic ethanol consumption has been associated with an increased DA turnover rate and decreased DA uptake (Mash *et al.*, 1996). Striatal dopamine deficit is correlated with ethanol craving. Dopaminergic D_2 receptor mechanisms are involved in the biology of ethanol dependence in man (Hietala *et al.*, 1994). Human genetic studies suggest that an association exists between ethanol addiction and both the DA D_2 receptor and the DA transporter. This is supported by brain imaging studies that have reported alterations in both DA D_2 receptor and DA transporter densities in the brain of ethanol addicts (Repo *et al.*, 1999). Reward-related impulsiveness may constitute a risk factor for ethanol dependence and that this core temperament could be partly mediated by the DA D_2 gene (Limosin *et al.*, 2003). Continuous chronic or repeated deprivations increase binding sites of D_1

and D_2 receptors in specific regions of the extended amygdala (EA) with greater sensitivity in the anterior regions (Sari *et al.*, 2006).

DA receptor gene expression and ethanol

The genes encoding DA receptor subtypes have received considerable attention for the past several years as a potential candidate that may affect susceptibility to addictive disorder, including ethanol addiction (Lee *et al.*, 2002). The genomic organizations of the DA receptors demonstrate that they are derived from the divergence of two gene families that mainly differ in the absence or the presence of introns in their coding sequences. DA D_1-like receptors genes do not contain introns in their coding regions, a characteristic shared with most G protein-coupled receptors. The genes encoding the DA D_2-like receptors are interrupted by introns (Marc *et al.*, 1998). Furthermore, most of the introns in the DA D_2-like receptor genes are located in similar positions. The DA D_2 receptor gene contains seven introns that are spliced out during mRNA transcription (Fischer *et al.*, 1989). ALDH genes are involved in dopamine metabolism and they interact with the DA D_2 receptor genes in alcohol dependence (Huang *et al.*, 2004). The constitutive expression of D_2 receptor short isoform also reduced the tumor cell growth rate (Sarkar *et al.*, 2005). Dopamine acts through G-protein-coupled D_2 receptors to affect the amount of intracellular cyclic AMP (Hayes *et al.*, 1992). The DA D_1 receptor gene, which lacks any introns, encodes a protein that extends for 446 amino acids (Caron *et al.*, 1991). In humans DA D_1 receptor gene has been localized to chromosome 5 (Kennedy *et al.*, 1990). Dopamine receptor genes responsive to alcohol exposure encode proteins which

are involved in growth hormone (GH) release and its expression is altered by chronic alcohol intake (Gerhard *et al.*, 2006). DA D_2 receptors activation inhibits norepinephrine gene expression and release in the arcuate nucleus and peripheral nerves (Carey *et al.*, 1983; Pelletier *et al.*, 1991). D_2 receptor gene A1 allele shows a significantly higher prevalence in ethanol users compared with nonusers (Comings *et al.*, 1994; Noble, 1996).

Serotonin

Serotonin (5-HT) is widely distributed in both the animal and the plant kingdoms and is found in such diverse locations as tunicates, molluscs, arthropods, fruits, nuts and venoms (Erspamer, 1996). The enormous range of this single brain chemical system may reflect the vast distribution of its fibers in brain, from a small group of large multipolar neurons. Serotonin is synthesized and released from neurons that originate within discrete regions, or nuclei, in the brain (Cooper & Bloom, 1991). 5-HT may be tied to the evolution of life itself, particularly through the role of tryptophan, its precursor molecule. Tryptophan is an indole-based, essential amino acid, which is unique in its light absorbing properties. In plants, tryptophan-based compounds capture light energy for use in metabolism of glucose, the generation of oxygen and reduced cofactors. Tryptophan, oxygen and reduced cofactors combine to form 5-HT. 5-HT-like molecules direct the growth of light-capturing structures towards the source of light. In plants, tryptophan produces receptor proteins which harness light and thus produce biologically important molecules (Josefsson & Rask, 1997). Chlorophyll, for example, captures light because it contains tryptophan, and then generates ATP, reduced cofactors (NADH), and oxygen. This entire process is blocked if tryptophan is substituted with another amino acid (Mogi *et al.*, 1989).

Serotonin has effects on other neurotransmitter systems. Ascending serotonergic systems from the median and dorsal raphe innervate areas of the brain rich in DA neurons, where they regulate the firing rate and release of DA. Liu *et al.*, (1992) have shown that serotonin, through regional effects on either raphe glia or mesencephalic glia, will promote nerve growth factors affecting maturity of serotonergic neurons. 5-HT is an endogenous amine involved in diverse biologic processes within the central and peripheral nervous system and the cardiovascular and gastrointestinal and respiratory systems (Hindle, 1994). It is reported that there is a hypothalamic serotonergic receptor functional regulation mediated through $5\text{-}HT_{2C}$ receptor during pancreatic regeneration (Mohanan *et al.*, 2005 a, b). Jackson & Paulose (1999) reported a decrease in brain 5-HT content during diabetes. 5-HT has been implicated more in behaviour, physiological mechanisms, and disease processes than any other brain neurotransmitter. This diversity of actions is made possible because of the existence of specific 5-HT cell surface receptor subtypes and their coupling to distinct intracellular messenger systems or ion channels (Hoyer *et al.*, 1994). Serotonin through $5\text{-}HT_2$ receptor caused a dose-dependent increase in DNA synthesis in primary cultures of rat hepatocytes (Sudha & Paulose, 1997). The synthesis and degradation of 5-HT is a very active process and it has been estimated that the total body pool of 5-HT is replaced every 24 hours. The synthesis of 5-HT occurs primarily by enzymatic hydroxylation of the benzene ring of tryptophan to form 5-hydroxytryptophan (5-HTP) and then through decarboxylation of the terminal carbon group of 5-HTP to form 5-HT. Once inside the cells, 5-HT is degraded by monoamine oxidase to form an aldehyde, which is then hydrolysed by ALDH to form 5-HIAA, the principal metabolite excreted in urine. The neurons form a collection of clustered cells termed the

raphe nuclei, located on the exact midline of the brainstem. Serotonergic fibers interact in complex ways with a variety of cell types-neurons, glial cells, endothelial cells, ependymal cells and others by binding to at least 14 distinct receptor proteins. Furthermore, 5-HT neurons are one of the first brainstem neurons to emerge during early development of the brain and spinal cord present by the sixth week of gestation in humans. In rats, 5-HT neurons in the brainstem raphe are among the first neurons to differentiate in the brain and play a key role in regulating neurogenesis (Kligman & Marshak, 1985). The 5-HT neurons are the first neuronal system to innervate the primordial cortical plate. During development, 5-HT fibers arrive at the cortical plate during the peak period of mitosis and maturation (Dori *et al.*, 1996). Lauder & Krebs (1978) reported that para-chlorophenylalanine (PCPA), a 5-HT synthesis inhibitor, retarded neuronal maturation. Since then, many other workers have shown a role for 5-HT in neuronal differentiation (Marois & Croll, 1992; Rodriguez, 1994).

5-HT receptor classification

5-HT receptors can be classified into seven classes from 5-HT_1 to 5-HT_7, based upon their pharmacological profiles, cDNA-deduced primary sequences and signal transduction mechanisms of receptors (Bradley *et al.*, 1986; Zifa & Fillion, 1992; Peroutka, 1993). All 5-HT receptors belong to the superfamily of G-protein coupled receptors containing a seven transmembrane domain structure except 5-HT_3 receptor, which forms a ligand-gated ion channel.

5-HT$_1$ Receptor

Five 5-HT$_1$ receptor subtypes have been recognised, 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$. All are seven transmembrane, G-protein coupled receptors encoded by intronless genes, of between 365 and 422 amino acids with an overall sequence homology of 40%. 5-HT$_{1A}$ receptor subtype which is located on human chromosome 5q11 is widely distributed in the CNS, particularly hippocampus (Hoyer *et al.*, 1994). The 5-HT$_{1B}$ receptor is located on human chromosome 6q13 and is concentrated in the basal ganglia, striatum and frontal cortex. The receptor is negatively coupled to adenylyl cyclase. The 5-HT$_{1D}$ receptor has 63% overall structural homology to 5-HT$_{1B}$ receptor and 77% amino acid sequence homology in the seven transmebrane domains. The receptor is located on human gene 1p36.3-p34.3 and is negatively linked to adenylyl cyclase. The 5-HT$_{1E}$ receptor was first characterized in man as a [^{3}H] 5-HT binding site in the presence of 5-carboxyamidotryptamine (5-CT) to block binding to the 5-HT$_{1A}$ and 5-HT$_{1D}$ receptors. It is reported that the brain 5-HT through 5-HT$_{1A}$ receptor has a functional role in the pancreatic regeneration through the sympathetic regulation (Mohanan *et al.*, 2005). Human brain binding studies have reported that 5-HT$_{1E}$ receptors are concentrated in the caudate putamen with lower levels in the amygdala, frontal cortex and globus pallidus (Hoyer *et al.*, 1994). This is consistent with the observed distribution of 5-HT$_{1C}$ mRNA (Hoyer *et al.*, 1994). The receptor has been mapped to human chromosome 6q14-q15, is negatively linked to adenylyl cyclase and consists of a 365 amino acid protein with seven transmembrane domains. 5-HT$_{1F}$ receptor subtype is closely related to the 5-HT$_{1E}$ receptor with 70% sequence homology

across the 7 transmembrane domains. mRNA coding for the receptor is concentrated in the dorsal raphe, hippocampus and cortex of the rat and also in the striatum, thalamus and hypothalamus of the mouse (Hoyer *et al.*, 1994).

5-HT_2 Receptor

The 5-HT_2 receptor family consists of three subtypes namely 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}. All three are single protein molecules of 458-471 amino acids with an overall homology of approximately 50% rising to between 70-80% in the seven transmembrane domains. 5-HT_{2A} receptor previously termed as $5HT_2$ receptor is located on human chromosome 13q14-q21 and is widely distributed in peripheral tissues. It mediates contractile responses of vascular, urinary, gastrointestinal and uterine smooth muscle preparations, platelet aggregation and increased capillary permeability in both rodent and human tissue (Hoyer *et al.*, 1994). 5-HT_{2C} was previously termed as 5-HT_{1C} before its structural similarity to the 5-HT_2 family members was recognized. All three are thought to be linked to the phosphoinositol hydrolysis signal transduction system *via* the α subunit of Gq protein. It is reported the involvement of serotonin, S_2 receptors in the DNA synthesis of primary culture of rat hepatocytes (Balasubramanian & Paulose, 1998). In human pulmonary artery endothelial cells, 5-HT_{2C} receptor stimulation causes intracellular calcium release *via* a mechanism independent of phosphatidylinositol hydrolysis (Hagan *et al.*, 1995). The 5-HT_{2B} receptor located on chromosome 2q36-2q37.1 mediates contraction of the rat stomach fundus and endothelium dependent relaxation of the rat and cat jugular veins and possibly of the pig pulmonary artery, *via* nitric oxide release

(Choi & Maroteaux., 1996). 5-HT_{2B} receptor mRNA has been detected throughout the mouse, rat and guinea pig colon and small intestine. 5-HT_{2C} specific antibodies have shown the presence of the receptor protein in the choroid plexus, in higher density and at a lower density in the cerebral cortex, hippocampus, striatum, and substantia nigra of rat and a similar distribution in man. The receptor has been mapped to human chromosome Xq24. No splice variants have been reported but the receptor is capable of post translational modification whereby adenosine residues can be represented as guanosine in the second loop to yield 4 variants.

5-HT_3 Receptor

Unlike other 5-HT receptors, 5-HT_3 receptor subunits form a pentameric cation channel that is selectively permeable to Na^+, K^+ and Ca^{2+} ions causing depolarisation. The 5-HT_3 receptor is a member of a superfamily of ligand-gated ion channels, which includes the muscle and neuronal nAChR, the gly and $GABA_A$ receptor (Unwin, 1993; Karlin & Akabas, 1995; Ortells & Lunt, 1995). The 5-HT_3 receptor binding site is widely distributed both centrally and peripherally and has been detected in a number of neuronally derived cells. The highest densities are found in the area postrema, nucleus tractus solitarius, substantia gelatinosa and nuclei of the lower brainstem. It is also found in higher brain areas such as the cortex, hippocampus, amygdala and medial habenula, but at lower densities. Like the other members of the gene superfamily, the 5-HT_3 receptor exhibits a large degree of sequence similarity and thus presumably structural homology with the AChR (Maricq *et al.*, 1991).

5-HT_4 Receptor

The receptor is functionally coupled to the G protein. Receptor binding studies have established that the 5-HT_4 receptor is highly concentrated in areas of the rat brain associated with DA function such as the striatum, basal ganglia and nucleus accumbens. These receptors are also located on GABAergic or cholinergic interneurons and/or on GABAergic projections to the subtstatia nigra (Patel *et al.*, 1995).

5-HT_5 Receptor

5-HT_5 receptors have thus been classified as 5-HT_{5A} and 5-HT_{5B} and their mRNAs have been located in man (Grailhe *et al.*, 1994). Two 5-HT receptors identified from rat cDNA and cloned were found to have 88% overall sequence homology, yet were not closely related to any other 5-HT receptor family (Erlander *et al.*, 1993). In cells expressing the cloned rat 5-HT_{5A} site, the receptor was negatively linked to adenylyl cyclase and may act as terminal autoreceptors in the mouse frontal cortex (Wisden *et al.*, 1993).

5-HT_6 Receptor

Rat and human 5-HT_6 mRNA is located in the striatum, amygdala, nucleus accumbens, hippocampus, cortex and olfactory tubercle, but has not been found in peripheral organs studied (Kohen *et al.*, 1996). Like the 5-HT_5 receptor, the 5-HT_6 receptor has been cloned from rat cDNA based on its homology to previously cloned G protein coupled receptors. The rat receptor consists of 438 amino acids with seven transmembrane domains and is positively coupled to

adenylyl cyclase *via* the Gs G protein. The human gene has been cloned and has 89% sequence homology with its rat equivalent and is coupled to adenylyl cyclase (Kohen *et al.*, 1996).

5-HT$_7$ Receptor

5-HT$_7$ receptor has been cloned from rat, mouse, guinea pig and human cDNA and is located on human chromosome 10q23.3-q24.4. Despite a high degree of interspecies homology (95%) the receptor has low homology (<40%) with other 5-HT receptor subtypes.

Effect of ethanol on brain 5-HT receptors

The serotonergic system, because of very diffuse projections throughout the central nervous system, has been implicated in numerous functions including nociception, analgesia, and autonomic regulation (Jolas & Aghajanian, 1997). 5-HT systems contribute to the discriminative properties of ethanol in animals and humans. Ethanol facilitates that activity of 5-HT$_{1B}$, 5-HT$_{2C}$, 5-HT$_3$ receptors, and it shares discriminative stimulus properties with drugs acting at these sites (Grant *et al.*, 1995 & 1997). Serotoninergic system appears to be involved in ethanol consumption and reinforcement by activating dopaminergic system (Koob & Weiss 1992). Levels of brain 5-HT receptor are inversely related to ethanol consumption (Pandey *et al.*, 1992; LeMarquand *et al.*, 1994; Himei *et al.*, 2000). The m-chlorophenylpiperazine (m-CPP) is a serotonin agonist which has been reported to elicit craving for ethanol (Benkelfat *et al.*, 1991; Krystal *et al.*, 1994). Ethanol is a positive modulator at the 5-HT$_3$ receptor, which has been implicated in ethanol drinking, anxiety and aggression (McKenzie *et al.*, 2005; Hayrapetyan *et al.*, 2005) but 5-HT$_{1D}$ receptor plays little role in the

pathophysiology of ethanol addiction (Bavanisha *et al.*, 2005). Acute ethanol exposure enhances the electrical signals generated by the 5-HT_3 receptor. When activated by serotonin binding, the 5-HT_3 receptor rapidly increases neuron activity by generating electrical signals (Lovinger & Peoples, 1993; Lovinger & Zhou, 1994). Chronic ethanol treatment may decrease serotonergic neurotransmission in selective brain regions. Serotonin receptor polymorphism reflects the pathogenesis of ethanol addiction (Yoshihara *et al.*, 2000).

5-HT receptor gene expression and ethanol

Ethanol and drugs of abuse indirectly induce the expression of a number of genes, which, in the context of protein synthesis, activate several biochemical pathways in brain neurons (German *et al.*, 1999). A common insertion-deletion polymorphism in the promoter region for the serotonin transporter gene alters *in vitro* gene transcription, (Lesch *et al.*, 1996) *in vitro* transporter availability (Stoltenberg *et al.*, 2002) and *in vivo* serotonin transporter density (Heinz *et al.*, 2001). There have been several associations of this polymorphism to behaviours and traits that relate to excessive alcohol intake and serotonin transporter gene promoter variation have been associated with alcohol consumption in human and animal populations (Christina *et al.*, 2004). 5-HT_{1D} receptor mRNA is found in the rat brain, predominantly in the caudate putamen, nucleus accumbens, hippocampus, cortex, dorsal raphe and locus coeruleus (Hoyer *et al.*, 1994). Genetic variability in the 5-HT_{2A} receptor is involved in the development of ethanol dependence (Nakamura *et al.*, 1999). The human 5-HT_{1B} receptor, encoded by the 5-HT_{1B} gene, is a presynaptic serotonin autoreceptor that plays a role in regulating serotonin synthesis and release. 5-HT_{1B} receptor is associated with alcohol dependence (Sun *et al.*, 2002). Hofmann *et al.*, (2002) reported that

prenatal ethanol exposure alters 5-HT_{1A} and 5-HT_{2A} receptor function in adulthood. 5-HT_6 receptor mutant mice demonstrated reduced responses to the sedative effects of ethanol (Bonasera *et al.*, 2006)

Brain neurotransmitters and ethanol

Brain is the major target for the actions of ethanol, and heavy ethanol consumption has long been associated with the brain damage. Brain neurotransmitters through their receptors play an important role in governing the cellular activities. The acute and chronic ethanol ingestion has been shown to induce significant changes in neurotransmitter systems (Nevo & Hamon, 1995). Ethanol can pass through cell walls and is distributed throughout the water content of tissues and cells. In its circulation through the body and reaches the brain. Multiple neurotransmitter systems play a role in mediating the behavioural effects of ethanol that have been linked to its abuse and dependence (Koob & Weiss, 1992). At the neurochemical level, the moderate consumption of ethanol selectively affects the function of GABA, glutamatergic, serotonergic, dopaminergic, cholinergic, and opioid neuronal systems. Ethanol can affect these systems directly, and/or the interactions between and among these systems become important in the expression of ethanol's actions (Eckardt *et al.*, 1998).

DA and 5-HT

Ethanol is similar to other abused substances in that it increases nucleus accumbens (NAcc) DA release, Furthermore, innate differences in central dopaminergic neurotransmission have been linked to high levels of ethanol drinking in selectively bred rodent lines (Li, 2000). Alterations of DA activity within the Etended Amygdala (EA) after chronic exposure to ethanol or

substances of abuse are considered a major mechanism for the development of ethanol addiction (Sari *et al.*, 2006). Neuronal DA receptors are widely distributed in the central (Kebabian *et al.*, 1979) and the peripheral nervous system at different levels. On the other hand, DA and 5-HT interact antagonistically in the dorsal striatum to control motor activity. Serotonin is one of the major neurotransmitter involved in ethanol addiction *in vivo* (Tank *et al.*, 1981). Along with other neurotransmitters serotonin play an important role in the brain process underlying ethanol abuse (David, 1999). 5-HT_2 agonists, as well as serotonin reuptake inhibitors, have been found to substitute for ethanol in drug discrimination tests (Signs & Schechter, 1988; Maurel *et al.*, 1997). 5-HT_3 activity is probably responsible for the nausea with excessive ethanol consumption (Wilde & Markham 1996). It is also likely to partially account for increased dopamine release as antagonists have been shown to block ethanol induced dopamine release (Carboni *et al.*, 1989; Badawy *et al.*, 1995). Serotonin can alter dopaminergic signal transmission in several ways. For example, by interacting with the 5-HT_2 receptor, serotonin stimulates the activity of dopaminergic neurons in a brain region called the VTA, thereby enhancing an ethanol-induced increase in the activity of these neurons (Brodie *et al.*, 1995). Serotonin also interacts with dopaminergic signal transmission through the 5-HT_3 receptor, which helps control dopamine release in the areas reached by VTA neurons, most notably the nucleus accumbens. Serotonin release in these brain regions can stimulate dopamine release, presumably by activating 5-HT_3 receptors located on the endings of dopaminergic neurons (Grant, 1995). 5-HT depletion resulted in increased ethanol consumption in animals and humans (Melchior & Tabakoff, 1986; Higley *et al.*, 1996; Jankowska *et al.*, 1994).

Dopamine's precise role in the development of ethanol addiction remains unclear (Rassnick *et al.*, 1993; Di Chiara, 1995).

Acetylcholine

Acetylcholine is the neurotransmitter of the parasympathetic system. Acetylcholine, acting on presynaptic nAChRs, modulates the release of neurotransmitters in the brain (Centeno *et al.*, 2006). Cholinergic receptors are classified as ionotropic nicotinic receptor and metabotropic muscarinic receptor. Muscarinic receptors are classified as M_1, M_2, M_3, M_4 and M_5. They are G-protein coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions that interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects. Ethanol enhances the activity of alpha4beta2 neuronal nicotinic acetylcholine receptor and support the possibility that a polymorphism of the nicotinic acetylcholine receptor alpha4 subunit gene (CHRNA4) modulates enhancement of nicotinic receptor function by ethanol (Kim *et al.*, 2004). Increased muscarinic M_1 and M_3 receptor activity at the time of pancreatic regeneration is reported to facilitate insulin secretion and beta cell proliferation (Renuka *et al.*, 2005). The striatum receives converging glutamatergic input from cortex and thalamus as well as dopaminergic input from the substantia nigra. Integration of these extrinsic inputs is modulated by the intrinsic actions of acetylcholine (ACh). Striatal ACh is supplied by large-sized cholinergic interneurons the functions of which are still not well characterized (Kawaguchi, 1993). At the cellular level, both striatal ACh and DA are potent neuromodulators that can affect activity-dependent changes in synaptic efficacy and may contribute to motor or habit learning (Wickens *et al.*, 1996; Calabresi *et al.*, 1992, 2000; Tang *et al.*, 2001).

The stimulatory, rewarding, and DA enhancing effects of ethanol involve central nAChR, especially those located in the ventral tegmental area (VTA) (Jerlhag *et al.,* 2006). AChRs are expressed at high levels in striatum. Muscarinic acetylcholine receptors are expressed both presynaptically and postsynaptically in striatum, and one of their actions is to decrease glutamatergic synaptic transmission (Malenka & Kocsis, 1988; Hersch *et al.,* 1994). nAChRs are expressed on dopaminergic terminals in the dorsal striatum (Clarke & Pert, 1985). Acute activation of these receptors stimulates DA release from striatal synaptosomes and in striatal slice preparations (Giorguieff *et al.,* 1976; Kulak *et al.,* 1997; Wonnacott *et al.,* 2000). The cholinergic system is yet another target for the actions of ethanol (Narahashi *et al.,* 1999) and has been found to act as a co-agonist with acetylcholine at the nAChRs, as well as to potentiate the effect of nicotine at this receptor, both of which ultimately results in an increase in mesolimbic dopamine (Soderpalm *et al.,* 2000).

Epinephrine and Norepinephrine

The sensitivity of noradrenergic systems to ethanol effects varies among brain regions (Tabakoff & Hoffman, 1996). Ethanol consumption increases central and peripheral levels of epinephrine (EPI) and norepinephrine (NE), which contributes to the stimulatory affects of ethanol, particularly in the ascending arm of the blood ethanol curve (Pohorecky, 1982), brain levels of norepinephrine have been shown to increase up to three-fold (Wang *et al.,* 1993). It is reported a significant increase in the NE content in the brainstem during diabetes (Jackson *et al.*, 1997, 1999). The locus coeruleus (LC) contains the cell bodies for the brain dorsal noradrenergic system (Grzanna & Molliver, 1980). LC basal activity and activation are reduced by ethanol, an action that may contribute

to sedative effects of ethanol (Aston-Jones *et al.,* 1982; Shefner & Tabakoff, 1985). These elevations occur primarily due to increased release and decreased clearance, rather than increases in synthesis (Howes *et al.,* 1986). A consequence of this is eventual depletion of epinephrine and norepinephrine in the adrenals after 4 days of ethanol intoxication (Adams & Hirst, 1984). This decrease contributes to the CNS depression that occurs with prolonged drinking. Changes in the levels of DA, 5-HT, NE, and their metabolites in several regions of the rodent brain, many of them involved ethanol treatment for a short period of time and withdrawal (Yan, 1999; Yoshimoto *et al.,* 2000). Ethanol activates the norepinephrine system in the limbic circuitry through an intercellular cascade that includes serotonin, opioid peptides and dopamine. Ethanol may also act directly through the production of neuroamines that interact with opioid receptors or with dopaminergic systems (Alvaksinen *et al.,* 1984; Blum & Kozlowski, 1990). Central alpha1-adrenergic receptors have a functional role in the pancreatic regeneration mediated through the sympathetic pathway (Ani *et al.,* 2006). Ethanol has a variety of effects on neuroendocrine function and there is a great deal of interest in investigating the effects of ethanol on the HPA axis (Rivier *et al.,* 1984). The by-products of ethanol metabolism include acetaldehyde, which may have an inhibitory effect on the adrenergic receptors. Increased cyclic adenosine monophosphate in neurons with long term ethanol exposure may increase norepinephrine receptor sensitivity and norepinephrine turnover (Keltner *et al.,* 1998). α-Adrenergic stimulation attenuates ethanol intoxication, whereas β-adrenergic blockade enhances intoxication (Alkana *et al.,* 1976 & 1977).

Gamma aminobutyric acid and glutamate

GABA system - the body's primary inhibitory pathway (Meldrum, 1982), ethanol potentiates GABA's activity (Suzdak *et al.,* 1986) acting through $GABA_A$ receptors. It likely has a biphasic effect on behaviour, with lower doses inhibiting inhibitory GABA interneurons on dopamine receptors in the VTA thus causing dopamine induced stimulation and euphoria, and higher doses producing widespread inhibition of CNS activity, thus overriding the stimulant effects (Kalivas *et al.,*1990; Grobin *et al.,* 1998). This is likely one of the major mechanisms through which it produces its sedative-hypnotic and anxiolytic actions. One of the most powerful actions of ethanol is to reduce the overall level of brain activity by a combination of effects on two key neurotransmitters, GABA and glutamate. Ethanol reduces the excitatory effects of glutamate. The n-Methyl-d-Aspartate (NMDA) receptor is one of three types of glutamate receptors - the body's primary excitatory neurotransmitter. It is named for NMDA, its synthetic, high-affinity ligand (Woodward, 2000), ethanol has been found to block the action of this receptor (Dildy & Leslie, 1989). The likely mechanism is by preventing glutamate's removal of a magnesium ion which blocks calcium influx into the cell (Collingridge & Bliss, 1995). This decreases the excitation of the cell, which, along with increased inhibition *via* GABA, results in the sedative-depressant effects of ethanol, particularly at higher doses. Chronic consumption of ethanol gradually makes the NMDA receptors hypersensitive to glutamate while desensitizing the GABAergic receptors.

Liver and ethanol

Ethanol effects on the human body and its health, the liver plays a particular important role (Yue *et al.*, 2006) and the hepatic enzymatic systems involved in ethanol metabolism are ADH, ALDH and microsomal P4502E1 (CYP2E1) (Gemma *et al.*, 2006). Acute ethanol intoxication may cause the changes of hepatic enzymes (Rakonczay *et al.*, 2003; Yue, 2006). The intragastric administration of ethanol induced some morphological disturbances in the liver (Zimatkin *et al.*, 1997). Ethanol metabolism causes oxidative stress (Rakonczay *et al.*, 2003) and lipid peroxidation not only in liver but also in extra-hepatic tissues. Ethanol administration has been shown to cause oxidative degradation and depletion of hepatic mitochondrial DNA (mtDNA) (Abdellah *et al.*, 2001). Chronic ethanol-induced decrease in the NAD dependant glycerol 3-phosphate dehydrogenase reaction was due to a decreased rate of NADH reoxidation in the liver (Manfred *et al.*, 1998). In the rat liver, both mitochondrial and cytosolic ALDH are functional (Klyosov *et al.*, 1996). Acetaldehyde, the first metabolite of ethanol, is produced in the liver following the first step of ethanol metabolism and is ten times more toxic than ethanol (Brien & Loomis, 1983). Acetaldehyde appears to mediate some of the behavioural & central neurotoxic effects of ethanol (Hunt, 1996).

Aldehyde dehydrogenase and ethanol

Mutations in ALDH genes cause inborn errors of metabolism such as the Sjogren-Larsson syndrome, type II hyperprolinaemia and gamma-hydroxybutyric aciduria and are likely to contribute to several complex diseases, including cancer and Alzheimer's disease. The ALDH gene products appear to be multifunctional

proteins, possessing both catalytic and non-catalytic properties (Vasiliou & Nebert, 2005). The aldehyde dehydrogenase, the primary enzyme responsible for acetaldehyde metabolism, is highly correlated with voluntary ethanol consumption in several strains of rats and mice (Schlesinger *et al.*, 1966; Sheppard *et al.*, 1968; Amir, 1978; Socaransky *et al.*, 1984). Also, this enzyme has been reported in mitochondria, microsomes and cytosol of rat liver (Tottmar *et al.*, 1973). In some oriental populations with a lowered genetic activity of ALDH, high blood concentrations of acetaldehyde are produced following ethanol ingestion (Enomoto *et al.*, 1991). As acetaldehyde is a highly toxic metabolite, it can cause adverse symptoms in susceptible individuals, including nausea, headache and palpitations (Enomoto *et al.*, 1991). These individuals consume less ethanol than people who have normal activity of ALDH (Higuchi *et al.*, 1992) and interestingly accumulation of acetaldehyde in blood following ethanol ingestion, due to a lower activity of ALDH, is believed to play a protective role against ethanol addiction (Harada *et al.*, 1982). Although there are several reports that ethanol preference may correlate with ALDH activity more in the brain than in the liver (Amir, 1978; Socaransky *et al.*, 1984), this mechanism is still relatively unknown (Minori *et al.*, 2002). As acetaldehyde itself has many pharmacological actions (Brien & Loomis, 1983), it may act on the central nervous system (Kinoshita *et al.*, 2001). Diadzin (*Radix puerariae*) an antidipsotropic agent could disturb an as-yet-undefined physiological pathway catalyzed by ALDH and alter the concentrations of some endogenous substrate(s) that regulate ethanol drinking behaviour. Rat liver mitochondrial preparations contained no detectable amounts of endogenous 5-HT, DA or any of their known metabolites (Wing, 1998). It is reported that oral treatment with the ALDH inhibitor disulfiram decreased ethanol preference (He *et al.*, 1997). Early interest

in biogenic aldehydes, the metabolic intermediate of ethanol, interferes in some way with the oxidative metabolism of the brain. Epidemiological studies also have associated low MAO and/or high ALDH activities with high ethanol consumption (von Knorring, 1985), where differences in acetaldehyde elimination may contribute to ethanol preference. Brain plays an important regulatory role in hepatic functions (Lautt, 1983). The liver is richly innervated (Rogers & Hermann, 1983). Acetaldehyde produced from ethanol is metabolized quickly to acetate by ALDH. Brain monoamines and ALDH level together plays a decisive role in the ethanol addiction and ethanol addiction. 5-HT and its metabolic intermediates differentially regulate ethanol drinking. Serotoninergic system appears to be involved in ethanol consumption and reinforcement by activating dopaminergic reward system (Weiss, 1992). With long-term use, adolescent rats have shown massive neuronal loss in their cerebellum, basal forebrain, and neocortex (Spear, 2002). Endogenous DA plays a modulatory role on sympathetic nerve terminals through these receptors. ALDH genes involved in dopamine metabolism and ALDH genes interact with the DA D_2 receptor gene and there is association between the DA D_2 receptor gene and alcohol dependence (Huang *et al.*, 2004). Strong ethanol preferences are associated with reduced serotonergic functions either directly or indirectly by increasing DA neurotransmission particularly in the ventral striatum (Koob, 1992). By speeding up the metabolism of ethanol to a toxic intermediate, acetaldehyde, or slowing down the conversion of acetaldehyde to acetate, genetic variants in the enzymes ADH or ALDH raise the level of acetaldehyde after drinking, causing symptoms that include flushing, nausea, and rapid heartbeat. The genes for these enzymes and the alleles, or gene variants that alter ethanol metabolism have been identified (Makimoto, 1998; Li, 2000). Ethanol metabolism is impaired by a

nonfunctional form of the enzyme aldehyde dehydrogenase (Wall & Ehlers, 1995) and consumption of even small amounts of ethanol may be severe (Goedde *et al.*, 1992). Cyanamide (CY), a potent ALDH inhibitor in the liver, as well as in the brain (Hellstrom & Tottmar, 1982). The brain inhibition may alter the metabolism of biogenic amines by promoting the formation of condensation products or by increasing the levels of biogenic aldehydes. Extracellular concentration of both DA and 5-HT significantly decreased in the nucleus accumbens after acute intraperitoneal injection of acetaldehyde to rats (Ward *et al.*, 1997).

Nervous system and hepatic functions

The autonomic nervous system influences many of the functions of the body, including those of cardiovascular system, kidneys, liver, pancreas, gastrointestinal tract and glands (Berthoud & Neuhuber, 2000). Brain plays an important regulatory role in hepatic functions (Lautt, 1983), signalling occurs between the liver and brain (Kerfoot *et al.*, 2006). Normal brain functioning depends on several aspects of normal liver functioning; the liver supplies certain nutrients to the brain that the brain itself cannot produce. The liver also cleanses the blood of substances that could damage brain cells (i.e., neurotoxins). Liver dysfunction is associated with more extensive brain dysfunction in liver cirrhosis patients (Tarter *et al.*, 1993). The autonomic nervous system directly innervates the hepatic parenchyma and has a role in metabolic control (Jungermann, & Stumpel, 1999). The autonomic nervous system plays a significant role in liver physiology and pathology (Stoyanova & Gulubova, 1998). After receiving information from afferent nerves, the hypothalamus sends signals to peripheral organs, including the liver, to keep homeostasis (Uyama *et al.*, 2004). The liver

has innervations of nerves from the central nervous system. In the liver, the autonomic nervous system plays an important role (Stoyanova & Gulubova, 2000). The degree of liver dysfunction was associated with increasing severity of autonomic dysfunction (Frokjaer *et al.*, 2006). Increased brain GABAergic neurotransmission is reported to regulate hepatic cell proliferation through the sympathetic stimulation (Biju *et al.*, 2002). Hypothalamus controls liver functions by neural and neuroendocrine connections. The hypothalamus consists of three major areas: lateral, medial, and periventricular. Each area has some nuclei. There are two important nuclei and one area in the hypothalamus that send out the neural autonomic information to the peripheral organs. In addition to direct neural connections, the hypothalamus can affect metabolic functions by neuroendocrine connections: the hypothalamus-pancreas axis, the hypothalamus-adrenal axis, and the hypothalamus-pituitary axis (Uyama *et al.*, 2004). Central nervous system modulates liver functions through the autonomic nervous system (Takayoshi, 2002). Miyajima *et al.*, (2001) & Pozzi *et al.*, (2001) reported that patients with liver cirrhosis have parasympathetic hypofunction and sympathetic hyperfunction. The hepatic parenchyma has been shown to have parasympathetic and sympathetic innervations (Nobin *et al.*, 1978; Carobi & Magni *et al.*, 1981; Rogers & Hermann, 1983). A selective 5-HT_2 receptor agonist, 1-(2, 5-di-methoxy-4-iodophenyl)-2-aminopropane (DOI) (Glennon, 1987) produced a tremendous increase in sympathetic nerve discharge (McCall *et al.*, 1987). Enhanced $GABA_B$ receptor was reported in neoplastic rat liver and hepatocyte cultures (Biju *et al.*, 2002). Sympathetic nervous system inhibition increases hepatic progenitors (Oben *et al.*, 2003). Mobilisation of 5-HT in intestine and its accumulation in liver and spleen tissues were observed at the initial periods after partial hepatectomy (Kulinskii *et al.*, 1983). One subset of central nervous system

5-HT receptors (5-HT_{1A}) can inhibit sympathetic nerve discharge while a second subset of receptors (5-HT_2) can increase sympathetic nervous discharge (McCall & Harris, 1988). Hypothalamic GABA receptor subtypes was suggested to regulate hepatic cell proliferation (Biju *et al.*, 2001). The central vagal connection with adrenergic and serotonergic innervations reaches the liver through the brainstem. The oxidation of fatty acids is the main energy source for the liver. Together with ethanol, isolated liver cells have a decreased oxidation of fatty acids. This is caused by the increased NADH: NAD^+ ratio which can result in a decreased activity of the enzymes responsible for the β-oxidation (Forsell, 1981). The activity of the citric acid cycle decreases if the level of the cofactor NAD^+ is too low. In that case, hydrogen equivalents from ethanol are used by the mitochondria instead of from the oxidation of fatty acids. This decrease of fatty acid oxidation may cause accumulation of fatty acids in the liver (Swanson & Sawchenko, 1980). These reports underlined the role of substantia nigra in modulating the outflow of both sympathetic and parasympathetic signals that ultimately reach the liver. Thyrotropin-releasing hormone (TRH) acts in the medulla, in particular in the left dorsal vagal complex, to induce stimulation of hepatic blood flow and hepatic proliferation, and protect against experimental liver injury through vagal and cholinergic pathways and neuropeptides such as beta-endorphin and bombesin in the brain modulate hepatic proliferation and bile secretion (Yoneda *et al.*, 2001). TRH acts in the brain to increase hepatic cAMP through vagal-cholinergic and prostaglandin-dependent pathways, suggesting that central TRH modulates hepatic functions through cAMP-mediated signalling pathways (Yoneda *et al.*, 2005). Hepatic encephalopathy is characterized by disturbances of motor and cognitive functions involving the basal ganglia (Sergeeva *et al.*, 2005). CRF acts in the brain to decrease hepatic surface

perfusion and elevate portal pressure through central CRF_2 receptor and sympathetic-noradrenergic pathways (Yoneda *et al.*, 2005).

Nervous System and ALDH

Rats consumed intoxicating quantities of ethanol when it was substituted for water (Lester, 1961; Senter & Sinclair, 1967; Everett & King, 1970; Falk *et al.*, 1972; Freed, 1972; Meisch & Thompson, 1972; Ogata *et al.*, 1972; Samson & Falk, 1974;). ALDH is responsible not only for the metabolism of exogenous ethanol, but also for the oxidation of biogenic aldehydes in the central nervous system and in the periphery (Mostofa *et al.*, 2003). It is known that a number of aldehydes occur in brain tissue (Blaschko *et al.*, 1937; Pugh & Quastel, 1937) first presented evidence that aldehydes arise in brain tissue by the oxidative deamination of monoamines. Brain ALDH plays an important role in the biosynthesis of biogenic amines (Tipton *et al.*, 1977), which may be one of the important factors in modifying ethanol-induced behaviour. Three types of nerve endings are reported with in the liver. They are the sympathetic, parasympathetic and peptidergic nerves. The neurotransmitters found in these nerves are catecholamines, serotonin, acetylcholine, vasoactive intestinal polypeptides and cholecystokinin respectively. The nerve fibres enter the liver in association with the vascular supply. The peptidergic nerves are present in both the exocrine and endocrine tissues of this gland and there is considerable interspecies variability as to which part receives a greater proportion of these fibres. The nerve terminals end approximately 20-30nm from the endocrine cells thus implying that neurotransmitters affect several cells by diffusing through the extracellular space. The substantia nigra is one autonomic area in the central nervous system which plays an important role in controlling structure and activity of liver. Adaptation

in the ethanol metabolizing enzymes, explicitly of those enzymes responsible for the metabolism of ethanol's primary metabolite acetaldehyde is the critical factor on inclination towards ethanol preference (Ewing *et al.*, 1974; Mizoi *et al.*, 1979; Zeiner *et al.*, 1979). Aldehyde dehydrogenase polymorphism results in change of effects of acetate and acetate-generated adenosine on the central nervous system and other organs during chronic ethanol consumption (Matsumoto, 1996). Hypothalamic origin of hypothyrodism and hypertension mediated through sympathetic stimulation was reported in pyridoxine deficient rats (Dakshinamurti *et al.*, 1986; Paulose *et al.*, 1988). The hypothalamic paraventricular nucleus has direct connections with the dorsal vagal complex mutation in the human fatty aldehyde dehydrogenase has been linked to a fetal neurological disorder called Sjogren-Larsson syndrome, and a change in ALDH activity has been observed in a number of tumors, including those of the liver, colon and breast. In short, ALDH is a vital enzyme involved with numerous processes of animal and plant health, most interestingly ALDH is involved in both biogenic amine metabolism (Berger & Weiner, 1977) and oxidation of biogenic aldehydes (Mostofa *et al.*, 2003).

Ethanol perfusion and ALDH

The liver perfusion model has a great advantage over isolated and cultured hepatocytes techniques, as the hepatic architecture, polarity and the integrity of the cytoskeleton is maintained (Shattuck *et al.*, 1993; Vom *et al.*, 1995). Perfused liver appears to be a useful system for studies of enzymes like ADH - independent oxidation of alcohol (Cronholm *et al.*, 1992). Desmoulin *et al.*, (1987) reported that the perfusion of the liver with 70 mM ethanol not

change the adenine nucleotide levels, while the Pi content is decreased by 10%. More than 80% of ethanol was taken into the isolated rat liver and recovered as free acetate in the perfusate (Yamashita *et al.*, 2001). The activity of ALDH in hepatic mitochondria was decreased by approximately 75% in carbon tetrachloride-intoxicated rat liver perfusion system (Yuki, *et al.*, 1984). Glucose production decreased as a result of infusion of an amino acid mixture (Ali *et al.*, 2000). It is reported that the ATP level significantly decreased at the beginning of the ethanol perfusion (Marie *et al.*, 2004). Infusion of amino acid solutions caused an increase in glucose concentration was also found in the rat liver. Hepatic glucose release increased with increased amino acid uptake (Freetly *et al.*, 1999). Liver infusion of glutamine or alanine alone increases glucose production by approximately 400% (Ali *et al.*, 2006). Ethanol perfusion induces an increase in the *in situ* mitochondrial ATP/O ratio in the whole liver (Marie *et al.*, 2002). The secretion of apoprotein B (ApoB) from the perfused liver was inhibited by noradrenaline or ATP (Yamauchi *et al.*, 1998). A study on hepatic respiration and glycolysis in perfused rat livers showed ethanol decreased the rate of lactate and pyruvate production reflecting an inhibition of glycolysis irrespective of whether glycogen or added glucose was the substrate (Thurman & Scholz, 1977). Acetaldehyde metabolism during ethanol oxidation has been studied in perfused rat livers and observed ethanol metabolism was regulated by both the ethanol and acetaldehyde oxidation rates (Eriksson, 1977).

Ethanol mediated electrophysiological changes

Ethanol ingestion has an effect on the CNS. The electroencephalogram (EEG) reading is a measure of spontaneous electrical activity in the brain (Tabakoff & Hoffman, 1988; Devor & Cloninger, 1989). Ethanol use impairs the

performance of a variety of frontal lobe-mediated tasks, like those that require planning, decision making, and impulse control (Weissenborn & Duka 2003; Burian *et al.,* 2003), but the underlying mechanisms are not known. Reports suggest that baseline blood flow to the frontal lobes increases during acute ethanol intoxication (Volkow *et al.,* 1988; Tiihonen *et al.* 1994), metabolism in the frontal lobes decreases (Wang *et al.,* 2000) and ethanol reduces the amount of activity that occurs when the frontal lobes are exposed to pulses from a strong magnetic field (Kahkonen *et al.,* 2003). The evidence suggests that acute intoxication alters the normal functioning of the frontal lobe. EEG patterns have been shown to be different in ethanol addicts and controls. Monozygotic twins have been shown to have almost identical EEG responses to ethanol (Tabakoff & Hoffman, 1988). Subjects at high risk for ethanol addiction can be differentiated from controls on the basis of their EEG alpha activity (Pollock *et al.,* 1983). Ethanol addicted subjects had greater increases of slow alpha activity and greater decreases of fast alpha activity after ethanol intake than controls. The high risk subjects also showed greater decreases in mean alpha frequency after ethanol intake. Neurophysiological measures, such as decreased P300 amplitude and altered EEG alpha activity, have been associated with increased ethanol addiction risk. The differences observed suggest that increased cortical P1 amplitude and altered cortical EEG activity in the 8-50 Hz frequency range may be neurophysiological risk factors associated with high ethanol consumption in mice (Slawecki, 2003). Kahkonen *et al.,* (2003) reported that ethanol-induced differences were most pronounced at anterior electrodes.

In the present study control and ethanol treated rats were used to study the functional correlation of dopamine and serotonin through DA D_2 & 5-HT_{2A} receptor subtypes on ALDH activity. Real-Time PCR studies were carried out to

confirm the DA D_2 & 5-HT_{2A} receptor binding parameters. Perfusion studies were done to analyse the effect of dopamine, serotonin and glucose on ALDH activity. Also, the brain activity in ethanol treated rats was studied using electroencephalogram to asses the functional difference in this animal model.

MATERIALS AND METHODS

MATERIALS AND METHODS

BIOCHEMICALS AND THEIR SOURCES

Biochemicals used in the present study were purchased from Sigma Chemical Co., St. Louis, U.S.A. All other reagents were of analytical grade purchased locally. Ethyl alcohol used for this study was purchased from Hayman Ltd. England. HPLC solvents were of HPLC grade obtained from MERCK, India.

Important chemicals used for the present study

i) Biochemicals: (Sigma Chemical Co., USA.)

5-Hydroxytryptamine (5-HT), (±)norepinephrine, sodium octyl sulphonate, ethylenediamine tetra acetic acid (EDTA), (±)epinephrine, dopamine, homovanillic acid (HVA), NAD^+, propionaldehyde, 5-hydroxytryptophan, 5-hydroxy indole acetic acid (5-HIAA), ethylene glycol bis (β-aminoethyl ether)-EGTA, ascorbic acid, pargyline, Tris buffer, calcium chloride, acetonitirle (HPLC grade), Tris HCl, perchloric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, sucrose.

ii) Radiochemicals.

[^{3}H] YM-09151-2 (cis-N-(1-benzyl-2-methylpyrrolidine-3-yl)-5-chloro-2-methoxy-4-methyl aminobenzamide (Sp.activity 85Ci/mmol) NEN Life Science Products, Inc. Boston, USA, [^{3}H](±)2, 3-dimethoxyphenyl-1-[2-(4-piperidine)-methanol] ([^{3}H]MDL100 907 (Sp.activity 82.0Ci/mmol) was purchased from Amersham Biosciences, UK.

iii) Molecular biology chemicals

Random hexamers, Taq DNA polymerase, human placental RNAse inhibitor, dNTPs were purchased from Bangalore Genei Pvt. Ltd., India. MuMLV was obtained from Amersham Life Science, UK. TRI-reagent kit was purchased from Sigma Chemical Co., USA. PCR primers used in this study was synthesised by Sigma Chemical Co., USA, Genemed Synthesis Inc., San Fransisco, USA. Real-Time PCR SyBr Green mix was purchased from Takara, Japan.

Animals

Adult Wistar rats of 180 to 200g body weight were purchased from Amrita Institute of Medical Sciences, Cochin and used for all experiments. All animals were housed in separate cages under 12 hours light and 12 hours dark periods and controlled temperature with free access to water/ethanol and food.

Ethanol treatment to animals

Ethanol treated rats were given free access of ethanol 15% and control rats were given water *ad libitum,* for 15 days .

Sacrifice and tissue preparation

The rats were sacrificed by decapitation on the 15^{th} day of the experiment. The brain dissection was carried out on a chilled glass plate into brainstem, cerebral cortex, corpus striatum, cerebellum and hypothalamus according to the procedure of Glowinski & Iversen (1966). The tissues were stored at $^{-}70^{\circ}C$ for various experiments.

Kinetic studies of aldehyde dehydrogenase in control and ethanol treated rats

Aldehyde dehydrogenase activity was assayed in brain regions and liver homogenates by the modified procedure of Gill *et al.*, (1996). ALDH activity was measured using a Shimadzu UV1201 Spectrophotometer at 25°C at 340 nm. 1 ml of assay mixture contained 0.1 M sodium pyrophosphate buffer pH 8.4, 1.0mM EDTA, and 5.0mM dithiothreitol, 1.0mM NAD^+, 10mM pyrazole. Propionaldehyde concentration varied with same NAD^+ concentration. One Unit of activity is defined as 1μmole NADH formed /minute. The results were expressed as Units/mg protein.

HPLC quantification of DA and 5-HT in liver and various brain regions of control and ethanol treated rats

Brain DA and 5-HT, their respective metabolites were estimated by HPLC connected with an electrochemical detector (Paulose *et al.*, 1988). The tissues from liver and brain regions were homogenized in 0. 4 N perchloric acid. The homogenate was centrifuged at 5000 x g for 10 minutes at 4°C (Heraeus Refrigerated Centrifuge, Japan) and the clear supernatant was filtered through 0.22μm HPLC grade filters and used for HPLC analysis in Shimadzu HPLC system with electrochemical detector fitted with C-18-CLS-ODS reverse phase column. Mobile phase was 75mM sodium dihydrogen orthophosphate buffer pH 3.25 containing 1mM sodium octyl sulphonate, 50mM EDTA and 7% acetonitrile, filtered through 0.22μM filter delivered at a flow rate of 1.0 ml/minute. Quantification was by electrochemical detection, using a glass carbon electrode set at + 0.80 V. The peaks were identified by relative retention time

compared with standards and concentrations were determined using a Shimadzu integrator interfaced with the detector.

Dopamine D_2 receptor binding studies using [^{3}H] YM-09151-2 in cerebral cortex and brainstem of control and ethanol treated rats.

Dopamine D_2 receptor binding assay was done according to the modified procedure of Unis *et al.*, (1997) & Madras *et al.*, (1988). The dissected cerebral cortex and brainstem were weighed and homogenized in 10 volumes of ice cold 50mM Tris-HCl buffer pH.7.4, along with 1mM EDTA, 5mM $MgCl_2$, 1.5 mM $CaCl_2$, 120mM NaCl, 5mM KCl. The homogenate was centrifuged at 48,000xg for 30 min. The pellet was washed and recentrifuged with 50 volumes of the buffer at 48,000xg for 30 min. This was suspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.25nM-2.0nM of [^{3}H] YM-09151-2 in 50mM Tris-HCl buffer, along with 1mM EDTA, 5mM $MgCl_2$, 1.5 mM $CaCl_2$, 120mM NaCl, 5mM KCl with 10μM pargyline and 0.1% ascorbic acid in a total incubation volume of 250μl containing 200-300μg of protein. Specific binding was determined using 5.0 μM unlabelled sulpiride. Competition studies were carried out with 0.25nM [^{3}H] YM-09151-2 in each tube with unlabelled sulpiride concentrations varying from 10^{-12} - 10^{-3} M. Tubes were incubated at 25^{0}C for 1 hr and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 5.0ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Dopamine D_2 receptor binding studies using [^{3}H] YM-09151-2 in corpus striatum, cerebellum and hypothalamus of control and ethanol treated rats.

Dopamine D_2 receptor binding assay was done according to the modified procedure of Madras *et al.*, (1988) & Green *et al.*, (1990). The dissected brain tissues corpus striatum, hypothalamus, and cerebellum were weighed, homogenised in 10 volumes of ice cold 0.32M sucrose in a Potter-Elvejhem homogeniser. The homogenate was centrifuged at 900xg for 10 min and the supernatant was again centrifuged at 17,000xg for 1hr. The pellet was washed twice and centrifuged at 17,000xg for 1hr with 50 volumes of 50mM Tris HCl, pH 7.5 and the final pellet was resuspended in a minimum volume of 50mM Tris HCl, pH 7.7 containing 4mM $CaCl_2$.

Binding assays were done using different concentrations i.e., 0.25nM - 2.5nM of [^{3}H]YM-09151-2 in 50mM Tris Buffer, pH 7.7 containing 4mM $CaCl_2$, 0.2% ascorbate and 10μM pargyline in a total incubation volume of 125μl. containing 100-150μg of protein. Specific binding was determined using 50μM unlabelled sulpiride. Competition studies were carried out with 1.0nM [^{3}H] YM-09151-2 in each tube with unlabelled sulpiride concentrations varying from 10^{-10} - 10^{-3} M.

Tubes were incubated at 25°C for 1 hr and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 3.0ml of ice cold 50mM Tris buffer, pH 7.7. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

5-HT_{2A} Receptor binding studies using [^{3}H] MDL 100907 radioligand in brain regions of control and ethanol treated rats.

5-HT_{2A} Receptor binding assay was done according to the modified procedure of Green *et al.*, (1990). The brain regions were homogenised in 10 volumes of ice cold 0.32M sucrose in a Potter-Elvejhem homogeniser. The homogenate was centrifuged at 900xg for 10 min and the supernatant was again centrifuged at 17,000xg for 1hour. The pellet was resuspended in 50 volumes of 50 mM Tris HCl, pH 7.5 and recentrifuged at 17,000xg for another 1hour. The final pellet was resuspended in a minimum volume of 50mM Tris HCl, pH 7.7 containing 4mM $CaCl_2$.

Binding assays were done using different concentrations i.e., 0.5nM-3.0nM of [^{3}H] MDL 100907 in 50mM Tris Buffer, pH 7.7 containing 4mM $CaCl_2$, 0.2% ascorbate and 10μM pargyline in a total incubation volume of 125μl containing 125-200μg of protein. Specific binding was determined using 100μM unlabelled ketanserin. Competition studies were carried out with 0.5 nM [^{3}H] MDL 100907 in each tube with unlabelled ketanserin concentrations varying from 10^{-10}-10^{-3} M. Tubes were incubated at 37 ^{0}C for 30 min. and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 3.0ml of ice cold 50mM Tris buffer, pH 7.7. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

5-HT_{2A} Receptor binding studies using [^{3}H] MDL 100907 radioligand in liver of control and ethanol treated rats.

5-HT_{2A} Receptor binding assay was done according to the modified procedure of Green *et* al., (1990). The liver was homogenised in 10 volumes of ice cold 0.32M sucrose in polytron homogeniser. The homogenate was centrifuged at 900xg for 10 min and the supernatant was again centrifuged at 17,000 x g for 1hour. The pellet was resuspended in 50 volumes of 50 mM Tris HCl, pH 7.5 and recentrifuged at 17,000xg for another 1hour. The washing step was repeated for 3-4 times. The final pellet was resuspended in a minimum volume of 50mM Tris HCl, pH 7.7 containing 4mM $CaCl_2$.

Binding assays were done using different concentrations i.e., 0.5nM-3.0nM of [^{3}H] MDL 100907 in 50mM Tris Buffer, pH 7.7 containing 4mM $CaCl_2$, 0.2% ascorbate and 10μM pargyline in a total incubation volume of 250μl containing 500-600μg of protein. Specific binding was determined using 100μM unlabelled ketanserin. Competition studies were carried out with 1.0 nM [^{3}H] MDL 100907 in each tube with unlabelled ketanserin concentrations varying from 10^{-12}-10^{-3} M. Tubes were incubated at 37 ^{0}C for 30 min. and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 3.0ml of ice cold 50mM Tris buffer, pH 7.7. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Protein Estimation

Protein concentrations were estimated according to Lowry *et al.*, (1951) using bovine serum albumin as standard.

ANALYSIS OF THE RECEPTOR BINDING DATA

Receptor Binding Parameters Analysis

The receptor binding parameters were determined using Scatchard analysis (Scatchard, 1949). The maximal binding (B_{max}) and equilibrium dissociation constant (K_d) were derived by linear regression analysis by plotting the specific binding of the radioligand on x-axis and bound/free on y-axis using Sigma plot computer software. This is called a Scatchard plot. The B_{max} is a measure of the total number of receptors present in the tissue and the K_d represents affinity of the receptors for the radioligand. The K_d is inversely related to receptor affinity or the "strength" of binding. Competitive binding data were analysed using non-linear regression curve-fitting procedure (GraphPad PRISM™, San Diego, USA).

Displacement Curve analysis

The data of the competitive binding assays are represented graphically with the - log of concentration of the competing drug on x-axis and percentage of the radioligand bound on the y-axis. The steepness of the binding curve can be quantified with a slope factor, often called a Hill slope. A one-site competitive binding curve that follows the law of mass action has a slope of -1.0. If the curve is more shallow, the slope factor will be a negative fraction (i.e., -0.85 or -0.60).

The slope factor is negative because curve goes downhill. If slope factor differs significantly from 1.0, then the binding does not follow the law of mass action with a single site, suggesting a two-site model of curve fitting. The concentration of competitor that competes for half the specific binding was defined as EC_{50}. It is same as IC_{50}. The affinity of the receptor for the competing drug is designated as K_i and is defined as the concentration of the competing ligand that will bind to half the binding sites at equilibrium in the absence of radioligand or other competitors (Cheng & Prusoff, 1973).

EXPRESSION STUDIES OF DOPAMINE D_2, 5-HT_{2A} RECEPTORS AND ALDH IN DIFFERENT BRAIN REGIONS OF CONTROL AND ETHANOL TREATED RATS

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

RT-PCR was carried out using Titan™ one tube RT-PCR system (Roche Diagnostics, Germany). cDNA synthesis was performed with MuMLV reverse transcriptase enzyme. The PCR step was carried out with a high fidelity enzyme blend consisting of Taq DNA Polymerase. Enzyme was stored in storage buffer (20mM Tris HCl, 100mM KCl, 0.1mM EDTA, 1mM Dithiothrietol (DTT), 0.5% Tween-20 (v/v), 0.5% Nonidet P40 (v/v), 50% Glycerol (v/v): pH 7.5.

Preparation of RNA

RNA was isolated from brain regions of control and ethanol treated rats using the Tri reagent kit from Sigma Chemical Co.,USA.

Isolation of RNA

Tissue (25-50 mg) homogenates were made in 0.5 ml TRI Reagent and was centrifuged at 12,000x*g* for 10 minutes at 4^0C. The clear supernatant was transferred to a fresh tube and it was allowed to stand at room temperature for 5 minutes. 100µl of chloroform was added to it, mixed vigorously for 15 seconds and allowed to stand at room temperature for 15 minutes. The tubes were then centrifuged at 12,000x*g* for 15 minutes at 4^0C. Three distinct layers appear after centrifugation. The bottom red organic layer contained protein, interphase contained DNA and a colourless upper aqueous layer contained RNA. The upper aqueous layer was transferred to a fresh tube and 250µl of isopropanol was added and the tubes were allowed to stand at room temperature for 10 minutes. The tubes were centrifuged at 12,000x*g* for 10 min at 4^0C. RNA precipitate forms a pellet on the sides and bottom of the tube. The supernatants were removed and the RNA pellet was washed with 500µl of 75% ethanol, vortexed and centrifuged at 12,000x*g* for 5 min at 4^0C. The pellets were semi dried and dissolved in minimum volume of DEPC-treated water. 2 µl of RNA was made up to 1 ml and absorbance was measured at 260nm and 280nm. For pure RNA preparation the ratio of absorbance at 260/280 was $\geq$ 1.7. The concentration of RNA was calculated as one absorbance$_{260}$ = 42µg.

Primers

The following primers were used for dopamine D_2, 5-HT_{2A} receptors and ALDH, β-actin mRNA expression studies.

5'- GCCAAACCAGAGAAGAATGG -3'	Forward Primer	DA D_2
5'- GATGTGCGTATGAAGGAAGG-3'	Reverse Primer	
5'-CAACTCCAGAGATGCTAACACTTCG- 3'	Forward Primer	5-HT_{2A}
5'-GGGTTCTGGATGGCGACATAG - 3'	Reverse Primer	
5'-CCCTTCAACCTCACCATCC- 3'	Forward Primer	ALDH
5' -GCGGCCATAACAATCTTCC-3'	Reverse Primer	
5'- CAACTTTACCTT GGC CAC TAC C -3'	Forward Primer	β-actin
5'- TACGACTGCAAACAC TCTACA CC -3'	Reverse Primer	

RT-PCR of dopamine D_2, 5-HT_{2A} receptors, ALDH and β-actin

RT-PCR was carried out in a total reaction volume of 20μl reaction mixture in 0.2ml tubes. RT-PCR was performed in an Eppendorf Personal

thermocycler. cDNA synthesis of 2mg RNA was performed in a reaction mixture containing MuMLV reverse transcriptase (40U/reaction), 2mM dithiothreitol, 4 units of human placental RNAse inhibitor, 0.5μg of random hexamer and 0.25mM dNTPs (dATP, dCTP, dGTP and dTTP). The tubes were then incubated at 42^0C for one hour. Then reverse transcriptase, MuMLV, was inactivated by heating at a temperature of 95°C.

Thermocycling profile for Real-Time PCR

For obtaining higher stringency conditions RT-PCR profile was adopted. PCR was carried out in a 25μl volume reaction mixture in the specially designed Real-Time PCR tubes provided by Takara, Japan, containing 2μl cDNA, 12.5μl reaction mixture and 1μl of primer and 9.5μl DEPC water. The reaction mixture, SyBr Premix EX Taq, of which the unit definition is one unit, is the amount of the enzyme that will incorporate 10nmol. of dNTP into acid insoluble products in 30 minutes at 74°C with activated salmon sperm DNA as the template-primer.

Thermocycling profile used for dopamine D_2 receptor

95°C -- 30 seconds	Initial denaturation	
95°C -- 10 seconds	Denaturation	
56°C -- 30 seconds	Annealing	45 cycles
72°C -- 30 seconds	Extension	

Thermocycling profile used for 5-HT_{2A} receptor

94°C -- 30 seconds	Initial denaturation	
94°C -- 10 seconds	Denaturation	
58°C -- 30 seconds	Annealing	45 cycles
72°C -- 30 seconds	Extension	

Thermocycling profile used for ALDH

94°C -- 30 seconds	Initial denaturation	
94°C -- 30 seconds	Denaturation	
60°C -- 30 seconds	Annealing	45 cycles
72°C -- 30 seconds	Extension	

Thermocycling profile used for β-actin

94°C -- 30 seconds	Initial denaturation	
94°C -- 30 seconds	Denaturation	
55°C -- 30 seconds	Annealing	45 cycles
72°C -- 30 seconds	Extension	

Analysis of Real -Time PCR product

The Crossing threshold (Ct) represents the comparative expression of the mRNA of the gene of interest from the samples used. The Ct values are taken from the graph directly in the software of the Real-Time PCR provided along with (Cephied Smart Cycler Software v2.0) and selected for each control and ethanol treated group. The lowest Ct value represents the higher expression of the mRNA isolated from the samples of the control and ethanol treated groups.

Liver perfusion with PBS in experimental rats

One set of rat liver was Perfused with ice cold phosphate buffered saline (PBS) for 10 min, PBS containing 5% ethanol for 5min, 5% ethanol + 4mM glucose for 5min, 5% ethanol + 20mM glucose for 5min. Another set of rat liver was Perfused with ice cold phosphate buffered saline (PBS) for 10 min, PBS containing 5% ethanol for 5min, 5% ethanol + 10^{-4} M DA for 5min, 5% ethanol + 10^{-4} M 5-HT for 5min. After each perfusion a portion of the liver was cut and used for ALDH enzyme analysis.

Electroencephalogram analysis in control and ethanol treated rats

Spontaneous electrical activity of brain regions of the control and ethanol treated rats were carried with Neurocare™ Wingraph Digital EEG system. EEG analysis was done by placing electrodes in right and left frontal, parietal, occipital and temporal areas of the scalp of experimental rat and electrode placed on the ear was considered as reference.Each electrode was placed 10-20 percent away from the neighbouring electrode. The EEG recording datas were analysed for the brain activity in different brain areas of ethanol treated and control rats.

Statistics

Statistical evaluations were performed by Student's t-test and ANOVA using InStat (Ver.2.04) computer programme. Linear regression Scatchard plots were made using SIGMA PLOT (Ver 2.03).

RESULTS

RESULTS

Body weight and ethanol consumption of experimental rats

There was an increased consumption during the ethanol treatment. A significant decrease in body weight ($p<0.05$) in ethanol treated rats were observed compared to the control (Fig - 1, 2 & Table - 1).

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in cerebral cortex

The kinetic parameters, V_{max} and K_m, were studied in enzyme preparations of ALDH in cerebral cortex. The results showed that there is a significant increase ($p<0.05$, $P<0.001$) in the V_{max} and K_m respectively in the ethanol treated condition when compared to control (Fig - 3 & Table- 2).

Real-Time PCR analysis of ALDH

The Real-Time PCR analysis in the cerebral cortex showed a decrease in Ct value of ethanol treated rats showing an increased expression in mRNA synthesis compared to control rats (Fig - 4 & Table - 3).

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in brainstem

The kinetic parameters, V_{max} and K_m, were studied in enzyme preparation of ALDH in brainstem. The results from enzyme preparations showed that there is a significant decrease ($p<0.001$) in the K_m in the ethanol treated rats when compared to control. The V_{max} did not show any significant change (Fig - 5 & Table - 4).

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in cerebellum

The kinetic parameters were studied in enzyme preparation of ALDH in cerebellum. The results from enzyme preparations showed that there is a significant decrease in V_{max} ($p<0.01$) with an increase in K_m ($p<0.001$) when compared to control (Fig - 6 & Table - 5).

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in plasma

The ALDH activity in the plasma of ethanol treated rats showed a significant increase ($p<0.05$) in V_{max} with a decrease in K_m ($p<0.01$) when compared to control. (Fig - 7 & Table - 6).

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in liver

The kinetic parameters, V_{max} and K_m, were studied in enzyme preparation of ALDH in liver. The results from enzyme preparations showed that there is a significant increase ($p<0.05$) in the V_{max} with a significant decrese in K_m ($p<0.01$) in the ethanol treated condition in liver when compared to control (Fig - 8 & Table - 7).

Real-Time PCR analysis of ALDH

The Real-Time PCR analysis in the liver showed a decrease in Ct value of ethanol treated rats showing an increased expression in mRNA synthesis compared to control rats (Fig - 9 & Table - 8).

Quantification of DA, 5-HT, and their metabolites by HPLC in the cerebral cortex of experimental rats

There was a significant decrease in 5-HT and DA content ($p<0.01$) in the cerebral cortex of ethanol treated rats. Turnover of 5-HIAA/5-HT as well as HVA/DA were significantly increased ($p<0.001$, $p<0.05$) in ethanol treated rats when compared to control (Table - 9).

Quantification of DA, 5-HT and their metabolites by HPLC in the brainstem of experimental rats

5-HT content was significantly decreased ($p<0.01$) and DA content was significantly decreased ($p<0.05$) in ethanol treated rats when compared to control. Turnover of 5-HIAA/5-HT was significantly increased ($p<0.001$) and HVA/DA significantly increased ($p<0.01$) in ethanol treated rats when compared to control (Table - 10).

Quantification of DA, 5-HT and their metabolites by HPLC in the Hypothalamus of control and ethanol treated rats

5-HT content significantly increased ($p<0.01$) and DA content was significantly decreased ($p<0.01$) in the hypothalamus of ethanol treated rats. Turnover of 5-HIAA/5-HT was also significantly decreased ($p<0.001$) but the HVA/DA was significantly increased ($p<0.001$) in ethanol treated rats compared to control (Table - 11).

Quantification of DA, 5-HT and their metabolites by HPLC in the corpus striatum of experimental rats

A significant decrease in 5-HT ($p<0.01$) and DA ($p<0.001$) contents were observed in the corpus striatum of ethanol treated rats. Turnover of 5-HIAA/5-HT and HVA/DA were significantly increased ($p<0.01$, $p<0.001$) in ethanol treated rats when compared to control (Table - 12).

Quantification of DA, 5-HT and their metabolites by HPLC in the liver of control and ethanol treated rats

A significant decrease in 5-HT and DA content ($p<0.001$) was observed in the liver of ethanol treated rats when compared to control. The turnover of 5-HIAA/5-HT and HVA/DA were significantly increased ($p<0.001$) in ethanol treated rats when compared to control (Table - 13).

Altered brain DA D_2 receptor binding parameters in control and ethanol treated rats.

Cerebral Cortex

Scatchard analysis of [^{3}H] YM-09151-2 against sulpiride in cerebral cortex of ethanol treated rats showed a significant decrease ($p<0.001$) in B_{max} with a significant decrease in K_d ($p<0.05$) compared to control (Fig - 10 & Table - 14).

Displacement analysis of [^{3}H] YM-09151-2 against sulpiride in cerebral cortex of control and ethanol treated rats.

In displacement analysis different concentrations of unlabelled sulpiride were used against [^{3}H] YM-09151-2. DA D_2 receptor affinity increased during ethanol treatment in cerebral cortex fitting the equation to a single-site model as seen in control. This was confirmed by the Hill slope value which is (-0.89) in ethanol treated group and in control (-0.93) near unity. The Log (EC_{50}) value in ethanol treated group decreased with an increase in affinity ie, decrease in K_i value (Fig - 11 & Table - 15).

Real-Time PCR analysis of D_2 receptor

The increase Ct value in cerebral cortex of ethanol treated rats showing a decreased expression in mRNA synthesis compared to control rats (Fig - 12 & Table - 16).

Brainstem

The B_{max} of [^{3}H] YM-09151-2 binding decreased ($p<0.05$) significantly in ethanol treated rats compared to control and the K_d value also decreased ($p<0.05$) significantly in ethanol treated group compared to control (Fig - 13 & Table - 17).

Displacement analysis of [^{3}H] YM-09151-2 against sulpiride in brainstem of control and ethanol treated rats.

In displacement analysis the competitive curve fitted for one site model with Hill slope value in control (-0.96) and ethanol treated (-0.99) near to unity.

The Log (EC_{50}) value in ethanol treated group decreased with an increase in affinity ie, decrease in K_i value (Fig - 14 & Table - 18).

Hypothalamus

Scatchard analysis of [^{3}H] YM-09151-2 against sulpiride in hypothalamus of ethanol treated rats showed a significant increase ($p<0.001$) in B_{max} with out any significant change in K_d compared to control (Fig- 15 & Table-19).

Displacement analysis of [^{3}H] YM-09151-2 against sulpiride in hypothalamus of control and ethanol treated rats.

In displacement analysis different concentrations of unlabelled ketanserin were used against [^{3}H] YM-09151-2 against sulpiride. DA D_2 receptor affinity increased during ethanol treatment in cerebral cortex fitting the equation to a single-site model as seen in control. This was confirmed by the Hill slope value which is (-0.97) in ethanol treated group and in control (-0.95) near unity. There was no change in the Log (EC_{50}) values, But the K_i value of ethanol treated rats increased compared with control indicating a shift in affinity to low affinity (Fig- 16 & Table - 20).

Real-Time PCR analysis of D_2 receptor

The decrease Ct value in hypothalamus of ethanol treated rats showing an increased expression in mRNA synthesis compared to control rats (Fig- 17 & Table - 21).

Corpus striatum

The B_{max} of [^{3}H] YM-09151-2 binding decreased significantly ($p<0.05$) in ethanol treated rats. K_d value also decreased significantly ($p<0.05$) in ethanol treated group compared to control (Fig -18 & Table - 22).

Displacement analysis of [^{3}H] YM-09151-2 against sulpiride in corpus striatum of control and ethanol treated rats.

In displacement analysis the competitive curve fitted for one site model with Hill slope value in control (-0.99) and ethanol treated (-0.99) near to unity. There is not much change in log (EC_{50}) value in ethanol treated group with increased affinity ie, decrease in K_i value (Fig - 19 & Table - 23).

Real-Time PCR analysis of D_2 receptor

The increased Ct value in corpus striatum of ethanol treated rats showing a decreased expression in mRNA synthesis compared to control rats (Fig - 20 & Table - 24).

Cerebellum

The B_{max} of [^{3}H] YM-09151-2 binding increased ($p<0.001$) significantly in ethanol treated rats, with significant increase in K_d ($p<0.01$) compared to control (Fig - 21 & Table - 25).

Displacement analysis of [^{3}H] YM-09151-2 against sulpiride in cerebellum of control and ethanol treated rats.

In displacement analysis the competitive curve fitted for one site model with Hill slope value in control (-0.95) and ethanol treated (-0.97) near to unity.. There was no change in the Log (EC_{50}) values, But the K_i value of ethanol treated rats increased compared with control indicating a shift in affinity of the receptors (Fig - 22 & Table - 26).

Real-Time PCR analysis of D_2 receptor

The decreased Ct value in cerebellum of ethanol treated rats showing an increased expression in mRNA synthesis compared to control rats (Fig - 23& Table - 27).

Altered brain 5-HT_{2A} receptor binding parameters in control and ethanol treated rats.

Cerebral Cortex

Scatchard analysis of [^{3}H] MDL 100907 against ketanserin in cerebral cortex of ethanol treated rats showed a significant decrease ($p<0.001$) in B_{max} without any significant change in K_d compared to control (Fig - 24 & Table - 28).

Displacement analysis of [^{3}H] MDL 100907 against ketanserin in cerebral cortex of control and ethanol treated rats.

In displacement analysis of different concentrations of unlabelled ketanserin were used against [^{3}H] MDL 100907. 5-HT_{2A} receptor affinity increased during ethanol treatment in cerebral cortex fitting the equation to a

single-site model as seen in control. This was confirmed by the Hill slope value which is (-0.98) in ethanol treated group and in control (-0. 91) near unity. The Log (EC_{50}) value in ethanol treated group increased with an increase in affinity ie, decrease in K_i value (Fig - 25 & Table - 29).

Real-Time PCR analysis of 5-HT_{2A} receptor

The increase Ct value in cerebral cortex of ethanol treated rats showing a decreased expression in mRNA synthesis compared to control rats (Fig - 26 & Table - 30).

Brainstem

Scatchard analysis of [^{3}H] MDL 100907 against ketanserin in brainstem of ethanol treated rats showed a significant increse ($p<0.001$) in B_{max} with out any significant change in K_d compared to control (Fig - 27 & Table - 31).

Displacement analysis of [^{3}H] MDL 100907 against ketanserin in brainstem of control and ethanol treated rats.

The competition curve for unlabelled ketanserin inhibited specific [^{3}H] MDL 100907 binding fitted for one site model with Hill slope value in control (-0.98) and ethanol treated(-0.99) near to unity. The K_i of ethanol treated rats decreased with out any change in log (EC_{50}) value compared with control indicating a shift in affinity to high affinity (Fig - 28 & Table - 32).

Hypothalamus

Scatchard analysis of [^{3}H] MDL 100907 against ketanserin in hypothalamus of ethanol treated rats showed a significant increase ($p<0.001$) in

B_{max} with significant increase in K_d ($p<0.001$) compared to control (Fig - 29 & Table - 33).

Displacement analysis of [^{3}H] MDL 100907 against ketanserin in hypothalamus of control and ethanol treated rats.

In displacement analysis different concentrations of unlabelled ketanserin were used against [^{3}H] MDL 100907. 5-HT_{2A} receptor affinity decreased during ethanol addiction in cerebral cortex fitting the equation to a single-site model as seen in control. This was confirmed by the Hill slope value which (-0.98) in ethanol treated group was near unity and in control had a hill slope value in unity (-0.99).The Log (EC_{50}) value in ethanol treated group increased with a decrease in affinity (Fig - 30 & Table - 34).

Real-Time PCR analysis of 5-HT_{2A} receptor

The decrease Ct value in hypothalamus of ethanol treated rats showing an increased expression in mRNA synthesis compared to control rats (Fig - 31 & Table - 35).

Corpus Striatum

The B_{max} of [^{3}H] MDL 100907 binding increased ($p<0.001$) significantly in ethanol treated rats compared to control and the K_d value also decreased ($p<0.01$) significantly in ethanol treated group compared to control (Fig - 32 & Table - 36).

Displacement analysis of [^{3}H] MDL 100907 against ketanserin in corpus striatum of control and ethanol treated rats.

In displacement analysis the competitive curve fitted for one site model with Hill slope value in control (-0.99) and ethanol treated (-0.99) near to unity. There was no change in the Log (EC_{50}) values, But the K_i value of ethanol treated rats increased compared with control indicating a shift in affinity (Fig - 33 & Table - 37).

Real-Time PCR analysis of 5-HT_{2A} receptor

The decrease Ct value in corpus striatum of ethanol treated rats showing an increased expression in mRNA synthesis compared to control rats (Fig - 34 & Table - 38).

Cerebellum

Scatchard analysis of [^{3}H] MDL 100907 against ketanserin in cerebellum of ethanol treated rats showed a significant decrease ($p<0.01$) in B_{max} with out any significant change in K_d compared to control (Fig - 35 & Table - 39).

Displacement analysis of [^{3}H] MDL 100907 against ketanserin in cerebellum of control and ethanol treated rats.

In displacement analysis different concentrations of unlabelled ketanserin were used against [^{3}H] MDL 100907. Since ketanserin has a higher potency than MDL 100907, 5-HT_{2A} receptor affinity decreased during ethanol addiction in cerebral cortex fitting the equation to a single-site model as seen in control. This was confirmed by the Hill slope value which (-0.96) in ethanol

treated group was near unity and in control had a hill slope value in unity (-0.98). The log (EC_{50}) value in ethanol treated group decreased with an increase in affinity (Fig - 36 & Table - 40).

Real-Time PCR analysis of 5-HT_{2A} receptor

The increase Ct value in cerebellum of ethanol treated rats showing a decreased expression in mRNA synthesis compared to control rats (Fig - 37 & Table - 41).

Liver

Scatchard analysis of [^{3}H] MDL 100907 against ketanserin in liver of ethanol treated rats showed a significant decrease ($p<0.001$) in B_{max} with a significant decrease in K_d ($p<0.05$) compared to control (Fig - 38 & Table - 42).

Displacement analysis of [^{3}H] MDL 100907 against ketanserin in liver of control and ethanol treated rats.

In displacement analysis different concentrations of unlabelled ketanserin were used against [^{3}H] MDL 100907. 5-HT_{2A} receptor affinity decreased during ethanol treatment in cerebral cortex fitting the equation to a single-site model as seen in control. This was confirmed by the Hill slope value which (-0.96) in ethanol treated group was near unity and in control had a hill slope value in unity (-0.98).The log (EC_{50}) value in ethanol treated group decreased with an increase in affinity (Fig - 39 & Table - 43).

Real-Time PCR analysis of 5-HT_{2A} receptor

The increased Ct value in liver of ethanol treated rats showing a decreased expression in mRNA synthesis compared to control rats (Fig - 40 & Table - 44).

Kinetic parameters of aldehyde dehydrogenase in perfused liver of rats

The V_{max} and K_m were studied in enzyme preparations of ALDH in perfused liver of rats. The results from enzyme preparations showed that there is a significant increase ($p<0.001$) in the V_{max} of the 5% ethanol perfused rat liver when compared to control and decreased significantly ($p<0.001$) near to control value in the liver of 5%ethanol + 4mM glucose and 5%ethanol + 20mM glucose perfused rats. 5% ethanol $+10^{-4}$ M 5-HT perfused rat liver showed an increased V_{max} ($p<0.001$) when compared to control with no significant change when compared with the 5%ethanol perfused rat liver. V_{max} decreased ($p<0.001$) near to control in 5% ethanol $+10^{-4}$ M DA perfused rat liver when compared to 5% ethanol perfused liver. There was a decreased affinity ($p<0.01$) in 5% ethanol perfused rats when compared to control and it reached near to control in the 5% ethanol + 4mM glucose perfused rats. Both, 5% ethanol + 20mM glucose, 5% ethanol + 10^{-4} M DA showed significant increase ($p<0.001$) in K_m compared to control. 5% ethanol $+10^{-4}$ M 5-HT perfused rat liver showed an increased affinity ($p<0.001$) when compared to 5% ethanol treated rats (Fig 41- 44 & Table 45-48).

Electroencephalogram analysis in control and ethanol treated rats

EEG electrogram analysis showed that there is a significant change in the brain activity in the frontal region compared to the control. The other regions studied did not show any prominent change in brain activity in ethanol treated rats compared to control (Fig 45 & 46).

DISCUSSION

DISCUSSION

The etiology of ethanol addiction is a complex interaction of psychosocial and biological factors. Ethanol freely diffuses across the blood–brain barrier and creates generalized effect all over the brain. Multiple neurotransmitter systems in various parts of brain alone as well as combined, play a prominent role in mediating the behavioural effects of ethanol that have been linked to its abuse and dependence (Koob, 1992). This undoubtedly reflects the fact that ethanol produces many pharmacological effects within the brain and body. ALDH is involved in biogenic amine metabolism (Berger & Weiner, 1977) as well as in ethanol metabolism. Brain monoamines and ALDH together plays a decisive role in ethanol addiction.

Ethanol is not stored in the body, but it is oxidized in preference over other fuels. It is reported that ethanol to a diet reduces lipid oxidation whereas oxidation of carbohydrate and protein are much less inhibited (Suter, 1992). Chronic prenatal ethanol exposure decrease cerebral cortex weights and increase locomotor activity (Abdollah *et al.*, 1993; Catlin *et al.*, 1993; Butters *et al.*, 2000, Craig, 2001). Ethanol-treatment resulted in increased foetal mortality and lipid peroxidation and decreased body weight (Tanaka, 1985). Decreased food consumption was observed after ethanol intake (Macho, 2003). In the present study a decrease in body weight was observed in adult male rats during ethanol treatment. Animal studies are consistent in reporting a decrease in the body weight of rats receiving ethanol solutions as the only source of liquid (Aguiar, 2004). Different concentrations of ethanol as low as 5% (v/v), or as high as 40% (v/v) are related to decreased body weight gain (Macieira, 1997). Similar results have been reported for 20% (v/v) ethanol solution (Laure, 1990). Ethanol-

induced energy intake has no clear correlation with body weight and it is reported that ethanol energy has a low biological value (Pirola, 1976, Lands 1991; Lieber, 1991). The rate of ethanol consumption is gradually inclined upward and reached a steady state and there is no correlation observed between the rate of ethanol consumption and the body weight.

Ethanol induced aldehyde dehydrogenase activity disparity in liver, plasma and brain regions.

Mechanism of ethanol craving has been related to the local level of brain acetaldehyde occurring in ethanol consumption and depending on the activities of the brain and liver ethanol and acetaldehyde-metabolizing systems (Bardina, 2003). There are several reports that ethanol preference may correlate with ALDH activity more in the brain than in the liver (Amir, 1978; Socaransky *et al.*, 1984) and this mechanism is still unknown. Oxidative deamination of monoamine neurotransmitters, catalyzed by the membrane-bound MAO generates reactive aldehyde intermediates. Aldehyde dehydrogenase, the primary enzyme responsible for acetaldehyde metabolism, is highly correlated with voluntary ethanol consumption in several strains of rats and mice (Amir, 1977). Both DOPAL and 5-HIAL are good substrates for ALDH (Ambroziak, 1991). Brain ALDH plays an important role in the biosynthesis of biogenic amines (Tipton *et al.*, 1977). Our results showed that there is a significant increase in kinetic parameters of ALDH in cerebral cortex and it is reported that ethanol preference is related to ALDH activity in the cerebral cortex (Yamazaki, 1984). The results from ALDH enzyme analysis of brainstem showed that there is a significant decrease in the K_m in brainstem without any change in V_{max}. There is a significant decrease in V_{max} with an increase in K_m in cerebellum. Disulfiram, an

ALDH inhibitor, treatment in the absence of ethanol, raises endogenous plasma and red blood cell acetaldehyde concentrations, possibly due to diminished catabolism of endogenously generated acetaldehyde (Eriksson, 1985; Rosman *et al.*, 2000). It was observed that plasma ALDH level of ethanol treated rats increased significantly when compared to control which is suggested to be due to the increased acetaldehyde level. The results from ALDH enzyme analysis showed that there is a significant increase in the V_{max} with a significant decrease in K_m in the ethanol treated condition in liver when compared to control. It has been reported that colonic mucosal ALDH activities are relatively low compared to liver (Koivisto & Salaspuro, 1996). As acetaldehyde itself has many pharmacological actions (Brien & Loomis, 1983), it may act on the central nervous system (Kinoshita *et al.*, 2001), where differences in acetaldehyde elimination may contribute to ethanol preference. Ethanol administration activates the HPA axis (Rivier & Lee, 1996). Acetaldehyde formed in brain is able to activate the HPA axis at a central level (Hiroshi *et al.*, 2001). Difference in acetaldehyde level exerted stress on HPA axis is mediated *via* brainstem and plays a role in peripheral system regulation. The expression pattern of aldehyde dehydrogenase in the liver and cerebral cortex were in concordance with the enzyme activity. DA and 5-HT induced variations in the ALDH activity plays an important role in acetaldehyde metabolism.

Brain DA and HVA changes during ethanol treatment

Neurotransmitters can activate different subtypes of the same receptor, producing different responses in different brain cells or in different parts of the brain (Shepherd, 1994). Receptor activation causes a change in the receiving neuron. These changes may consist of a transient increase or decrease in the

neuron's responsiveness to further messages (Grant, 1994). Due to these changes, activating mechanisms in the central nervous system prevail. The ability of ethanol to diffuse throughout the water contained in the brain and body suggested that there were probably multiple sites of ethanol action. Ethanol may produce some of its effects by interfering with signal transduction (Alling, 1993; Davis, 1996). Repeated exposure to ethanol can produce long-lasting changes in adolescent behaviour and brain function. Ethanol ingestion has been shown to induce significant change in neurotransmitter systems (Imperato, 1986; Nevo, 1995). DA and 5-HT have received special attention because of their putative role in the motivational effects of ethanol (Cloninger, 1986; Sellers, 1992; Wallis, 1993). Administration of ethanol induces DA release (Imperato, 1986; Di Chiara, 1985; O'Brien, 1995) in the caudate nucleus and nucleus accumbens of freely moving rats. DA levels in the striatum remained almost unchanged following chronic treatment with ethanol and acteldehyde (Myers *et al.,* 1985; Matsubara *et al.,* 1987). Ethanol acts on the dopaminergic neurons, producing lasting changes on the system. Altered central DA function has also been implicated as influencing the propensity for ethanol consumption in humans, at least in some populations (Cowen, 1999). Changes in turnover of neurotransmitters in specific brain regions may reflect alterations in neuronal activity resulting from varied aldehyde dehydrogenase activity. This undoubtedly reflects the fact that ethanol produces many pharmacological effects within the brain. Blocking the effects of DA reduces ethanol intake by animals (Koob, 1992).

DA content decreased significantly in the cerebellum of ethanol treated rats with an increased HVA/DA turnover rate. With long-term use, adolescent rats have shown massive neuronal loss in their cerebellum and basal forebrain

(Spear, 2002). Prolonged ethanol exposure directs to neurotransmitters changes. There is a significant decrease in DA content in the cerebral cortex of ethanol treated rats with significantly increased turnover of HVA/DA. Recent studies in animals have found that as little as 2–4 days of ethanol intoxication can lead to neuronal loss in several brain areas including entorhinal cortex and hippocampal dentate gyrus (Collins, 1998). DA content was significantly decreased in the hypothalamus of ethanol treated rats. HVA/DA was significantly increased in ethanol treated rats. It indicates the alterations of the biogenic amine contents in different regions of the brain after chronic ethanol ingestion. DA content was significantly increased in brainstem of ethanol treated rats with significantly decreased HVA/DA turnover ratio in ethanol treated rats when compared to control. There is a stimulated release of biogenic amines in some brain regions and decrease in other regions due to the biphasic effect of ethanol. This has been implicated in the alterations of aldehyde dehydrogenase kinetic parameters. Vasconcelos *et al.*, (2004) reported that duration of ethanol treatment seems to be important regarding changes in monoamine levels. Budygin *et al.*, (2001) reported that ethanol exerts a profound effect on DA neurons, resulting in the suppression of DA neurotransmission in the striatum at high doses. DA content decreased significantly in corpus striatum of ethanol treated rats with an increased HVA/DA turnover rate. It is reported that striatal DA deficit correlated with ethanol craving (Heinz, 2005). Microdialysis experiments in rodents indicate that ethanol promotes DA release predominantly in the nucleus accumbens, a phenomenon implicated in the reinforcing effect of the drug. In humans, ethanol also promotes DA release, with a preferential effect on the ventral striatum (Boileau, 2003). It was reported (Tuomainen, 2003) that the application of ethanol to the nucleus accumbens temporarily increased DA levels

in a dose-dependent manner. Rothblat *et al.*, (2001) demonstrated that DA and DOPAC levels were significantly decreased in the striatum of rats chronically receiving ethanol. Ethanol-induced stimulation of dopaminergic neurotransmission may encode the reinforcing properties of ethanol consumption (Heinz, 2000). Acetaldehyde increases DA neuronal activity (Marzia, 2004). The observed discrepancy in the metabolic rate of DA at different brain regions is due to ethanol induced brain alterations in the ALDH system resulting in difference in acetaldehyde elimination.

Brain 5-HT and 5-HIAA changes during ethanol treatment

Neurons connect with thousands of adjacent neurons. Berggren *et al.*, (2002) reported a negative correlation between prolonged and excessive ethanol consumption and central serotonergic neurotransmission due to a toxic effect of ethanol on 5-HT neurons. A significant decrease in 5-HT content was observed in the corpus striatum of ethanol treated rats and the turnover of 5-HIAA/5-HT significantly increased when compared to control. Striatal dopamine deficit is correlated with ethanol craving (Heinz, 2005). Chronic ethanol treatment decrease serotonergic neurotransmission in selective brain regions. Human studies reported damage to entorhinal cortex (Ibanez, 1995) and significant hippocampal shrinkage in ethanol addicts (Harding, 1997). It was observed a significant decrease in 5-HT content in the cerebral cortex with a significant increase in 5-HIAA/5-HT turnover rate in ethanol treated rats when compared to control. The decreased level of 5-HT observed was due to enhanced metabolic rate of 5-HT by the activated ALDH enzyme. 5-HT and its metabolic intermediates differentially regulate ethanol drinking behaviour (Wing, 1998). Ethanol has a biphasic effect on 5-HT, first raising the levels and then lowering

them (LeMarquand, 1994). Ethanol administration eventually results in depressed 5-HT levels, and thus the activity, due to increased peripheral metabolism of its precursor, l-tryptophan (Badawy, 1995). 5-HT levels remained largely the same in the nucleus accumbens following acute exposure to ethanol (Heidbreder & De, 1993). 5-HT content increased in hypothalamus with a decreased 5-HIAA/5-HT turnover rate of ethanol treated rats compared to control. Reduced density of 5-HT transporter binding in the brain might reflect reductions in the density of 5-HT terminals that might contribute to reduced central 5-HT function (Tiihonen, 1997; Chen, 1991). Chronic ethanol administration altered the serotonergic system in a time dependent manner (Uzbay *et al.*, 1998). 5-HT content was significantly decreased in brainstem of ethanol treated rats when compared to control. Turnover rate of 5-HIAA/5-HT significantly increased in ethanol treated rats when compared to control. These results indicate alterations of the biogenic amine contents in brain regions after chronic ethanol ingestion. Stimulated release of biogenic amines in some brain regions and decreased in other regions is due to the biphasic effect of ethanol and has been implicated in the regulation of aldehyde dehydrogenase kinetic parameters. Decrease in serotonergic activity might be involved in the early phase of ethanol withdrawal (Syvalahti *et al.*, 1988). The alterations of brain 5-HT function in the brainstem, hypothalamus, corpus striatum, cerebral cortex play an important role in the sympathetic control of ALDH enzyme regulation in liver. McBride (1995) has reported that levels of brain 5-HT is lower in ethanol-preferring rats than in non-preferring ones. 5-HT and its metabolite 5-HIAA changes at different brain regions are due to ethanol induced brain alterations in ALDH system resulting in the difference in acetaldehyde elimination.

Liver DA, 5-HT and their metabolite changes during ethanol treatment.

Aldehydes in the metabolic pathways of ethanol, DA and 5-HT are substrates for ALDH. Acetaldehyde is the initial metabolite of ethanol, which is produced in the liver following ethanol administration. Aldehyde dehydrogenase oxidizes a broad class of aldehydes to their carboxylic acids (Lindahl, 1992), involved in biogenic amine metabolism (Berger and Weiner, 1977). Ethanol intake significantly changes the liver cytosolic redox potential by increasing the $NADH/NAD^+$ ratio (Smith, 1959). Although the ethanol feeding did not influence the stomach ADH and ALDH activity levels, these enzymes in the liver were affected (Wei, 1988). Decreased DA and 5-HT content in liver with an increased HVA/DA and 5-HIAA/5-HT turnover rate observed in ethanol treated rats compared to control. Over activity has been supposed to contribute to the morphological and functional degeneration of rat peripheral sympathetic nervous system. It has been observed that in patients in the preliminary stage of addiction show only functional disturbances in the liver: the increase of ethanol dehydrogenase activity with evidences for the induction of its synthesis (Kharchenko, 2001). Most of the acetaldehyde produced from ethanol is metabolized quickly to acetate by liver ALDH and hence acetaldehyde concentration in blood following ethanol administration is very low (Eriksson, 1973; Eckardt *et al.*, 1998). Our results suggest that decreased DA and 5-HT level and increased turnover rate of metabolites may be due to the ethanol induced neurotransmitter mediated changes on aldehyde dehydrogenase.

DA D_2 receptor alteration in brain regions

Determining the specific neurotransmitters and receptor subtypes that may be involved in the development of the effects of ethanol addiction is the first step in developing medications to treat ethanol addiction (Hunt, 1993; Deitrich, 1996). Neuronal DA receptors are widely distributed in the central and the peripheral nervous systems at different levels. DA D_2 receptor-selective agonist, quinpirole, increases renal sympathetic firing (Szabo, 1992). Compared to normal rats, the alcohol-preferring rats have a reduced supply of DA in the nucleus accumbens and a lower density of DA D_2 receptors in certain areas of the limbic system (Russell *et al.*, 1988; McBride *et al.* 1990; McBride *et al.* 1993). From our analysis we observed a decreased receptor activity in cerebral cortex, brainstem and corpus striatum in ethanol treated rats with an increased affinity. This is a mechanism to compensate the decreased DA D_2 status. The brain reduces the number of DA binding sites on neurons to protect itself from a persistent oversupply of the neurotransmitter. Jan *et al.*, (1994) suggests that severely ethanol-dependent subjects with reduced DA D_2 receptor function. It is reported that striatal DA D2 receptor density is decreased in ethanol-dependent patients (Tiihonen, 1997; Volkow, 1996). Serotonergic neurotransmitter pathways have all been shown to interact at various points along the mesolimbic dopaminergic pathway to modulate its activity (Denise & Sellers, 2001). Increased YM-09151-2 binding to DA D_2 receptor was observed in cerebellum and hypothalamus of ethanol treated rats compared to control. Increased density of DA D_2 receptors may be a predictor of vulnerability to relapse in ethanol-dependent patients (Guardia, 2000). Repeated deprivations increase binding sites of DA D_1 and DA D_2 receptors in specific regions of the extended amygdala (Sari *et al.*, 2006). The functional alterations in the DA D_2 receptor kinetics in

different brain regions is due to ethanol induced central neurotransmitter system changes occurring during ethanol treatment.

5-HT_{2A} receptor alteration in brain regions

Change in receptor function results from direct action of ethanol on the receptor protein or molecules closely associated with the receptor in the cell membrane (Lovinger, 1993, 1994). Ethanol exposure inhibits the function of a neurotransmitter receptor; the cells may attempt to compensate for continuous inhibition by increasing the receptor numbers or by altering the molecular makeup of receptors or cell membranes so that ethanol no longer inhibits receptor function. The 5-HT_2 receptor appears to undergo such adaptive changes (Pandey, 1995). 5-HT_{2A} receptor kinetics showed a functional decrease in cerebral cortex, cerebellum and liver of ethanol treated rats compared to control. There are lowered levels of 5-HT_{2A} binding sites in the cingulate cortex, the frontal cortex and in the agranular insular cortex (Fedeli, 2002) in 7 days of high doses of ethanol treated rats. It was suggested that this decrease in 5-HT_{2A} receptor density represented a down regulation of the receptors due to an activation of serotonergic transmission in these regions. Ethanol reduces the normal formation and growth of 5-HT neurons in the midbrain. Furthermore, the projection of 5-HT fibers, in density as well as in distribution, is reduced in the major trajectory bundle. This may affect the amount of 5-HT fibers available to the forebrain (Youssef, 2001). 5-HT_{2A} receptor kinetics showed a functional increase in corpus striatum, hypothalamus and brainstem. Altered regulation of brain serotonergic mechanisms; changes in 5-HT_{2A} receptor density and functioning have been observed in ethanol abuse. Dense projections from the subgenual cingulate cortex to the dorsal raphe (Freedman *et al.*, 2000) raises the tantalizing

possibility that the subgenual cortex plays some role in regulating overall serotonergic activity (Ursula, 2004). Altered 5-HT function in fronto-cortical areas could be linked to the genetic predisposition to high voluntary ethanol intake (Ciccocioppo *et al.,* 1999). Preuss *et al.,* (2001) reported an association of 5-HT_{2A} promoter polymorphism and impulsive behaviour in ethanol dependents. The serotonergic neurons that innervate neuroendocrine control regions in the hypothalamic paraventricular nucleus send collaterals to other limbic brain regions, notably the amygdala (Petrov *et al.,* 1994). Hence the alterations of the serotonergic system mediated changes during ethanol treatment calls for special attention.

Hepatic 5-HT_{2A} receptor alterations

Brain plays an important regulatory role in hepatic function (Lautt, 1983). The relationship between the functional status of the liver and that of the brain has been known for centuries (Frerichs, 1860). The liver is richly innervated (Rogers & Hermann, 1983). 5-HT facilitates central sympathetic nerve activity (Kuhn *et al.,* 1980). Autonomic nervous system has an important role in the process of hepatic cell proliferation (Tanaka *et al.,* 1987). The role of 5-HT in regulating cortisol secretion has long been recognized (Dinan, 1996), and evidence suggests that cortisol secretion is regulated by central 5-$HT_{2A/2C}$ receptors (Rittenhouse, 1994). During acute stress, the HPA axis - modulate the brain's response to stress - is activated, increasing the adrenocorticotropic hormone (ACTH), which in turn increases cortisol, clearly indicating the interaction between serotonergic system and HPA axis. During ethanol intoxication and ethanol withdrawal, ACTH and cortisol are also increased. In hepatic encephalopathy and other liver diseases, neurotransmission in the brain is

reported to be altered (Basile *et al.*, 1991; Jones, 1995; Butterworth, 1995). 5-HT_{1A} agonists act centrally inhibiting sympathetic nerve discharge (McCall *et al.*, 1987). Hypothalamic and autonomic nervous regulation of carbohydrate and amino acid metabolism was observed in the liver (Shimazu, 1981). Brainstem has direct connection with liver through the vagus nerve (Tanaka *et al.*, 1987) and plays a regulatory role in liver function. Ethanol induced serotonergic activity alterations over ALDH enzyme leads to the increased activity of ALDH enzyme. The 5-HT system itself is altered and the number of receptor binding sites in liver is greatly reduced with an increase in affinity as a compensatory mechanism. Decrease in 5-HT_{2A} receptor protein level with increased affinity is observed in our model which clearly establishes its unambiguous role in ethanol mediated receptor changes and its regulatory aspects during ethanol treatment.

Ethanol induced ALDH, DA D_2 and 5-HT_{2A} receptor gene expression changes

Ethanol exposure affects multiple genes and various receptor-associated signalling pathways which regulate the expression of a multitude of downstream genes (Fan *et al.*, 2004). The human DA D_2 receptor gene is an important candidate gene for ethanol addction and/or for the modification of its severity (Blum *et al.*, 1995; Noble, 2000; Finckh, 2001; Lu *et al.*, 2001). Neuroadaptive changes in DA D_2 receptor levels occur following alcohol drinking and withdrawal. The Real-Time PCR analysis of DA D_2 in the hypothalamus and cerebellum of ethanol treated rats showed an increased expression in mRNA

synthesis compared to control rats. There is evidence that over expression of DA D_2 attenuates alcohol drinking (Thanos *et al.*, 2004). A modification of gene expression is the crucial component of risk that predisposes an individual towards ethanol addiction. The Real-Time PCR analysis of DA D_2 in the cerebral cortex and corpus striatum of ethanol treated rats showed a decreased expression in mRNA synthesis compared to control rats. The DA D_2 receptor genes are interacting with ALDH genes, there is association between the DA D_2 receptor gene and alcohol dependence. Also ALDH genes are involved in dopamine metabolism (Huang *et al.*, 2004). The Real-Time PCR analysis of ALDH in the liver and cerebral cortex of ethanol treated rats showed an increased expression in mRNA synthesis compared to control rats. Exposure to ethanol changes the patterns of gene expression in such a manner that drinking session continued and ultimately, addiction. The Real-Time PCR analysis of 5-HT_{2A} in the liver, cerebral cortex and cerebellum of ethanol treated rats showed a decreased expression in mRNA synthesis compared to control rats. The diverged pattern of gene expression that portrays the perturbed nervous system assumes a new set point in the face of constant exposure to alcohol. The Real-Time PCR analysis of 5-HT_{2A} in the hypothalamus, corpus striatum of ethanol treated rats showed an increased expression in mRNA synthesis compared to control rats. The differential expression DA D_2 and 5-HT_{2A} receptor genes suggests the involvement of the dopaminergic and serotonergic receptor subtype alterations during ethanol treatment in conferring functional regulation on ALDH activity.

Central, Peripheral DA, 5-HT and Liver ALDH activity

Levels of ethanol consumption are correlated with brain and liver aldehyde-oxidizing capacity (Amir, 1978; Socaransky, 1984). Alteration in the

ethanol metabolizing enzymes, specifically those enzymes responsible for the metabolism of ethanol's primary metabolite acetaldehyde, is the critical factor in the predisposition towards ethanol addiction (Haranda *et al.*, 1983; Mizoi *et al.*, 1983). The high ethanol preferring rats showed significantly lower DA and 5-HT release in the striatum and nucleus accumbens than low alcohol preferring rats (Minori, 2002). It is reported that ALDH is involved in biogenic amine metabolism (Berger & Weiner, 1977). Endogenous DA plays role in modulating norepinephrine release by human sympathetic nerves *in vivo* (Massimo, 1999). ALDH plays this role by regulating the levels of acetaldehyde in brain (Karen, 1987) and liver. Dopamine and serotonin content decreased in brain regions - cerebral cortex and corpus striatum of ethanol treated rats with an increased HVA/DA, 5-HIAA/5-HT turnover rate. Most ethanol elimination occurs by ADH and ALDH systems *via* oxidation of ethanol to acetaldehyde and acetic acid (Crabb, 1995). It has been observed that ethanol preferences in rats vary with the levels of brain ALDH activity (Amir, 1977; Amit *et al.*, 1980). Dopamine content increased in brainstem with an increased HVA/DA turnover rate and serotonin content decreased with an increased 5-HIAA/5-HT turnover rate in ethanol treated rats compared to control. Brain ALDH activity was significantly higher in rats preferring ethanol than in rats not preferring ethanol. With respect to implications for a biological regulator of ethanol intake, the most exciting aspect of cerebral ALDH is its apparent noninducible character in response to ethanol or acetaldehyde exposure (Socaransky *et al.*, 1984). Although the precise mechanism by which ALDH regulates voluntary ethanol intake is yet to be elucidated, these studies support the decrease in DA synthesis. The enhanced clearance of synaptic DA may cause DA hypofunction during ethanol dependence (Rothblat *et al.*, 2001) which will eventually affect the ALDH

kinetic function. Serotonin content increased in hypothalamus with a decreased 5-HIAA/5-HT turnover rate and dopamine content decreased with an increased HVA/DA turnover rate of ethanol treated rats compared to control. A significant decrease in 5-HT and DA content was observed in the liver with significantly increased turnover rate of 5-HIAA/5-HT and HVA/DA in ethanol treated rats when compared to control. These results suggest that sympathetic nerves directly involve in ethanol metabolism in the rat liver. Augmented kinetic rate of ALDH is suggested to be due to the differential regulation of DA and 5-HT system through sympathetic stimulation and peripheral control at the hepatic level. Thus, brain and liver 5-HT and their metabolic rate, 5-HT_{2A} receptor affinity shift differentially regulates ALDH function during ethanol addiction. Monoamine neurotransmitter system alterations induce the activation of ALDH in the liver oxidation of acetaldehydes.

Dopaminergic and serotonergic regulation on kinetic parameters of aldehyde dehydrogenase

The perfusion model technique could help in identifying neurotransmitters acting as messengers in signal transfer and it is vital to identify those contributing to ALDH regulation. Lower activity of ALDH, is believed to play a preventive role against ethanol (EtOH) addiction (Goedde, 1982). Tae *et al.*, (2006) reported a time dependent decrease in plasma acetaldehyde concentration without changing plasma ethanol concentrations observed when rats are treated with Rosiglitazone - peroxisome proliferator-activated receptor (PPAR)-ɣ agonist - mediated by receptor-dependent activation of the PPAR-ɣ–

retinoid X receptor (RXR) complex. Thus, the expression of aldehyde dehydrogenase could potentially be regulated by rosiglitazone by acting on PPAR response elements (PPREs) in ALDH promoter site. Administration of substances that increase the supply of 5-HT at the synapse or that directly stimulate DA D_2 receptors reduce craving for ethanol (McBride *et al.* 1993). DA D_2 receptor agonists reduce the intake of ethanol among rats that prefer ethanol, whereas DA D_2 receptor antagonist increases the drinking of ethanol in these inbred animals (Dyr *et al.*, 1993). Selective serotonin reuptake inhibitors (SSRIs) have been reported to reduce drinking in animals and also in some heavy drinking individuals (Liskow & Goodwin, 1987). Ethanol metabolism is impaired by a nonfunctional form of the enzyme aldehyde dehydrogenase (Wall & Ehlers, 1995). More than 80% of ethanol taken into the isolated rat liver recovered as free acetate in the perfusate (Yamashita, 2001). Sympathetic-nerve stimulation stimulates glycogenolysis in perfused liver (Iwai & Jungermann, 1989). The DA induced decrease in liver ALDH enzyme level represents an activation of the whole DA receptor-signalling cascade in the liver and the functional changes of 5-HT mediated affinity shift in ALDH during EtOH perfusion clearly shows the involvement of serotonergic and dopaminergic system in ALDH regulation.

Ethanol mediated electrophysiological changes

Ethanol interferes with synaptic firing. Central effect of ethanol is mainly based on their effect on GABAergic, glutamatergic and serotonergic transmission (Pietrzak, 2005). A characteristic feature of the EEG recording after ethanol

administration is a deceleration of the rhythms obtained from the cortex and an increase in the amplitude (Klemm & Stevens, 1974; Perrin *et al.*, 1974). Alpha rhythm is more significant and it can be recorded in different parts of the brain. Human study suggested that ethanol decreases alpha rhythm frequency and increases its amplitude (Klemm *et al.*, 1976; Noldy & Carlen, 1990). Acetaldehyde produces electrophysiological actions on VTA neurons *in vivo*, similar to those produced by ethanol, and significantly participate in ethanol-induced increment in DA neuronal activity (Marzia *et al.*, 2004). EEG studies in the frontal region showed a prominent brain activity difference in the ethanol treated rats. Ciccocioppo *et al.*, (1999) reported that altered 5-HT function in fronto-cortical areas could be linked to high voluntary ethanol intake. The EEG findings suggested that ethanol induced changes made rats physiologically more sensitive than control rats. Ethanol interferes with synaptic firing. Acetaldehyde also have role in electrophysiological changes. Discrepancy in the acetaldehyde metabolism is suggested to differentially stimulate electrophysiological indices. Increased cortical P1 amplitude and altered cortical EEG activity may be the neurophysiological 'risk factors' associated with high ethanol consumption in mice (Slawecki *et al.*, 2003). It is reported that reduced central 5-HT function causes poor impulse control in ethanol addicts (Sander *et al.*, 1995; Nielsen *et al.*, 1994). Kahkonen *et al.*, (2003) reported that ethanol-induced differences were most pronounced at anterior electrodes. The prefrontal cortex has been linked to impulse control because damage to this region of the brain can lead to loss of inhibitions, which is prominent in ethanol addicts. The hyper activity at the frontal cortical region observed during the EEG analysis supports the central effects of ethanol especially at the frontal region.

Thus the results suggests that DA and 5-HT through their DA D_2 and 5-HT_{2A} receptor subtypes functionally regulate the ALDH activity in the brain regions and liver tissue of ethanol treated rats. Real-Time PCR studies confirm the DA D_2 & 5-HT_{2A} receptor binding parameters. Perfusion studies data show that dopamine, serotonin and glucose can regulate the ALDH activity in the liver of rats. EEG studies in the frontal region showed a prominent brain activity difference in the ethanol treated rats. DA and 5-HT functional regulation of ALDH has immense clinical significant in the management of ethanol addiction.

CONCLUSION

Neuronal dopamine and serotonin receptors are widely distributed in the central and the peripheral nervous systems at different levels. Dopaminergic and serotonergic systems have crucial role in aldehyde dehydrogenase regulation. Stimulation of autonomic nervous system during ethanol treatment is suggested to be an important factor in regulating the ALDH function. The ALDH enzyme activity was increased in plasma, cerebral cortex, and liver but decreased in cerebellum. The ALDH enzyme affinity was decreased in plasma, brainstem and liver and increased in cerebral cortex and cerebellum. The difference in ALDH activity in brain regions shows the functional regulation of ALDH by the dopaminergic and serotonergic systems at the central level. There is also peripheral level regulation in plasma and liver. Dopamine and serotonin content decreased in liver and brain regions - cerebral cortex, corpus striatum of ethanol treated rats with an increased HVA/DA, 5-HIAA/5-HT turnover rate. Dopamine content decreased in brainstem with an increased HVA/DA turnover rate and serotonin content decreased with an increased 5-HIAA/5-HT turnover rate in the brainstem of ethanol treated rats compared to control. Serotonin content increased in hypothalamus with a decreased 5-HIAA/5-HT turnover rate where as dopamine content decreased in hypothalamus with an increased HVA/DA turnover rate of ethanol treated rats compared to control. Dopamine, serotonin and their metabolic intermediates differentially regulate ethanol craving. Dopamine D_2 receptor binding parameters showed a functional increase in cerebellum, hypothalamus, and a decrease in brainstem, cerebral cortex and corpus striatum of ethanol treated rats compared to control. 5-HT_{2A} receptor binding parameters showed a functional increase in corpus striatum, hypothalamus, brainstem and a decrease in cerebral cortex, cerebellum and liver

of ethanol treated rats compared to control. The alterations of DA D_2 and 5-HT_{2A} receptor function and gene expression in the cerebellum, hypothalamus, corpus striatum, cerebral cortex play an important role in the sympathetic regulation of ALDH enzyme in ethanol addiction. The differences between ethanol treated and control rats in disposition of DA D_2 and 5-HT_{2A} receptors give a clear change in the presynaptic monoamine synthesis and postsynaptic receptor availability during ethanol treatment. The hyperactivity at the frontal cortical region is observed during the EEG analysis support the central effects of ethanol especially at the frontal region. The gene expression pattern of DA D_2 and 5-HT_{2A} receptors in the brain regions were in concordance with the receptor alterations. The results from ethanol perfusion study in liver show the dopaminergic and serotonergic functional regulation on ALDH. These alterations in the DA D_2 and 5-HT_{2A} receptors of the brain are suggested to play a regulatory role in the liver through sympathetic innervation. In addition, receptor binding studies and Real-Time PCR analysis revealed that DA D_2, 5-HT_{2A} receptor functional alterations observed during ethanol treatment clearly gives indication to the ethanol induced gene expression changes, functional interaction between DA D_2 and 5-HT_{2A} receptors and their role in ALDH regulation. Brain activity studies using EEG showed a prominent difference in the frontal region of ethanol treated rats.

Thus it is concluded that there is a serotonergic and dopaminergic functional regulation of ALDH activity in the brain regions and liver of ethanol treated rats. Gene expression studies of DA D_2 and 5-HT_{2A} studies confirm these observations. Perfusion studies using DA, 5-HT and glucose showed ALDH regulatory function. Brain activity measeurement using EEG showed a prominent

frontal brain wave difference. This will have immense clinical significance in the management of ethanol addiction.

SUMMARY

1) Ethanol treated rats were used as a model system to study the dopaminergic and serotonergic functional regulation on the aldehyde dehydrogenase at the molecular level.

2) Ethanol induced aldehyde dehydrogenase activity was observed in liver, plasma and the different brain regions when compared to control.

3) Dopamine and serotonin content decreased in liver and brain regions - cerebral cortex, corpus striatum of ethanol treated rats with an increased HVA/DA, 5-HIAA/5-HT turnover rate.

4) Dopamine content increased in brainstem with an increased HVA/DA turnover rate and serotonin content decreased in brainstem with an increased 5-HIAA/5-HT turnover rate of ethanol treated rats compared to control.

5) Serotonin content increased in hypothalamus with a decreased 5-HIAA/5-HT turnover rate and dopamine content decreased in hypothalamus with an increased HVA/DA turnover rate of ethanol treated rats compared to control.

6) Dopamine D_2 receptor binding parameters showed a functional increase in cerebellum, hypothalamus, and decrease in brainstem, cerebral cortex and corpus striatum of ethanol treated rats compared to control.

7) 5-HT_{2A} receptor binding parameters showed a functional increase in corpus striatum, hypothalamus, brainstem and decrease in cerebral cortex, cerebellum and liver of ethanol treated rats compared to control.

8) Real-Time PCR analysis of DA D_2, 5-HT_{2A} receptor confirmed the receptor data.

9) Real-Time PCR analysis of ALDH showed an increased expression in liver and cerebral cortex of ethanol treated rats compared to control.

10) Dopaminergic and serotonergic functional regulation of kinetic parameters of aldehyde dehydrogenase was observed in ethanol treated rats compared to control.

11) A prominent brain activity difference was observed in the frontal cortical region in ethanol treated rats compared to control by EEG analysis.

Thus it is observed that there is a functional regulation of dopamine and serotonin through DA D_2 and 5-HT_{2A} receptors in brain regions and liver on ALDH activity. The data suggests the importance of brain neurotrasmitter regulatory role on ALDH activity in ethanol tereated rats.

REFERENCES

REFERENCES

Abdellah Mansouri., Christine Demeilliers., Sabine Amsellem., Dominique Pessayre. & Bernard Fromenty. (2001). Acute Ethanol Administration Oxidatively Damages and Depletes Mitochondrial DNA in Mouse Liver, Brain, Heart, and Skeletal Muscles: Protective Effects of Antioxidants *J. Pharmacol. Exp. Ther.*, **298**, 737-743.

Abdollah. S., Catlin. M. C. & Brien. J. F. (1993). Ethanol neuro-behavioural teratogenesis in the guinea pig: behavioural dysfunction and hippocampal morphological change. *Can J Physiol Pharmacol,* **71**, 776-782.

Adams. M. A. & Hirst. M. (1984). Adrenal and urinary catecholamines during and after severe ethanol intoxication in rats: a profile of changes. *Pharmacol Biochem Behav,* **21**, 125-31

Addolorato. G., Caputo. F. & Capiristo. (2002). Baclofen efficacy in reducing alcohol craving and intake: A preliminary double-blind randomized controlled study. *Alcohol and Alcoholism.,* **37**, 504–508.

Aguiar. A. S., Da-Silva. V. A. & Boaventura. G. T. (2004). Can calories from ethanol contribute to body weight preservation by malnourished rats. *Braz J Med Biol Res.*, **37**, 841-846.

Ambroziak. W. & Pietruszko. R. (1991). Human aldehyde dehydrogenase activity with aldehyde metabolites of monoamines, diamines, and polyamines. *J. Biol. Chem.,* **266**, 13011-13018.

Amir. S. (1977). Brain and liver aldehyde dehydrogenase. Relations of ethanol consumption in Wistar rats.*Neuropharmacology.*, 16, 781-784.

Amir. S. (1978). Brain and liver aldehyde dehydrogenase activity and voluntary ethanol consumption by rats: relations to strain, sex and age. *Psychopharmacology.*, *57*, 97–102.

Ani Das. V., Savitha. B. & Paulose. C. S. (2006). Decreased alpha1-adrenergic receptor binding in the cerebral cortex and brain stem during pancreatic regeneration in rats. *Neurochem Res.*, **31**,727-734.

Ali. A. M., Rossouw. H. C., Silove. M. & Walt. J. G. (2000). Development of an improved technique for the perfusion of the isolated caudal lobe of sheep liver. *Experimental Physiology.*, **85**, 469-478.

Ali. A. M., Mohamed. H. E., van der Walt. J. G., Rossouw H.C & Engelbrecht Ovine H.(2006). Hepatic Metabolism. The Effect of Portal Amino Acid Concentration on the Metabolism of Glucose and Urea *Journal of Animal and Veterinary Advances.*, **5**, 16-19.

Alkana. R. L., Parker. E. S. & Cohen. H. B., (1976). Reversal of ethanol intoxications in humans: an assessment of the efficacy of propranolol. *Psychopharmacology.*, **51**, 29–37.

Alkana. R. L., Parker. E. S. & Cohen. H.B., (1977). Reversal of ethanol intoxication in humans: an assessment of the efficacy of L-dopa, aminophylline, and ephedrine. *Psychopharmacology.*, **55**, 203–212.

Alvaksinen. M. N. V., Saano. H., Juvonene. A., Huhtikangas. & Gunther. J. (1984). Binding of beta-carbolines and tetrahydroisoquinolines by opiate receptors of the d-type. *Acta Pharmacologica et Toxicologica.*, **55**, 380-385.

Aston-Jones. G., Foote. S.` & Bloom. F. (1982). Low doses of ethanol disrupt sensory responses to brain noradrenergic neurons. *Nature.*, **296**, 857–860.

Badawy. A. A., Morgan. C. J., Lovett. J. W., Bradley. D. M. & Thomas. R. (1995). Decrease in circulating tryptophan availability to the brain after acute ethanol consumption by normal volunteers: implications for alcohol-induced aggressive behaviour and depression. *Pharmacopsychiatry.*, **28**, 93-97.

Balasubramanian. S. & Paulose. C. S. (1998). Induction of DNA synthesis in primary cultures of rat hepatocytes by serotonin: Possible involvement of serotonin S_2 receptor. *Hepatology*, **27**, 62-66.

Baldessarini. R. J. & Tarazi. F. I. (1996). Brain dopamine receptors: A primer on their current status, basic and clinical. *Harvard Rev Psychiatry*, **3**, 301-325.

Baldessarini. R. J. & Tarazi. F. I (2001). Drugs and the treatment of psychiatric disorders. In: Hardman, J.G., Limbird L.E., Eds. *Goodman and Gilman's. In The Pharmacologic Basis of Therapeutics, McGraw-Hill, New York*, pp. 485-520.

Bardina. L. R., Pronko. P. S., Satanovskaia. V. I. & Kuzmich. A. B. (2003). Effect of acetaldehyde on ethanol- and aldehyde-metabolising systems of the liver and brain of rats. *Ukr Biokhim Zh.*, **75**, 129-133.

Basile. A. S., Jones. E. A. & Skolnick. P. (1991). The pathogenesis and treatment of hepatic encephalopathy: evidence for the involvement of benzodiazepine receptor ligands. *Pharmacol. Rev.*, **43**, 27-71.

Bavanisha Vythilingum, Charmaine Hugo. J., Stefan Maritz. J., Willie Pienaar & Dan Stein. J. (2005). Pharmacological challenge with a serotonin 1D agonist in alcohol dependence. *BMC Psychiatry.*, **5**, 31-34.

Benkelfat. C., Murphy. D. L., Hill. J. L., George. D. T., Nutt. D., Linnoila. M., (1991). Ethanol- like properties of the serotonergic partial agonist mchlorophenylpiperazine in chronic alcoholic patients. *Archivesof General Psychiatry*, **48**, 333-336.

Berger. D. & Weiner. H. (1977) Relationship between alcohol preference and biogenic aldehyde metabolizing enzymes in rats. *Biochemical Pharmacology*, **26**, 841–846.

Berggren. U., Eriksson. M., Fahlke. C. & Balldin. J. (2002). Is long-term heavy alcohol consumption toxic for brain serotonergic neurons? Relationship between years of excessive alcohol consumption and serotonergic neurotransmission. *Drug and Alcohol Dependence*, **65**, 159-165.

Berthoud. H. R., Neuhuber. W. L., (2000). Functional and chemical anatomy of the afferent vagal system. *Auton Neurosci.*, **85**, 1–17.

Biju. M. P., Pyroja. S., Rajeshkumar. N. V. & Paulose. C. S. (2001). Hypothalamic GABA receptor functional regulation and liver cell proliferation. *Mol. Cell. Biochem.*, **216**, 65-70.

Biju. M. P., Pyroja. S., Rajeshkumar. N. V. & Paulose. C. S. (2002). Brainstem GABA receptor functional regulation during rat liver cell proliferation. *Neurochem Res.*, **27**, 905-910.

Biju. M. P., Pyroja. S., Rajeshkumar. N. V. & Paulose. C. S. (2002). Enhanced GABA(B) receptor in neoplastic rat liver: induction of DNA synthesis by baclofen in hepatocyte cultures. *J Biochem Mol Biol Biophys.*, **6**, 209-214

Blaschko. H., Richter. D. & Schlossmann. H. (1937). The oxidation of adrenaline and other amines, *Biochem J.*, **31**, 2187-2196.

Blum. K., Noble. E. P., Sheridan. P. J., Montgomery. A., Ritchie. T., Jagadeeswaran. P., Nogami. H., Briggs. A. H. & Cohn. J. B. (1990), Allelic

association of human dopamine D2 receptor gene in alcoholism. *JAMA*, **263**, 2055-2060.

Blum. K, Sheridan. P. J., Wood. R. C., Braverman. E. R., Chen. T. J. & Comings D. E. (1995). Dopamine D2 receptor gene variants: association and linkage studies in impulsive-addictive-compulsive behaviour. *Pharmacogenetics*, 5, 121-141.

Blum. K. & Kozlowski. G. P. (1990). Ethanol and neuromodulator interactions: a cascade model of reward. *Progress in Alcohol Research*, 2, 131-149.

Boileau. I., Assaad. J. M., Pihl. R. O., Benkelfat. C., Leyton. M., Diksic. M., Tremblay. R. E. & Dagher. A. (2003). Alcohol promotes dopamine release in the human nucleus accumbens. *Synapse*, **49**, 226-231.

Bonasera. S. J., Chu. H. M., Brennan. T. J. & Tecott. L. H. (2006). A null mutation of the serotonin 6 receptor alters acute responses to ethanol. *Neuropsychopharmacology*, **31**, 1801-1813.

Bouthenent. M. L., Souil. E., Martres. M. P., Sokoloff. P., Giros. B. & Schwartz J. C. (1991). Localization of dopamine D_3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D_2 receptor mRNA. *Brain Res.*, **564**, 203-219.

Bradley. P. B., Engel. G., Feniuk. W., Fozard. J. R., Humphrey. P. P. A., Middlemiss. D. N., Mylecharane. E. J., Richardson. B. P. & Saxena. P. R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology*, **25**, 563-576.

Brien. J. F. & Loomis. C. W. (1983). Pharmacology of acetaldehyde. *Canadian Journal of Physiology and Pharmacology*, **61**, 1–22.

Brodie. M. S., Trifunovic. R. D. & Shefner. S. A. (1995). Serotonin potentiates ethanol-induced excitation of ventral tegmental area neurons in brain slices from three different rat strains. *J. Pharmacol. and Exp. Ther.*, **273**, 1139-1146.

Brown. Z. W., Amit. Z. & Smith. B. (1980). Intraventricular self-administration of acetaldehyde and voluntary consumption of ethanol in rats. *Behavioural and Neural Biology*, **28**, 150-155.

Buck. K. J. & Finn, D.A. (2000). Genetic factors in addiction: QTL mapping and candidate gene studies implicate GABAergic genes in alcohol and barbiturate withdrawal in mice. *Addiction*, **96**, 139–149.

Budygin. E. A., Phillips. P. E., Robinson. D. L., Kennedy. A. P., Gainetdinov. R. R. & Wightman. R. M. (2001). Effect of acute ethanol on striatal dopamine neurotransmission in ambulatory rats. *Journal of Pharmacology and Experimental Therapeutics*, **297**, 27-34.

Burinan. S. E., Hhensberry. R. & Liguori. A. (2003). Differential effects of alcohol and alcohol expectancy on risk-taking during simulated driving. *Human Psychopharmacology*, **18**, 175-184.

Butters. N. S., Gibson. M. A. S., Reynolds. J. N. & Brien. J. F. (2000). Effects of chronic prenatal ethanol exposure on hippocampal glutamate release in the postnatal guinea pig. *Alcohol,* **21**, 1-9.

Butterworth. R. F. (1995). The neurobiology of hepatic encephalopathy. *Semin. Liver Dis.*, **16**, 235-244.

Cagetti. E., Liang. J., Spigelman. I. & Olson. R. W. (2003). Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioural responses to positive allosteric modulators of GABAA receptors. *Molecular Pharmacology,* **63**, 53–64.

Calabresi. P., Maj. R., Pisani. A., Mercuri. N. B. & Bernardi. G. (1992). Long-term synaptic depression in the striatum: physiological and pharmacological characterization. *J. Neurosci.*, **12**, 4224-4233.

Calabresi. P., Centonze. D., Gubellini. P., Pisani. A. & Bernardi. G. (2000). Acetylcholine mediated modulation of striatal function. *Trends Neurosci.*, **23**,120-126.

Carboni. E., Acquas. E., Frau. R. & Di Chiara. G. (1989). Differential inhibitory effects of a 5-HT3 antagonist on drug-induced stimulation of dopamine release. *Eur J Pharmacol.*, **164**, 515-519.

Carey. R. M., Van Loon. G. R., Baines. A. D. & Kaiser. D. L. (1983). Suppression of basal and stimulated noradrenergic activities by the dopamine agonist bromocriptine in man. *J Clin Endocrinol Metab.*, **56**, 595–602.

Carlsson. A. (1993). Thirty years of dopamine research. *Adv Neurology*, **60**, 1-17,

Carobi. C. & Magni. F. (1981). The afferent innervation of the liver: A horseradish peroxidase study in the rat. *Neurosci Lett.*, **23**, 269–274.

Castro. S. W. & Strange. P. (1993). Differences in ligand binding properties of the short and long versions of the dopamine D2 receptor. *J. Neurochem.*, **360**, 372-375.

Catlin. M. C., Abdolloh. S. & Brien. J. F. (1993). Dose-dependent effects of prenatal ethanol exposure in the guinea pig. *Alcohol*, **10,** 90-115.

Centeno. M. L., Henderson. J. A., Pau. K. Y. & Bethea. C. L. (2006). Estradiol increases alpha7 nicotinic receptor in serotonergic dorsal raphe and noradrenergic locus coeruleus neurons of macaques. *J.Comp Neurol.*, **497**, 489-501.

Chen. Y. & Prusoff. W. H. (1973). Relationship between the inhibition constant (K_i) and the concentration of an inhibitor that causes a 50% inhibition of an enzymatic reaction.*Biochem.Pharmacol.*, **22**, 3099-3108.

Chen. H. T., Casanova. M. F. & Kleinman. J. E. (1991). ^{3}H-paroxetine binding in brains of alcoholics. *Psychiatry Res.*, **38**, 293–299.

Choi. D. S. & Maroteaux. L. (1996). Immunohistochemical localisation of the serotonin 5-HT2B receptor in mouse gut, cardiovascular system, and brain. *FEBS Lett.*, **391**, 45-51.

Christina. S., Barr. V. M. D., Timothy. Newman K., Stephen Lindell., Courtney Shannon., Maribeth Champoux., Klaus Peter Lesch., Stephen Suomi. J., David Goldman. & Dee Higley J. (2004). Interaction Between Serotonin Transporter Gene Variation and Rearing Condition in Alcohol Preference and Consumption in Female Primates. *Arch Gen. Psychiatry.*, **61**, 1146-1151.

Ciccocioppo. R., Angeletti. S., Colombo. G., Gessa. G. & Massi. M. (1999). Autoradiographic analysis of 5-HT2A binding sites in the brain of Sardinian alcohol-preferring and nonpreferring rats. *Eur J Pharmacol.*, **373**, 13-9.

Civelli. O., & Bunzow. J. (1993). Molecular diversity of the dopamine receptor. *Ann Rev Pharmacol Toxicol.*, **32**, 281-307.

Clarke. P. B. & Pert. A. (1985). Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. *Brain Res.*, **348**, 355-358.

Cloninger. C. R. (1987). Neurogenetic and adaptive mechanisms in alcoholism. *Science*, **236**: 410-416.

Collingridge. G. L. & Bliss. T. V. (1995). Memories of NMDA receptors and LTP.*Trends Neurosci.*, **18**, 54-56

Collins. M. A., Zou. J. Y. & Neafsey. E. J. (1998). Brain damage due to episodic alcohol exposure in vivo and in vitro: Furosemide neuroprotection implicates edema-based mechanism. *FASEB J.*, **12**, 221–230.

Cooper. J. R. & Bloom. F. E. (1991). The biochemical basis of Neuropharmacology, Sixth. Oxford University Press.

Corringer. P. J., Le Novère. N. & Changeux. J. P. (2000). Nicotinic receptors at the amino acid level. *Annu Rev Pharmacol Toxicol.*, **40**, 431–458.

Comings. D. E., Muhleman. D., Ahn. C., Gysin. R. & Flanagan. S. D. (1994). The dopamine D_2 receptor gene: a genetic risk factor in substance abuse. *Drug Alcohol Depend.*, **34**, 175–180.

Cowen. M. S & Lawrence. A. J. (1999). The role of opioid-dopamine interactions in the induction and maintenance of ethanol consumption. *Prog Neuropsychopharmacol Biol Psychiatry,* **23**, 1171-1212.

Crabb. D. W. (1995). Ethanol oxidizing enzymes: roles in alcohol metabolism and alcoholic liver disease. *Prog. Liver Dis.*, **13**,151-172.

Craig Bailey. D. C., James Brien. F. & James Reynolds. N. (2001).Chronic Prenatal Ethanol Exposure Increases $GABA_A$ Receptor Subunit Protein Expression in the Adult Guinea Pig Cerebral Cortex. *The Journal of Neuroscience*, **21**, 4381-4389.

Crews. F. T., Morrow. A. L., Criswell. H. & Breese. G. (1996). Effects of ethanol on ion channels. *Int Rev Neurobiol.*, **39**, 283–367.

Cristina. M. S., Russel.N., Susan. W. R., Mohamed. J. & Marc. G. C. (1998). Dopamine Receptors: From Structure to Function. *Physiol. Rev.*, **78**, 189-225.

Cronholm. T., Norsten-Hoog. C., Ekstrom. G., Handler. J. A., Thurman. R. G. & Ingelman- Sundberg M. (1992). Oxidoreduction of butanol in deermice (Peromyscus maniculatus) lacking hepatic cytosolic alcohol dehydrogenase *European Journal of Biochemistry*, **204**, 353-357.

Dakshinamurti. K., Paulose. C. S., Viswanathan. M. & Siow. Y. L. (1988). Neuroendocrinology of pyridoxine deficiency. *Neurosci Biobehav Rev.*, **12**,189-193.

David. Lovinger M. (1999). The Role of Serotonin in Alcohol'sEffects on the Brain. *Current Seperations*, **18**, 1-3.

Davis-Cox. M. I., Fletcher. T. L., Turner. J. N., Szarowski. D. & Shain. W.(1996). Three-day exposure to low-dose ethanol alters guanine nucleotide binding protein expression in the developing rat hippocampus. *Journal of Pharmacology and Experimental Therapeutics*, **276**, 758-764.

Daws. L. C., Montanez. S., Munn. J. L., Owens. W. A., Baganz. N. L., Boyce-Rustay. J. M., Millstein. R. A., Wiedholz. L. M., Murphy. D. L. & Holmes. A. (2006). Ethanol inhibits clearance of brain serotonin by a serotonin transporter-independent mechanism. *J Neurosci.*, **26**, 6431-6438

Deitrich. R. A., Dunwiddie. T. V., Harris. R. A. & Erwin. V. G (1989). Mechanism of action of ethanol; initial central nervous system actions. *Pharmacological Reviews*, **41**, 489–537.

Deitrich. R. A., & Erwin. V. G. (1996). Pharmacological effects of ethanol on the nervous system. Boca Raton, FL: CRC Press. pp. 269-290.

Delin. C. R. & Lee. T. H. (1992). Drinking and the brain: Current evidence. *Alcohol and Alcoholism*, **27**, 117– 126.

Denise Tomkins. M. & Edward Sellers. M. (2001). Addiction and the brain: the role of neurotransmitters in the cause and treatment of drug dependence.*CMAJ*, **164**, 817-21.

Desmoulin. F., Cozzone. P. J. & Canioni. P. (1987). Phosphorus-31 nuclear-magnetic-resonance study of phosphorylated metabolites compartmentation, intracellular pH and phosphorylation state during normoxia, hypoxia and ethanol perfusion, in the perfused rat liver. *European Journal of Biochemistry*, **162**, 151-159.

Devor. E. J. & Cloninger. C. R. (1989). Genetics of alcoholism. *Annual Review of Genetics*, **23**, 19-36.

Di Chiara. G. (1995). The role of dopamine in drug abuse viewed from the perspective of its role in motivation. *Drug and Alcohol Dependence*, **38**. 95-137.

Di Chiara. G. & Imperato. A. (1985). Ethanol preferentially stimulates dopamine release in the nucleus accumbens of freely moving rats. *European Journal of Pharmacology*, **115**, 131-132.

Dildy. J. E. & Leslie. S. W. (1989). Ethanol inhibits NMDA-induced increases in free intracellular Ca^{2+} in dissociated brain cells. *Brain Res.*, **499**, 383-387.

Dinan. T. G. (1996). Serotonin and the regulation of hypothalamic-pituitary-adrenal axis function, *Life Sci.*, **58**, 1683-1694.

Dolhman. H. G., Thorne. J., Caron. M. G. & Lefkowitz. R. J. (1991). Model system for the study of seven transmembrane segment receptors. *Annual Review of Biochemistry*, **60**, 653-688,

Dori. I., Dinopoulos. A., Blue. M. E. & Parnavelas. J. G. (1996). Regional differences in the ontogeny of the serotonergic projection to the cerebral cortex. *Exp. Neurol.*, **138**, 1-14.

Dowd. B. F. (1993). Structure of dopamine receptors. *J. Neurochem.*, **60**, 804-816,

Eckardt. M. J., File. S. E., Gessa. G. L., Grant. K. A., Guerri, C., Hoffman, P. L., Kalant, H., Koob, G. F., Li, T. K. & Tabakoff, B. (1998). Effects of moderate alcohol consumption on the central nervous system. *Alcoholism: Clinical and Experimental Research*, **22**, 998–1040.

Ellis. F. W. (1966). Effects of ethanol on plasma corticosterone levels. *Journal of Pharmacology and Experimental Therapeutics*, **153**, 121–128.

Enjalbert. A., Sladeczek. F., Guillon. G., Bertrand. P., Shu. C. & Epelbaum. J., (1986). Angiotensin II and dopamine modulate both cAMP and inositol phosphate productions in anterior pituitary cells. *J. Biol Chem.*, **261**, 4071-4075.

Enomoto. N., Takase. S., Yasuhara. M. & Takada. A. (1991). Acetaldehyde metabolism in different aldehyde dehydrogenase-2 genotypes. *Alcoholism: Clinical and Experimental Research*, **15**, 141–144.

Eriksson. C. J. P. (1973). Ethanol and acetaldehyde metabolism in rat strains genetically selected for their ethanol preference. *Biochemical Pharmacology*, **22**, 2283–2292.

Eriksson. C. J. P. (1977), Regulation of acetaldehyde metabolism during ethanol oxidation in perfused rat liver. *Adv Exp Med Biol.*, **85**, 225-236.

Eriksson. C. J. P. (1985). Endogenous acetaldehyde in rats. Effects of exogenous ethanol, pyrazole, cyanamide and disulfiram. *Biochemical Pharmacology*, **34**, 3979–3982.

Erlander. M. G., Lovenberg. T. W., Baron. B. M., de Lecea. L., Danielson. P. E., Racke. M., Slone. A. L., Siegel. B. W., Foye. P. E. & Cannon. K. (1993). Two members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat brain. Proc. *Natl. Acad. Sci.*, **90**, 3452-3456.

Erspamer. V. (1996). Occurence of indolealkylamines in nature. 5-hydroxytryptamine and realetd alkylamines. *In Handbuch der Experimentellen Pharmakologic. ed. Erspamer.V.* pp. 132-181.

Evert. D. L. & Oscar-Berman. M. (1995). Alcohol-related cognitive impairments: An overview of how alcoholism may affect the workings of the brain. *Alcohol Health & Research World,* **19**, 89–96,

Everett. P. B. & King. R. A. (1970). Schedule-induced alcohol ingestion. *Psychonomic Science*, **18,** 278-279.

Evrard. S. G., Duhalde-Vega. M., Tagliaferro. P., Mirochnic. S., Caltana. L. R. & Brusco. A. (2006). A low chronic ethanol exposure induces morphological changes in the adolescent rat brain that are not fully recovered even after a long abstinence: An immunohistochemical study. *Exp Neurol.*, **200**, 438-59.

Ewing. J. A., Rouse. B. A. & Pellizzari. E. D. (1974). Alcohol sensitivity and ethnic background. *American Journal of Psychiatry*, **131**, 206-210.

Falk. J. L., Samson. H. H. & Winger. G. (1972). Behavioural maintenance of high concentration of blood ethanol and physical dependence in the rat. *Science*, **177**, 811-813.

Fan. L., Bellinger. F. P., Ge. Y. L. & Wilce. P. A. (2004). Genetic study of alcoholism and novel gene expression in the alcoholic brain.*Addict Biol.*, **9**, 11–18.

Fedeli. A., Ciccocioppo. R., Economidou. D., Angeletti. S. & Massi. M . (2002). Autoradiographic analysis of 5-hydroxytryptamine 5-HT2A binding sites in the rat brain after chronic intragastric ethanol treatments. *Res Commun Mol Pathol Pharmacol.*, **112**, 113-27.

Finckh. U. (2001). The dopamine D2 receptor gene and alcoholism. Association studies. In: DP Agarwal, HK Seitz (Eds): *Alcohol in Health and Disease. New York: Marcel Dekker, Inc.* pp 151-176.

Florijn. W. J., Tarazi. F. I. & Creese. I. (1997) Dopamine Receptors. In: Bittar EE, Bittar N, Eds, *Principles of Medical Biology*, JAI Press, New York, 73–94.

Forsell. L. (1981). Studies on the metabolic effects of ethanol in rat, dissertation. 54 *Opuscula medica*, Supplementum LV, Stockholm.

Freed. E. X. (1972). Alcohol polydipsia in the rat as a function of caloric need. *Quarterly Journal of Studies on Alcohol*, **33,** 504-507.

Freedman. L. J., Insel. T. R. & Smith. Y. (2000). Subcortical projections of area 25 (subgenual cortex) of the macaque monkey. *J Compar Neurol.*, **421**, 172-188

Freetly. H. C. & Ferrell. C. L. (1999). Relationship of portal-drained viscera and liver net flux of glucose, lactate, volatile fatty acids and nitrogen metabolism to milk production in the ewe. *J. Dairy Sci.*, **83**, 597-604.

Frerichs. F. T. (1860). A clinical treatise on diseases of the liver by Dr Friedrich Theodor Frerichs; translated by Charles Murchison. London. *The New Sydenham Society*, 193–246.

Frokjaer. V. G., Strauss. G. I., Mehlsen. J., Knudsen. G. M., Rasmussen. V. & Larsen. F. S. (2006). Autonomic dysfunction and impaired cerebral autoregulation in cirrhosis, *Clin Auton Res.*, **16**, 208-216. Epub

Gelernter. J., Kennedy. J. L., Van. T. H. H. M., Civelli. O. & Kidd. K. K. (1992). The D_4 dopamine receptor (DRD4) maps to distal 11p close to HRAs. *Genomics*, **13**, 208-210.

Gemma. S., Vichi. S., Testai. E. (2006). Individual susceptibility and alcohol effects:biochemical and genetic aspects. *Ann Ist Super Sanita*, **42**, 8-16.

Gerhard Wiesbeck. A., Kenneth. M., Dürsteler-MacFarland , Friedrich Martin Wurst, Marc Walter , Sylvie Petitjean , Sandra Muller , Norbert Wodarz & Jobst Boning. (2006). No association of dopamine receptor sensitivity *in vivo* with genetic predisposition for alcoholism and DRD2/ DRD3 gene polymorphisms in alcohol dependence. *Addiction Biology*, **11**, 72–75.

German Torres & Judith Horowitz M. (1999). Drugs of Abuse and Brain Gene Expression. *Psychosomatic Medicin*, **61**, 630-650.

Gill. K., Amit. Z. & Smith. B. R. (1996). The regulation of alcohol consumption in rats: the role of alcohol-metabolising enzymes-catalase and aldehyde dehydrogenase. *Alcohol*, **13**, 347–353.

Gingrich. J. A. & Marc. G. C. (1993). Recent advances in the molecular biology of dopamine receptors. *Annu. Rev. Neurosci.*, **16**, 299-321.

Giorguieff. M. F., Le Floch M. L., Westfall. T. C., Glowinski. J. & Besson. M. J. (1976). Nicotinic effect of acetylcholine on the release of newly synthesized (3H) dopamine in rat striatal slices and cat caudate nucleus. *Brain Res.*, **106**, 117-131.

Giros. B., Sokoloff. P., Martres. M. P., Riou. J. F., Emorine. L. J. & Schwartz. J. C. (1989). Alternative splicing directs the expression of two D_2 dopamine receptor isoforms. *Nature,* **342**, 923-6.

Giros. B., Sokoloff. P., Martres. M. P., Riou. J. F., Emorine. L. J. & Schwartz. J. C. (1990).Cloning of the human D_3 dopaminergic receptor and chromosome identification. *C R Acad Sci., Paris,* **311**, 501-508.

Glennon. R. A. (1987). Central serotonin receptors are targets for drug research. *J. Med. Chem.*, **30**, 1-12.

Glowinski. J. & Iversion. L. L. Regional studies of catecholamines in the rat brain: The disposition of [^{3}H] Norepinephrine, [^{3}H] DOPA invarious regions of the brain.*J Neurochem.*, **13,** 655-699.

Goedde. H. W., Agarwal D. P, Fritze G., Meier Tackmann D., Singh S., Beckmann G. (1992). Distribution of ADH_2 and ALDH2 genotypes in different populations. *Human Genetics*, **88**, 344-346.

Grailhe. R., Amlaiky. N., Ghavami. A., Ramboz. S., Yocca. F., Mahle. C., Margouris. C., Perrot. F. & Hen. R. (1994). Human and mouse 5-HT5A and 5-HT5B receptors: Cloning and functional expression. *Soc. Neurosci. Abstr.*, **20**, 1160-1168.

Grandy. D. K., Litt. M., Allen. L., Bunzow. J. R., Marchionni. M. & Makam. H. (1989). The human dopamine D_2 receptor gene is located on chromosome 11 at q22-q23 and identifies a TaqI RFLP. *Am J Hum Genet.*, **45**, 778-785.

Grandy. D. K., Marchionni. M. A., Makam. H., Stofko. R. E., Alfano. M. & Fischer. J. B. (1989).Cloning of the cDNA and gene for a human D_2 dopamine receptor. *Proc Natl Acad Sci.*, **86**, 9762-9766.

Grandy. D., Zhoung. Y., Bouvier. C., Zhou. Q., Johnson. R. & Allen. L. (1991). Multiple human D_5 dopamine receptor genes: a functional receptor and two pseudogenes. *Proc Natl Acad Sci.*, **88**, 9175-9179.

Grant. K. A. (1995. The role of 5HT-3 receptors in drug dependence. *Drug and Alcohol Dependence*, **38**,155-171.

Grant. K. A. (1994).Emerging neurochemical concepts in the actions of ethanol at ligand-gated ion channels. *Behavioural Pharmacology*, **5**, 383-404.

Grant. K. A., Colombo. G. & Gatto. G. J. (1997).Characterization of the ethanol-like discriminative stimulus effects of 5-HT receptor agonists as a function of ethanol training dose. *Psychopharmacology*, **133**, 133–141.

Grant. K. A., Lovinger. D. M. (1995).Cellular and behavioural neurobiology of alcohol: receptor-mediated neuronal processes. *Clin Neurosci.*, **3**,155–164.

Green. A. R., DeSouza. R. J., Davis. E. M. & Cross. A. J. (1990). The effects of Ca^{2+} antagonists and hydralazine on central 5-Hydroxytryptamine biochemistry biochemistry and function in rats and mice.*Br. J. Pharmacol..*, **99**, 41-46.

Grobin. A. C., Matthews. D. B., Devaud. L. L. & Morrow. A. L. (1998). The role of GABA(A) receptors in the acute and chronic effects of ethanol. *Psychopharmacology*, **139**, 2-19.

Grzanna. R. & Molliver. M. E. (1980). The locus coeruleus in the rat: an immunohistochemical delineation. *Neuroscience*, **5**, 21-40.

Gusella. J. F. (1989).Location cloning strategy for characterizing genetic defects in Huntington's disease and Alzheimer's disease. *FASEB*, **3**, 2036-2041.

Guardia. J., Catafau. A. M., Battle. F., Martin. J. C., Segura. L. & Gonzalvo. B. (2000). Striatal dopaminergic D_2 receptor density measured by [^{123}I] iodobenzamide SPECT in the prediction of treatment outcome of alcohol-dependent patients. *Am J Psychiatry*, **157**, 127-129.

Hagan. J. J., Hatcher. J. P. & Slade. P. D. (1995). The role of 5-HT1D and 5-HT_{1A} receptors in mediating 5-hydroxytryptophan induced myoclonic jerks in guinea pigs. *Eur. J. Pharmacol.*, **294**, 743-751.

Harada. S., Agarwal. D. P., Goedde. H. W., Tagaki. S. & Ishikawa. B. (1982). Possible protective role against alcoholism for aldehyde dehydrogenase isozyme deficiency in Japan. *Lancet*, **2**, 827-832.

Haranda. S., Agarwal. D. P., Goedde. H. W., & Ishikawa. B. (1983). Aldehyde dehydrogenase isoenzyme variation and alcoholism in Japanese. *Pharmacology Biochemistry & Behaviour*, **18**, 151-154.

Harding. A. J., Wong. A., Svoboda. M., Kril. J. J. & Halliday. G. M. (1997). Chronic alcohol consumption does not cause hippocampal neuron loss in humans. *Hippocampus*, **7**, 78–87.

Hasselmo. M. E. (1995). Neuromodulation of cortical function: modeling the physiological basis of behaviour. *Behav Brain Res.*, **67**, 1–27.

Hayes. G., Biden. T. J., Selbie. L. A. & Shine. J. (1992). Structural subtypes of the dopamine D2 receptor are functionally distinct: expression of the cloned D2A and D2B subtypes in a heterologous cell line. *Mol Endocrinol.*, **6**, 920–6.

Hayrapetyan. V., jenschke. M, Dillon. G. H. & Machu T. K. (2005). Co-expression of the 5-HT_{3B}subunit with the 5-HT_{3A} receptor reduces alcohol sensitivity. *Brain Res Mol Brain Res.*, **142**,146-150.

He. X. X., Nebert. D. W., Vasiliou. V., Zhu. H. & Shertzer .H. G. (1997).Genetic differences in alcohol drinking preference between inbred strains of mice. *Pharmacogenetics*, **7**, 223-33.

Heidbreder. C. & De Witte. P. (1993). Ethanol differentially affects extracellular monoamines and GABA in the nucleus accumbens. *Pharmacology, Biochemistry and Behaviour, 46*, 477-481.

Heinz. A. & Goldman. D. (2000). Genotype effects on neurodegeneration and neuroadaptation in monoaminergic neurotransmitter systems. *Neurochemistry International*, **37**, 425-432.

Heinz. A., Mann. K., Weinberger. D. R. & Goldman. D. (2001), Serotonergic dysfunction, negative mood states, and response to alcohol. *Alcohol Clin Exp Res.*, **25**, 487- 495.

Heinz. A., Siessmeier. T., Wrase. J., Buchholz. H. G., Grunder. G., Kumakura. Y., Cumming. P., Schreckenberger. M., Smolka. M. N., Rosch. F., Mann. K. & Bartenstein. P. (2005).Correlation of alcohol craving with striatal dopamine synthesis capacity and D2/3 receptor availability: a combined [18F]DOPA and [18F]DMFP PET study in detoxified alcoholic patients. *Am J Psychiatry*, **162**, 1515-1520.

Hellstrom. E. & Tottmar. O. (1982). Effects of aldehyde dehydrogenase inhibitors on enzymes involved in the metabolism of biogenic aldehydes in rat liver and brain. *Biochemical Pharmacology*, **31**, 3899-3905.

Hemmings. H. & Greengrade. P. (1986). DARPP-32, a dopamine and 3'-5' monophosphate-regulated phospho protein: regional, tissue and phylogenetic distribution. *J Neurosci.*, **6**, 1469-1481.

Hemmings. H., Walaas. S., Ouiment. C. & Greengard. P. (1987). Dopaminergic regulation of protein phosphorylation in the striatum: DARPP-32. *Trends Neurosci.*, **10**, 377-383.

Hersch. S. M., Gutekunst. C. A., Rees. H. D., Heilman. C. J. & Levey. A. I . (1994). Distribution of m1-m4 muscarinic receptor proteins in the rat striatum: light and electron microscopic immunocytochemistry using subtype-specific antibodies. *J Neurosc.*, **14**, 3351-3363.

Hietala. J., West. C., Syvalahti. E., Nagren. K., Lehikoinen. P., Sonninen. P. & Ruotsalainen. U. (1994). Striatal D2 dopamine receptor binding characteristics in vivo in patients with alcohol dependence *Psychopharmacology*, **116**, 285-290.

Higley. J. D., Suomi. S. J. & Linnoila. M. (1996).A nonhuman primate model of type II excessive alcohol drinking consumption? Part I. Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentrations and diminished social competence correlate with excessive alcohol consumption. *Alcohol Clin. Exp. Res.*, **20**, 629–642.

Higuchi. S., Muramatsu. T., Shigemori. K., Saito. M., Kono. H., Dufour. M. & Harford. T. (1992). The relationship between low Km aldehyde dehydrogenase phenotype and drinking behaviour in Japanese. *Journal of Studies on Alcohol*, **53**, 170–175.

Himei. A., Kono. Y., Yoneda. H., Sakai. T., Koh. J., Sakai. J., Inada. Y. & Imamichi. H. (2000). An association study between alcoholism and the serotonergic receptor genes. Alcoholism: *Clinical and Experimental Research.* **24**, 341–342.

Hindle. A.T. (1994). Recent developments in the physiology and pharmacology of 5-hydroxytryptamine. *Br. J. Anaesth.*, **73**, 395-407.

Hio. C., Drong. R., Riley. D., Gill. G., Slightom. J. & Huff. R. (1994).D_4 dopamine receptor-mediated signalling events determined in transfected Chinese hamster ovary cells. *J Biol Chem.*, **269**, 11813-11819.

Hiroshi Kinoshita., David Jessop. S., David Finn. P., Toni Coventry. L., David Roberts. J., Kiyoshi Ameno., Iwao Jiri. & Michael Harbuz. S. (2001). Acetaldehyde, a metabolite of ethanol, activates the hypothalamic–pituitary–adrenal axis in the rat. *Alcohol and Alcoholism*, **36**, 59-64.

Hofmann. C. E., Simms. W., Yu. W. K. & Weinberg. J. (2002). Prenatal ethanol exposure in rats alters serotonergic-mediated behavioural and physiological function.*Psychopharmacology*, **161**, 379-386.

Howes. L. G., MacGilchrist. A., Hawksby. C., Sumner. D. & Reid. J. L. (1986). Plasma [3H]-noradrenaline kinetics and blood pressure following regular, moderate ethanol consumption. *Br J Clin Pharmacol.*, **22**, 521-526.

Hoyer. D., Clarke. K. E., Fozard. J. R., Hartig. P. R., Martin. G. R., Mylecharane. E. J., Saxena. P. R. & Humphrey. P. P. (1994). AInternational Union for Pharmacology Classification of Receptors for 5-Hydroxytryptamine. *Pharmacol Rev.*,**46**,157-203.

Huang. S. Y., Lin. W. W., Ko. H. C., Lee. J. F., Wang. T. J., Chou. Y. H., Yin. S. J. & Lu. R. B. (2004). Possible interaction of alcohol dehydrogenase and aldehyde dehydrogenase genes with the dopamine D_2 receptor gene in anxiety-depressive alcohol dependence. *Alcohol Clin Exp Res.*, **28**, 374-384.

Hunt. W. A (1993).Neuroscience research: How has it contributed to our understanding of alcohol abuse and alcoholism? A review. *Alcohol Clin Exp Res.*, **17**, 1055–1065.

Hunt. W. A. (1996).Role of acetaldehyde in the actions of ethanol on the brain-a review, *Alcohol*, **13**, 147-151.

Ibanez J., Herrero. M. T., Insausti. R., Balzunegui. T., Tunon. T., Garcia-Bragado. F. & Gonzalo. L. M. (1995). Chronic alcoholism decreases neuronal nuclear size in the human entorhinal cortex. *Neurosci Lett.*, **183**, 71–74.

Imperato. A. & Di Chiara. G. (1986). Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *Journal of Pharmacology and Experimental Therapeutics*, **239**, 219-228.

Iwai. M. & Jungermann. K. (1989). Mechanism of action of cysteinyl leukotrienes on glucose and lactate balance and on flow in perfused rat liver. Comparison with the effects of sympathetic nerve stimulation and noradrenaline. *Eur. J. Biochem.*, **180**, 273-281.

Jackson. J. & Paulose. C. S. (1999). Enhancement of [m-methoxy 3H] MDL 100 907 binding to $5HT_{2A}$ receptors in cerebral cortex and brain stem of streptozotocin induced diabetic rats. *Mol. Cell. Biochem.*, **199**, 81-85.

Jackson. J., Padayatti. P. S., Paul. T. & Paulose. C. S. (1997). Platelet monamine

changes in diabetic patients and streptozotocin -induced diabetic rats. *Current Science*, **72,** 137-139.

Jackson. D. M. & Westlind. D. A. (1994). Dopamine receptors: molecular biology, biochemistry and behavioural aspects. *Pharmacol. Ther.*, **64**, 291-370.

Jan Balldin Berggren. U., Lindstedt. G. & Oreland L. (1994). Alcoholics With Reduced Dopamine D_2 Receptor Function Possess Normal Platelet Monoamine Oxidase Activity. *Alcohol and Alcoholism*, **29**, 659-661.

Jankowska. E., Bidzinski. A. & Kostowski. W. (1994). Alcohol drinking in rats treated with 5, 7-dihydroxytryptamine: effect of 8-OH-DPAT and tropisetron (ICS 205-930). *Alcohol*, **11**, 283–288.

Jerlhag. E., Grotli. M., Luthman. K., Svensson. L. & Engel. J. A. (2006). Role of the Subunit composition of central nicotinic acetylcholine recepotros for the stimulatory and dopamine-enhancing effects of ethanol. *Alcohol Alcohol*, Jun 23; [Epub ahead of print].

Jolas. T. & Aghajanian. G. K. (1997). Neurotensin and the serotonergic system. *Prog Neurobiol.*, **52**, 455-68.

Jones. E. A. (1995). Fatigue associated with chronic liver disease: a riddle wrappped in a mystery inside an enigma. *Heaptology*, **22**, 1606-1608.

Josefsson. L. G. & Rask. L. (1997). Cloning of a putative G-protein-coupled receptor from Arabidopsis thaliana. *Eur. J. Biochem.*, **249**, 415-420.

Julius. D., Livelli. T., Jessell. T. M. & Axel. R. (1989). Ectopic expression of the serotonin 1c receptor and the triggering of malignant transformation. *Science*, **244**, 1057-62.

Jungermann. K. & Stumpel. F. (1999). Role of hepatic, intrahepatic and hepatoenteral nerves in the regulation of carbohydrate metabolism and hemodynamics of the liver and intestine. *Hepatogastroenterology*, **46**, 1414–1417.

Kahkonen. S., Wilenius. J., Nikulin. V. V., Ollikainen. M. & Ilmoniemi. R. J. (2003). Alcohol reduces prefrontal cortical excitability in humans: a combined TMS and EEG study. *Neuropsychopharmacology*, **28**, 747-754.

Kalivas. P. W., Duffy. P. & Eberhardt. H. (1990). Modulation of A10 dopamine neurons by gamma-aminobutyric acid agonists. *J Pharmacol Exp.*, **253**, 858-866.

Kanterman. R. Y., Mahan.L. C., Briley. E. M., Monsma. F. J. Jr., Sibley. D. R. & Axelrod. J. (1991). Transfected D_2 dopamine receptors mediate the potentiation of arachidonic acid release in chinese hamster ovary cells. *Mol Pharmacol.*, **39**, 364-369.

Karen Spivak., Carlos. M. G., Aragon. & Zalman Amit. (1987). Alterations in Brain Aldehyde Dehydrogenase Activity Modify Ethanol-induced Conditioned Taste Aversion Alcoholism.*Clinical and Experimental Research*, **11**, 513-517.

Karlin. A. & Akabas. M. H. (1995). Toward a structural basis for the function of nicotinic acetylcholine receptors and their cousins. *Neuron*, **15**, 1231-1244.

Kawaguchi. Y. (1993). Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. *J Neurosci.*, **13**, 4908-4923.

Kebabian. J. & Calne. D. (1979). Multiple receptors for dopamine. *Nature*, **277**, 93-96.

Keltner. D., Young. R. C., Heerey. E. A. & Oemig. C. (1998). Teasing in hierarchical and intimate relations. *Journal of Personality and Social Psychology*, **75**, 1231-1247.

Kerfoot. S. M., Mello. D. C., Nguyen. H., Ajuebor. M. N., Kubes. P., Le. T. & Swain. M. G. (2006). TNF-alpha-secreting monocytes are recruited into the brain of cholestatic mice. *Hepatology*, **43**, 154-62.

Kharchenko. N. K., Synyts'kyi. V. N. & Kovtun. T. V. (2001). Comparative analysis of the effects of alcoholism and opium addiction on liver function *Fiziol Zh.*, **47**, 81-86.

Kim. S. A., Kim. J. W., Song. J. Y., Park. S., Lee. H. J. & Chung. J. H. (2004). Association of polymorphisms in nicotinic acetylcholine receptor alpha 4 subunit gene (CHRNA4), mu-opioid receptor gene (OPRM1), and ethanol-metabolizing enzyme genes with alcoholism in Korean patients. *Alcohol*, **34**, 115-120.

Kinoshita. H., Jessop. D. S., Finn. D. P., Coventry. T. L., Roberts. D. J., Ameno. K., Ijiri. I. & Harbuz. M. S. (2001). Acetaldehyde, a metabolite of ethanol, activates the hypothalamic–pituitary–adrenal axis in the rat. *Alcohol and Alcoholism*, **36**, 59–64.

Klemm. W. R., Mallari. C. G., Dreyfus. L. R., Fiske. J. C., Forney. E. & Mikeska. J. A. (1976). Ethanol induced regional and oseresponse differences in multiple-unit activity in rabbits. *Psychopharmacology*, **49**, 235–244.

Klemm. W. R. & Stevens. R. E. (1974). Alcohol effects on EEG on multiple-unit activity in various brain regions of rats. *Brain Res.*, **70**, 361–368.

Kligman. D. & Marshak. D. R. (1985). Purification and characterization of aneurite extension factor from bovine brain. *Proc. Natl. Acad. Sci.*, **82**, 7136-7139.

Klyosov. A. A., Rashkovetsky. L. G., Tahir. M. K. & Keung. W. M. (1996). Possible role of liver cytosolic and mitochondrial aldehyde dehydrogenases in acetaldehyde metabolism. *Biochemistry*, **35**, 4445–4456.

Knauf. C., Cani. P. D., Perrin. C., Iglesias. M. A., Maury. J. F., Bernard. E., Benhamed. F., Gremeaux. T., Drucker. D. J., Kahn. C. R., Girard. J., Tanti. J. F., Delzenne. N. M., Postic. C. & Burcelin. R. (2005). Brain glucagon-like peptide-1 increases insulin secretion and muscle insulin resistance to favor hepatic glycogen storage. *J Clin Invest.* ,**115,** 3554-3563.

Kohen. R, Metcalf. M. A., Khan. N., Druck. T., Huebner. K., Lachowicz. J. E., Meltzer. H. Y., Sibley. D. R., Roth. B. L. & Hamblin. M. W. (1996). Cloning, characterization, and chromosomal localization of a human 5-HT6 serotonin receptor. *J. Neurochem.*, **66**, 47-55.

Koivisto. T. & Salaspuro. M. (1996). Aldehyde dehydrogenase of the rat colon: comparison with other tissues of the alimentary tract and the liver. *Alcoholism: Clinical and Experimental Research*, **20**, 551–555.

Konishi. T., Calvillo. M., Leng. A. S., Lin. K. M. & Wan. Y. J. (2004). Polymorphisms of the dopamine D2 receptor, serotonin transporter, and GABA (A) receptor beta(3) subunit genes and alcoholism in Mexican-Americans. *Alcohol.* , **32**, 45-52.

Koob. G. F. & Weiss. F. (1992). Neuropharmacology of cocaine and ethanol dependence. In: Galanter M, editor. *Recent developments in alcoholism*, **10**, 201-233.

Kuhn. D. M., Wolf. W. A. & Lovenberg.W. (1980). Review of the role of the central serotonergic neruonal system in blood pressure regulation. *Hypertension*, **2**, 253-255.

Kulak. J. M., Nguyen. T. A., Olivera. B. M. & McIntosh. J. M. (1997). α-Conotoxin MII blocks nicotine-stimulated dopamine release in rat striatal synaptosomes. *J Neurosci.*, **17**, 5263-5270.

Kulinskii.V. I., Udovitsina. T. I., Vstavskaia. Iu. A. & Rykov. S. A. (1983). Comparison of the changes in mitotic activity and in serotonin concentration in regenerating liver. *Vopr. Med. Khim.*, **29**, 104-107.

Lands. W. E. & Zakhari. S. (1991). The case of the missing calories. *Am J Clin Nutr.*, **54**, 47-48.

Lauder. J. M. & Krebs. H. (1978). Serotonin as a differentiation signal in early neurogenesis. *Dev. Neurosci.*, **1**, 15-30.

Laure-Achagiotis. C., Poussard. A. M. & Loui-Sylvestre. J. (1990). Alcohol drinking, food and fluid intakes and body weight gain in rats. *Physiology and Behaviour*, **47**, 545-548.

Lautt. W. W. (1983). Afferent and efferent neural roles in liver function. *Prog. Neurobiol.*, **21**, 323-348.

Lee., Min-Seo ab., Ryu & Sung-Ho. (2002). No association between the dopamine D3 receptor gene and Korean alcohol dependence *Psychiatric Genetics*, **12**, 173-176.

Le Marquand. D., Pihl. R. O. & Benkelfat. C. (1994). Serotonin and alcohol intake, abuse, and dependence: clinical evidence. *Biol Psychiatry*, **36**, 326-37.

Lesch. K. P., Bengel. D., Heils. A., Sabol. S. Z., Greenberg. B. D., Petri. S., Benjamin. J., Muller. C. R., Hamer. D. H. & Murphy. D. L. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, **274**, 1527-1531.

Lester. D. (1961). Self-maintenance of intoxication in the rat. *Quarterly Journal of Studies on Alcohol*, **22**, 223-231.

Levesque. D., Diaz. J., Pilon. C., Martres. M., Giros. B. & Souil. E. (1992). Identification, characterization and localization of the dopamine D_3 receptor in rat brain using 7-[^{3}H] hydroxy-N,N-di-n-propyl-2-aminotetralin. *Proc Natl Acad Sci.*, **89**, 8155-8159.

Levesque. D., Marteres. M., Diaz. J., Griffon. N., Lammers. C. & Sokoloff. P. (1995). A paradoxical regulation of the dopamine D_3 receptor expression

suggests the involvement of an anterograde factor from dopamine neurons. *Proc Natl. Acad. Sci.*, **92**, 1719-1723.

Li. T. K. (2000). Pharmacogenetics of responses to alcohol and genes that influence alcohol drinking. *Journal of Studies on Alcohol,* **61**, 5–12.

Limosin. F. A., Loze. J. Y. A., Dubertret. C. B., Gouya. L. C., Ades. J. B D., Rouillon. & Gorwood. F. A. (2003). Impulsiveness as the intermediate link between the dopamine receptor D2 gene and alcohol dependence. *Psychiatric Genetics*, **13**, 127-129.

Liu. J. & Lauder. J. (1992). Serotonin promotes region-specific glial influences on cultured serotonin and dopamine neurons. *Glia*, **5**, 306–17.

Lu. R. B., Lee. J. F., Ko. H. C. & Lin. W. W. (2001). Dopamine D2 receptor gene (DRD2) is associated with alcoholism with conduct disorder. *Alcohol Clin Exp Res.*, **25**, 177-184.

Luo. J. & Miller. M. W. (1998). Growth factor-mediated neural proliferation; target of ethanol toxicity. *Brain Res Rev.*, **27**, 157–167.

Liappas. I., Piperi. C., Malitas. P. N., Tzavellas. E. O., Zisaki. A., Liappas. A. I., Kalofoutis. C. A., Boufidou. F., Bagos. P., Rabavilas. A. & Kalofoutis. (2006). A Interrelationship of hepatic function, thyroid activity and mood status in alcohol-dependent individuals. *In Vivo*, **20**, 293-300.

Lieber. C. S. (1991).Perspectives: do alcohol calories count? *Am J Clin Nutr.*, **54**, 976-982.

Lindahl. R. (1992). Aldehyde dehydrogenases and their role in carcinogenesis. *Critical Reviews in Biochemistry and Molecular Biochemistry*, **27**, 283–335.

Lingford-Hughes. A., Potokar. J. & Nutt. D. (2002). Treating anxiety complicated by substance misuse. *Advances in Psychiatric Treatment,* **8**, 107-116.

Liskow. B. I. & Goodwin. D. W. (1987). Pharmacological treatment of alcohol intoxication, withdrawal and dependence: A critical review. *Journal of Studies on Alcohol,* **48**, 356-370.

Lovinger. D. M., & Peoples. R. W. (1993). Actions of alcohols and other sedative/hypnotic compounds on cation channels associated with glutamate and Alling. C., Diamond. I., Leslie. S. W., Sun. G. Y. & Wood. W.G. eds. Alcohol,

Cell Membranes and Signal Transduction in Brain. New York: Plenum Press,. pp. 157–168.

Lovinger. D. M. & Zhou. Q. (1994). The Role of Serotonin in Alcohol's Effects on the Brain *Neuropharmacol*, **33,** 1567-1572.

Lowry. O. H., Rosenbrough. N. H., Farr. A. L. & Randall. R. J. (1951). Protein measurement with folin Phenol reagent. *J Biol Chem,* **193,** 265-275.

Macho. L., Zorad. S., Radikova. Z., Patterson-Buckedahl. P. & Kvetnansky. R. (2003). Ethanol consumption affects stress response and insulin binding in tissues of rats. *Endocr Regul*, **37**, 195-202.

Macieira. M. S., Almeida. W. G., Silva. E. A., Schenberg. L. C. & Nakamura-Palacios. E. M. (1997). Alcohol dependence induced in rats by semivoluntary intermittent intake. *Brazilian Journal of Medical and Biological Research*, **30**, 1107-1111.

Madras. B. K., Michele. A., Fahey. D. R. C. & Roger. D. S. (1988). D1 and D2 dopamine receptors in caudate-putamen of nonhuman primates (Macaca *fascicularis*). *Journal of Neurochemistry,* **51**, 934-943.

Mahan. L., Burch. R., Monsma. F. & Sibley. D., (1990). Expression of striatal D_1 dopamine receptors coupled to inositol phosphate production and Ca^{2+} mobilization in Xenopus oöcytes. *Proc Natl Acad Sci.* **87**, 2196-2200.

Makimoto. K. (1998). Drinking patterns and drinking problems among Asian-Americans and Pacific Islanders. *Alcohol Health & Research World.* **22**, 270–275.

Malenka. R. C. & Kocsis. J. D. (1988). Presynaptic actions of carbachol and adenosine on corticostriatal synaptic transmission studied in vitro. *J Neurosci.*, **8**, 3750-3756.

Manfred Brauer., Wuhua Lu., & Mingfu Ling. (1998). Chronic ethanol administration alters hepatic rates of glycerol phosphorylation and glycerol 3-phosphate oxidation: a dynamic in vivo ^{31}P magnetic resonance spectroscopy study. *Biochem. Cell Biol.*, **76**, 542–552.

Marie-Christine Beauvieux., Patrice Couzigou., Henri Gin., Paul Canioni. & Jean-Louis Gallis. (2004). Some processes of energy saving and expenditure occurring during ethanol perfusion in the isolated liver of fed rats; a Nuclear Magnetic Resonance study. *BMC Physiol.*, **4**, 3-5.

Marie Christine Beauvieux , Pierre Tissier, Patrice Couzigou, Henri Gin' Paul Canioni & Jean-Louis Gallis.(2002). Ethanol perfusion increases the yield of oxidative phosphorylation in isolated liver of fed rats. *Biochimica Biophysica Acta,* **1570**, 135-140.

Maricq. A. V., Peterson. A. S., Brake. A. J., Myers. R. M. & Julius. D. (1991). Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. *Science,* **254**, 432-7.

Marois. R. & Croll. R. P. (1992). Development of serotonin like immunoreactivity in the embryonic nervous system of the snail Lymnaea stagnalis. *J. Comp. Neurol.*, **322,** 255-265.

Marzia Foddai, Gabriella Dosia, Saturnino Spiga & Marco Diana. (2004). Acetaldehyde Increases Dopaminergic Neuronal Activity in the VTA. *Neuropsychopharmacology,* **29**, 530-536.

Mash. D. C., Staley. J. K., Doepel. F. M., Young. S. N., Ervin. F. R. & Palmour. R. M. (1996). Altered dopamine transporter densities in alcohol-preferring vervet monkeys. *Neuroreport.*, **7**, 457-62.

Massimo Mannelli, Lucia Ianni, Chiara Lazzeri, Walter Castellani, Cinzia Pupilli, Giorgio La Villa, Giuseppe Barletta, Mario Serio & Franco Franchi. (1999). *In Vivo* Evidence That Endogenous Dopamine Modulates Sympathetic Activity in Man. *Hypertension,* **34**, 398-402.

Matsubara. K., Fukushima. S. & Fukui. Y. (1987). A systematic regional study of brain salsolinol levels during and immediately following chronic ethanol ingestion in rats. *Brain Research,* **413**, 336–343.

Matsumoto. H., Matsubayashi. K. & Fukui. Y. (1996).Mitochondrial ALDH polymorphism affects ethanol-derived acetate disposition in Wistar rats. *Alcohol Clin Exp Res.*, **20**, 284-288.

Maurel. S., Schreiber. R. & De Vry. J. (1997). Substitution of the selective serotonin reuptake inhibitors fluoxetine and paroxetine for the discriminative stimulus effects of ethanol in rats. *Psychopharmacology*, **130**, 404-6.

McBride. W. J., Bodart. B., Lumeng. L. & Li. T. K. (1995). Association between low contents of dopamine and serotonin in the nucleus accumbens and high alcohol preference. *Alcoholism: Clinical and Experimental Research,* **19**,1420-1422.

Mc Bride. W. J., Guan. X. M., Chernet. E., Lumeng. L. & Li. T. K. (1990). Regional differences in the densities of serotonin 1A receptors between P and NP rats. *Alcoholism: Clinical and Experimental Research,* **14**, 316-319.

Mc Call. R. B. & Harris. L. T. (1987). Characterization of the central sympathoinhibitory action of ketanserin. *J. Pharmacol. Exp. Ther.*, **241**, 736-740.

Mc Call. R. B., Patel. B. N. & Harris. L. T. (1987). Effects of serotonin1 and serotonin2 receptor agonists and antagonists on blood pressure, heart rate and sympathetic nerve activity. *J. Pharmacol. Exp. Ther.*, **242**, 1152-1159.

Mc Call. B. R. & Harris. L. T. (1988). 5-HT2 receptor agonists increases spontaneous sympathetic nerve discharge. *Eur. J. Pharm.*, **151**, 113-116.

Mc Kenzie-Quirk. S. D., Girasa. K. A., Allan. A. M. & Miczek. K. A. (2005). 5-HT(3) receptors, alcohol and aggressive behaviour in mice. *Behav Pharmacol.*, **16**, 163-169.

Meador-Woodruff. J. G. D., Van Tol. H., Damask. S., Little. K. & Civelli. O. (1994). Dopamine receptor gene expression in the human medial temporal lobe. *Neuropsychopharmacology*, 10, 239-248.

Mehta. A. K. & Ticku. M. K. (1999). An update on GABAA receptors. *Brain Research Reviews,* **29**, 196–217.

Meisch. R. A. & Thompson. T. (1972). Ethanol intake during schedule-induced polydipsia. *Physiology & Behaviour*, **8**, 471-475.

Melchior. C. L. & Tabakoff. B. (1986). The effect of 5,7-dihydroxytryptamine treatment on response to ethanol in mice.*Pharmacol. Biochem. Behav.*, **24**, 955–961.

Meldrum. B. (1982). Pharmacology of GABA. *Clin Neuropharmacol.*, **5**, 293-316.

Miller. M. W. (1992). Effects of prenatal exposure to ethanol on cell proliferation and neuronal migration; in Miller MW (ed): Development of the Central Nervous System: Effects of Alcohol and Opiates. New York, Wiley-Liss, 47–69.

Minori Nishiguchi, Hiroshi Kinoshita, Jamal Mostofa1, Tadaaki Taniguchi, Harumi Ouchi, Takako Minami, Katsuhiko Hatake, Takao Utsumi, Hiroyuki Motomura & Shigeru Hishida (2002). Different blood acetaldehyde concentration following ethanol administration in a newly developed high

alcohol preference and low alcohol preference rat model system. *Alcohol and Alcoholism*, **37**, 9-12.

Miyajima. H., Nomura. M., Muguruma. N., Okahisa. T., Shibata. H., Okamura. S., Honda. H., Shimizu. I., Harada. M., Saito. K., Nakaya. Y. & Ito. S. (2001). Relationship among gastric motility, autonomic activity, and portal hemodynamics in patients with liver cirrhosis. *J Gastroenterol Hepatol.*, **16**, 647–659.

Miyazawa. A., Fujiyoshi. Y., Stowell. M. & Unwin. N. (1988). Nicotinic acetylcholine receptor at 4.6 A resolution: transverse tunnels in the channel wall. *J Mol Biol.*, **288**, 765–786.

Mizoi. Y., Ijiri. I., Tatsumo. Y., Kijima. T., Fujiwara. S., Adachi. J., & Hishida. S. (1979). Relationship between facial flushing and blood acetaldehyde levels after alcohol intake. *Pharmacology Biochemistry & Behaviour*, **10**, 303-311.

Mizoi. Y., Tatsumo. Y., Adachi. J., Kogame. M., Fukunasa. T., Fujiwara. S., Hishida. S. & Ijiri. I. (1983). Alcohol sensitivity related to polymorphism of alcohol-metabolizing enzymes in Japanese. *Pharmacology Biochemistry & Behaviour*, **18**, 127-134.

Mogi. T., Marti. T. & Khorana. H. G. (1989). Structure-function studies on bacteriorhodopsin. IX. Substitutions of tryptophan residues affect protein-retinal interactions in bacteriorhodopsin. *J. Biol. Chem.*, **264**, 14197-14201.

Mohanan[a]. V. V., Khan. R. & Paulose. C. S. (2006) Hypothalamic 5-HT functional regulation through 5-HT1A and 5-HT2C receptors during pancreatic regeneration. *Life Sci.*, **78**, 1603-1609.

Mohanan[b]. V. V., Chathu. F. & Paulose. C. S. (2005). Decreased 5-HT_{2C} receptor binding in the cerebral cortex and brain stem during pancreatic regeneration in rats. *Mol Cell Biochem.*, **272**, 165-170.

Mohanan. V. V., Kaimal. S. B., Paulose. C. S. (2005). Decreased 5-HT_{1A} receptor gene expression and 5-HT_{1A} receptor protein in the cerebral cortex and brain stem during pancreatic regeneration in rats. *Neurochem Res.*, **30**, 25-32

Monsma. F., Mahan. L., McVittie. L., Gerfen. C. & Sibley. D. (1990). Molecular cloning and expression of a D_1 dopamine receptor linked to adenylyl cyclase activation. *Proc Natl Acad Sci*, **87**, 6723-6727.

Moore. K. & Lookingland. K. (1995). Dopaminergic neuronal systems in the hypothalamus. In: Bloom F, Kupfer D, Eds. *Psychopharmacology*: Fourth Generation of Progress, Raven Prss, New York, 245-456.

Mostofa Jamal, Kiyoshi Ameno, Takako Kubota, Setsuko Ameno, Xia Zhang, Mitsuru Kumihashi & Iwao Ijiri. (2003). *In vivo* formation of salsolinol INDUCED by high acetaldehyde concentration in rat striatum employing microdialisis. *Alcohol and Alcoholism,* **38**, 197-201.

Myers. W. D., Kim. T. N., Singer. G., Smythe. G. A. & Duncan. M. W. (1985). Dopamine and salsolinol levels in rat hypothalami and striatum after schedule-induced self injection (SISI) of ethanol and acetaldehyde. *Brain Research,* **358**, 122–128.

Nakamura. T., Matsushita. S., Nishiguchi. N., Kimura. M., Yoshino. A., Higuchi. S. (1999). Association of a polymorphism of the 5HT2A receptor gene promoter region with alcohol dependence. *Mol. Psychiatry.*, **4**, 85-88.

Narahashi. T., Aistrup. G. L., Marszalec. W. & Nagata. K. (1999). Neuronal nicotinic acetylcholine receptors: a new target site of ethanol. *Neurochem Int.*, **35**, 131-141

Nevo. I. & Hamon. M. (1995). Neurotransmitter and neuromodulatory mechanisms involved in alcohol abuse and alcoholism. *Neurochemistry International*, **26**: 305-336.

Nicole Barbarich. C. & Walter Kaye. H. (2004). Altered 5-HT_{2A} Receptor Binding after Recovery from Bulimia-Type Anorexia Nervosa: Relationships to Harm Avoidance and Drive for Thinness. *Neuropsychopharmacology,* 29, 1143-1155.

Nobin. A., Baumgarten. H. G., Falck. B., Ingemansson. S., Moghimzadeh. E., Rosengren. E. & Fausto. N. (1978). Organization of the sympathetic innervation in liver tissue from monkey and man. *Cell Tissue Res.*, **195**, 371–380.

Noble. E. P. (2000). The DRD_2 gene in psychiatric and neurological disorders and its phenotypes. *Pharmacogenomics.*, **1**, 309-333.

Noble. E. P. (1996). The gene that rewards alcoholism. *Sci Am Sci Med.*, **3**, 52–61.

Noldy. N. E. & Carlen. P. L. (1990). Acute, withdrawal, and chronic alcohol effects in man: event-related potential and quantitative EEG techniques. *Ann Med.*, **22**, 333–339.

Oben. J. A., Roskams. T., Yang. S., Lin. H., Sinelli. N., Li. Z., Torbenson. M., Huang. J., Guarino. P., Kafrouni. M. & Diehl. A. M. (2003). Sympathetic nervous system inhibition increases hepatic progenitors and reduces liver injury. *Hepatology*, **38**, 664-73.

O'Brien. C. P., Eckardt. M. J. & Linnoila. V. M. I. (1995). Pharmacotherapy of alcoholism. In: Bloom FE & Kupfer DJ (Editors), *Psychopharmacology*: The Fourth Generation of Progress. Raven Press, New York, 1745-1755.

Ogata. H., Ogata. F., Mendelson. J. H. & Mello. N. K. (1972). A comparison of techniques to induce alcohol dependence and tolerance in the mouse. *Journal of Pharmacology and Experimental Therapeutics*, **180**, 216-230.

Ogawa. N. (1995). Molecular and chemical neuropharmacology of dopamine receptor subtypes. *Acta Med. Okayama*, **49**, 1-11.

Ogilvie. K., Lee. S. & Rivier. C. (1997). Effects of three different modes of alcohol administration on the activity of the rat hypothalamic-pituitary-adrenal axis. *Alcoholism: Clinical and Experimental Research*, **21**, 467–476.

Ortells. M. O. & Lunt. G. G. (1995). Evolutionary history of the ligand-gated ion-channel superfamily of receptors. *Trends Neurosci.*, **18**, 121-127.

Ortiz. J., Fitzgerald. L. W., Charlton. M., Lane. S., Trevisan. L., Guitart. X., Shoemaker. W., Duman. R. S. & Nestler. E. J. (1995). Biochemical actions of chronic ethanol exposure in the mesolimbic dopamine system. *Synapse*, **21**: 289-298.

Pandey. S. C., Davis. J. M. & Pandey. G. N. (1995). Phosphoinositide system-linked serotonin receptor subtypes and their pharmacological properties and clinical correlates *J. Psychiatry and Neurosci.*, **20**, 215-225.

Pandey. S. C., Piano. M. R., Schwertz. D. W., Davis. J. M. & Pandey. G. N. (1992). Effect of ethanol administration and withdrawal on serotonin receptor subtypes and receptor-mediated phosphoinositide hydrolysis in rat brain. *Alcoholism: Clinical And Experimental Research*, **16**, 1110–1116.

Patel. S., Roberts. J., Moorman. J. & Reavill. C. (1995). Localization of serotonin-4 receptors in the striatonigral pathway in rat brain. *Neuroscience*, **69**, 1159-1167.

Paulose. C. S., Dakshinamurthi. K., Packer. L. & Stephens. N. L. (1988). Symapathetic stimulation and hypertension in pyridoxine deficient adult rat. *Hypertension*, **11**, 387-391.

Pelletier. G. & Simard. J. (1991). Dopaminergic regulation of pre-proNPY mRNA levels in the rat arcuate nucleus. *Neurosci Lett.*, **127**, 96–98.

Peroutka. S. J. (1993). 5 -Hydroxytryptamine receptors. *J. Neurochem.*, **60**, 408-416.

Perrin. R. G., Hockman. C. H., Kalant. H. & Livingston. K. E. (1974). Acute effects of ethanol on spontaneous and auditory evoked electrical activity in cat brain. *Electroencephalogr Clin Neurophysiol.*, **36**, 19–31.

Petrov. T., Krukoff. T. L. & Jhamandas. J. H. (1994). Chemically defined collateral projections from the pons to the central nucleus of the amygdala and hypothalamic paraventricular nucleus in the rat. *Cell Tissue Res.*, **277**, 289-295.

Pietrzak. B. & Czarnecka. E. (2005). Effect of the combined administration of ethanol and acamprosate on rabbit EEG. *Pharmacol Rep.*, **57**, 61-69.

Pirola. R. C. & Lieber. C. S. (1976). Hypothesis: energy wastage in alcoholism and drug abuse: possible role of hepatic microsomal enzymes. *Am J Clin Nutr.*, **29**, 90–93.

Pohorecky. L. A. (1982) Influence of alcohol on peripheral neurotransmitter function. *Fed Proc.*, **41**, 2452-2455.

Plum. L., Schubert. M. & Bruning. J. C. (2005). The role of insulin receptor signalling in the brain. *Trends Endocrinol Metab.*, **16**, 59-65.

Pollock. V. E., Volavka. J., Goodwin. D. W., Mednick. S. A., Gabrielli. W. F., Knop. J. & Schulsinger. F. (1983). The EEG after alcohol administration in men at risk for alcoholism. *Archives of General Psychiatry*, **40**, 857-861

Pozzi. M., Grassi. G., Redaelli. E., Dell'oro. R., Ratti. L., Redaelli. A., Foglia. G., Di Lelio. A., & Mancia. G. (2001). Patterns of regional sympathetic nerve traffic in preascitic and ascitic cirrhosis. *Hepatology*, **34**, 1113–1118.

Preuss. U. W., Koller. G., Bondy. B., Bahlmann. M. & Soyka. M. (2001). Impulsive Traits and 5-HT2A Receptor Promoter Polymorphism in Alcohol Dependents: Possible Association but No Influence of Personality Disorders. *Neuropsychobiology*, **43**,186-191.

Pugh. C. E. M. & Quastel. J. H. (1937). Oxidation of amines by animal tissues *Biochem. J.*, **31**, 2306-2321.

Rakonczay. Z. Jr., Boros. I., Jarmay. K., Hegyi. P., Lonovics. J. & Takacs. T. (2003). Ethanol administration generates oxidative stress in the pancreas and liver, but fails to induce heat-shock proteins in rats. *J. Gastroenterol Hepatol.*, **18**, 858-867.

Rassnick. S., D'Amico. E., Riley. E., & Koob. G. F. (1993). GABA antagonist and benzodiazepine partial inverse agonist reduce motivated responding for ethanol. *Alcoholism: Clinical and Experimental Research*, **17**, 124-130.

Reed. T. E., Kalant. H., Gibbons. R. J., Kapur. B. M., & Rankin. J. C. (1976). Alcohol and acetaldehyde metabolism in Caucasians, Chinese and Amerinds. *Canadian Medical Association Journal*, **115**, 851-855.

Renuka. T. R., Savitha. B. & Paulose. C. S. (2005). Muscarinic M1 and M3 receptor binding alterations in pancreas during pancreatic regeneration of young rats. *Endocr Res.*, **31**, 259-270.

Repo. E., Kuikka. J. T., Bergstron. K. A., Karhu. J., Hiltunen. J. & Tiihonen. J. (1999). Dopamine transporter and D2-receptor density in late-onset alcoholism. *Psychopharmacology*, **147**, 314-318.

Rittenhouse. P. A., Bakkum. E. A., Levy. A. D., Li. Q., Carnes. M. & van de Kar. LD. (1994). Evidence that ACTH secretion is regulated by serotonin2A/2C (5-HT2A/2C) receptors. *J Pharmacol Exp Ther.*, **271**, 1647-1655.

Rivier. C. (1996). Alcohol stimulates ACTH secretion in the rat: Mechanisms of action and interactions with other stimuli. *Alcoholism: Clinical and Experimental Research*, **20**, 240–254.

Rivier. C., Bruhn. T. & Vale. W. (1984). Effects of ethanol on the hypothalamo-pituitary-adrenal axis in the rat: Role of corticosterone-releasing factor (CRF). *Journal of Pharmacology and Experimental Therapeutics*, **229**, 127–131.

Rivier. C. & Lee. S. (1996). Acute alcohol administration stimulates the activity of hypothalamic neurons that express corticotropin-releasing factor and vasopressin. *Brain Research*, **726**, 1–10.

Rivier. C. & Vale. W. (1988). Interaction between ethanol and stress on ACTH and beta-endorphin secretion. *Alcoholism: Clinical and Experimental Research,* **12**, 206–210.

Roberta Ward. J., Wendy Kest., Philippe Bruyeer., Frédéric Lallemand & Philippe De Witte. (2001). Taurine modulates catalase, aldehyde dehydrogenase, and ethanol elimination rates in rat brain. *Alcohol and Alcoholism*, **36**, 39-43.

Rodriguez. J. H. (1994). Serotonin as a neurotrophic factor in the fetal brain: Binding, capture and release in centers of axonal growth. *Gac. Med. Mex.*, **130**, 246-252.

Rogers. R. C. & Hermann. G. E. (1983). Central connections of the hepatic branch of the vagus nerve horse radish peroxidase histochemical study. *J. Auto. Nerv. Sys.*, **7**, 165-174.

Rosman. A. S., Afsar. W., Baraona. E. & Lieber. R. (2000). Disulfiram treatment increases plasma and red blood cell acetaldehyde in abstinent alcoholics. *Alcoholism: Clinical and Experimental Research,* **24**, 958–964.

Rothblat. D. S., Rubin. E. & Schneider. J. S. (2001). Effects of chronic alcohol ingestion on the mesostriatal dopamine system in the rat. *Neuroscience Letters*, 300, 63-66.

Russell. V. A., Lanin. M. C. L. & Taljaard. J. F. (1988). Effect of ethanol on 3H-dopamine release in rat nucleus accumbens and striatal slices. *Neurochemical Research,* **13**, 487-492.

Samson. H. H. & Harris. R. A. (1992). Neurobiology of alcohol abuse. *Trends in Pharmacological Sciences*, **13**, 206-211.

Samson. H. H. & Falk. J. L. (1974). Alteration of fluid preference in ethanol-dependent animals. *Journal of Pharmacology and Experimental Therapeutics*, **190**, 365-376.

Sander. T., Harms. H. & Podschus. J. (1995). Dopamine D1, D2 and D3 receptor genes in alcohol dependence. *Psychiatr Genet.*, **5**, 171–176.

Sari. Y., Bell. R. L. & Zhou. F. C. (2006). Effects of chronic alcohol and repeated deprivations on dopamine D1 and D2 receptor levels in the extended amygdala of inbred alcohol- preferring rats. *Alcohol Clin Exp Res.*, **30**, 46-56.

Sarkar. D. K., Chaturvedi. K., Oomizu. S., Boyadjieva. N. I. & Chen. C. P. (2005). Dopamine, Dopamine D2 Receptor Short Isoform, Transforming Growth

Factor (TGF)-ß1, and TGF-ß Type II Receptor Interact to Inhibit the Growth of Pituitary Lactotropes. *Endocrinology*, **146**, 4179-4188.

Scatchard. G. (1949). The attraction of proteins for small molecules and ions.*Anh. N. Y. Acad.Sci.*, 660-672.

Schlesinger. K., Kakihana. R., & Bennett. E. L. (1966). Effects of tetraethylthiuram disulfide (Antabuse) on the metabolism and consumption of ethanol in mice. *Psychonomic Science*, **28**, 514-520.

Schwartz. J. C., Giros. B., Martres. M. P. & Sokoloff. P. (1992). The Dopamine Receptor Family: Molecular Biology and Pharmacology. *Seminars in the Neurosciences*, **4**, 99-108.

Seeburg. P. H. (1989). The dopamine D_2 receptor: two molecular forms generated by alternative splicing. *EMBO J.*, **8**, 4025-4034.

Seeman. P. (1980). Brain dopamine receptors. *Pharmacol Rev.*, **32**, 229-313.

Sellers. E. M., Higgins. G. A. & Sobell. M. B. (1992). 5-HT and alcohol abuse. *Trends in Pharmacological Sciences*, **13**, 69-75.

Senter. R. J. & Sinclair. J. D. (1967). Self-maintenance of intoxication in the rat: A modified replication. *Psychonomic Science*, **9**, 291-292.

Sergeeva. O. A., Schulz. D., Doreulee. N., Ponomarenko. A. A., Selbach. O., Borsch. E., Kircheis. G., Huston. J. P., Haussinger. D. & Haas. H. L. (2005). Deficits in cortico-striatal synaptic plasticity and behavioural habituation in rats with portacaval anastomosis. *Neuroscience*, **134**, 1091-8.

Shattuck. K. E., Grinnell. C. D. & Rassin. D. K. (1993). Amino acid infusions induced reversible, dose related decreases in bile flow in the isolated rat liver. *J. Parenter. Enterat. Nutr.*, **17**, 171-176.

Shepherd. G. M. (1994) *Neurobiology*, 3rd ed. New York: Oxford University Press.Pp 133-169.

Sheppard. J. E., Albersheim. P. & Mc Clearn. G. E. (1968). Enzyme activities and ethanol preference in mice. *Biochemical Genetics*, **2**, 205-212.

Shefner. S. & Tabakoff. B. (1985). Basal firing of rat locus coeruleus neurons affects sensitivity to ethanol. *Alcohol*, **2**, 239-243.

Shimazu. T. (1981). Central nervous system regulation of liver and adipose tissue metabolism. *Diabetologia*, Publisher: Springer Berlin / Heidelberg, **20**, 343 - 356.

Sibley. D., Monsama. F. & Shen. Y. (1993). Molecular neurobiology of dopaminergic receptors. *Intl Rev Neurobiol.*, **35**, 391-415.

Sibley. D. R. (1999). New insights into dopaminergic receptor function using antisense and genetically altered animals. *Annu. Rev. Pharmacol. Toxicol.*, **39**, 313-341.

Signs. S. A. & Schechter. M. D. (1988). The role of dopamine and serotonin receptors in the mediation of the ethanol interoceptive cue. *Pharmacol Biochem Behav.*, **30**, 55-64.

Slawecki. C. J., Grahame. N. J., Roth. J., Katner. S. N. & Ehlers. C. L. (2003). EEG and ERP profiles in the high alcohol preferring (HAP) and low alcohol preferring (LAP) mice: relationship to ethanol preference. *Brain Res.*, **961**, 243-54.

Smith. M. E. & Newman. H. W. (1959). The rate of ethanol metabolism in fed and fasted animals. *J. Biol. Chem.*, **234**, 1544-1548.

Socaransky. S. M., Aragon. C. M., Amit. Z. & Blander. A. (1984). Higher correlation of ethanol consumption with brain than liver aldehyde dehydrogenase in three strains of rats. *Alcohol*, 84, 250-253.

Soderpalm. B., Ericson. M., Olausson. P., Blomqvist. O. & Engel. J. A. (2000). Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. *Behav Brain Res.*, **113**, 85-96.

Sokoloff. P., Giros. B., Martres. M., Bouthenet. M. & Schwartz. J. (1990). Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. *Nature*, **347**, 146-51.

Spear. L. P. (2002). Alcohol's effects on adolescents. *Alcohol Res Health*, **26**, 287-291.

Stoltenberg. S. F., Twitchell. G. R., Hanna. G. L., Cook. E. H., Fitzgerald. H. E., Zucker. R. A. & Little. K. Y. (2002), Serotonin transporter promoter polymorphism, peripheral indexes of serotonin function, and personality measures in families with alcoholism. *Am J Med Genet.*, **114**, 230-234.

Stoyanova. I. I. & Gulubova. M. V. (1998). Peptidergic nerve fibres in the human liver. *Acta Histochem.*, **100**, 245-256.

Stoyanova. I. I. & Gulubova. M. V. (2000). Immunocytochemical study on the liver innervation in patients with cirrhosis. *Acta Histochem.,*. **102**, 391-402

Strange. P. G. (1996). Dopamine Receptors. Studies on Structure and Function. *Adv. in Drug Res.*, **28**, 314-351.

Sudha. B. & Paulose. C. S. (1997). Induction of DNA synthesis in primary cultures of rat hepatocytes by serotonin: Possible involvement of serotonin S2 receptor. *Hepatology*, **27**, 62-67.

Sun. H. F., Chang. Y. T., Fann. C. S., Chang. C. J., Chen. Y. H., Hsu. Y. P., Yu. W. Y. & Cheng. A. T. (2002). Association study of novel human serotonin 5-HT (1B) polymorphisms with alcohol dependence in Taiwanese Han. *Biol Psychiatry*, **51**, 896-901.

Sunahara. R., Niznik. H., Weiner. D., Sturmann. T., Brann. M. & Kennedy. J. (1990). Human dopamine D_1 receptor encoded by an intronless gene on chromosome 5. *Nature*, **347**, 80-83

Suter. P. M., Schutz. Y. & Jequier. E. (1992).The effect of ethanol on fat storage in healthy subjects. *N Engl J Med.*, **326**, 983–987.

Suzdak. P. D., Schwartz. R. D., Skolnick. P. & Paul. S. M.(1986). Ethanol stimulates gamma-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes. *Proc Natl Acad Sci.*, 83, 4071-4075

Swanson. L.W. & Sawchenko. P. E. (1980). Paraventricular nucleus: A site for the integration of neuroendocrine and autonomic mechanisms, (Progress in Neuroendocrinology), *Neuroendocrinol.*, **31**, 410-417.

Syvalahti. E. K., Hietala. J., Roytta. M. & Gronroos. J. (1988). Decrease in the number of rat brain dopamine and muscarinic receptors after chronic alcohol intake. *Pharmacology and Toxicology*, **62**, 210-212.

Szabo. B., Crass. D. & Starke. K. (1992). Effect of the dopamine D2 receptor agonist quinpirole on renal sympathetic nerve activity and renal norepinephrine spillover in anesthetized rabbits. *J Pharmacol Exp Ther.*, **263**, 806-815.

Tae Woo Jung, Ji Young Lee, Wan Sub Shim, Eun Seok Kang, Soo Kyung Kim, Chul Woo Ahn, Hyun Chul Lee & Bong Soo Cha. (2006). Rosiglitazone relieves

acute ethanol-induced hangover in sprangue- dawley rats. *Alcohol and Alcoholism,* **41**, 231-235.

Tabakoff. B. & Hoffman. P. L. (1996). Effect of alcohol on neurotransmitters and their receptors and enzymes. In: Begleiter H, Kissin B, eds. *The pharmacology of alcohol and alcohol dependence*. New York:Oxford University Press.

Tabakoff. B. & Hoffman. P. L. (1988). Genetics and biological markers of risk for alcoholism. *Public Health Reports,* **103,** 690-698.

Tanaka. K., Ohkawa. S., Nishino. T., Niijima. A. & Inoue. S. (1987). Role of the hepatic branch of the vagus nerve in liver regeneration in rats. *Am. J. Physiol.*, **253**, 439-444.

Takayoshi Kiba. (2002). The Role of the Autonomic Nervous System in Liver Regeneration and Apoptosis - *Recent Developments. Digestion*, **66**, 79-88.

Tanaka. H., Iwasaki. S., Arima. M. & Nakazawa. K. (1985). Effects of combinations of maternal agents on the fetal cerebrum in rat-ethanol or caffeine with X-irradiation in utero. *Brain Dev.*, 7, 10-20.

Tang. K. C., Low. M. J., Grandy. D. K. & Lovinger. D. M. (2001). Dopamine-dependent synaptic plasticity in striatum during in vivo development. *Proc Natl Acad Sci.*, **98**, 1255-1260.

Tank. A. W. (1981). Enzymology and subcellular localization of aldehyde oxidation in rat liver.Oxidation of 3, 4-dihydroxyphenylacetaldehyde derived from dopamine to 3, 4-dihydroxyphenylacetic acid. *Biochem.Pharmacol.*, **30**, 3265-3275.

Tarter. R. E., Arrie. A. & Van Thiel. D. H. (1993). Liver–brain interactions in alcoholism. In: Hunt, W.A., and Nixon, S.J., eds. *Alcohol-Induced Brain Damage*. NIAAA Research Monograph No. 22. Rockville, MD: National Institute on Alcohol Abuse and Alcoholism. pp. 415–429.

Thanos. P. K., Taintor. N. B., Rivera. S. N., Umegaki. H., Ikari. H., Roth. G., Ingram. D. K., Hitzemann. R., Fowler. J. S., Gatley. S. J., Wang. G. J. & Volkow N. D. (2004). DRD2 gene transfer into the nucleus accumbens core of the alcohol preferring and nonpreferring rats attenuates alcohol drinking. *Alcohol Clin Exp Res.*, **28**, 720-728.

Thiagarajan. A. B., Mefford. I. N. & Eskay. R. L. (1989). Single-dose ethanol administration activates the hypothalamic-pituitary-adrenal axis: exploration of the mechanism of action. *Neuroendocrinology*, **50**, 427–432.

Thurman. R. G. & Scholz. R. (1977). Interaction of glycolysis and respiration in perfused rat liver.Changes in oxygen uptake following the addition of ethanol *Eur.J.Biochem.*, **75**, 13-21.

Tipton. K. F., Houslay. M. D. & Turner. A. J. (1977). Metabolism of aldehydes in brain. *Essays in Neurochemistry and Neuropharmacology*, **1**, 103–138.

Tiihonen. J., Kuikka. J. T. & Bergstrom. K. A. (1997). Single-photon emission tomography imaging of monoamine transporters in impulsive violent behaviour. *Eur J Nucl Med.*, **24**, 1253–1260.

Tiihonen. J., Kuikka. J. & Hakola. P. (1994), Acute ethanol-induced changes in cerebral blood flow. *American Journal of Psychiatry*, **151**, 1505-1508.

Tottmar. S. O. C., Pettersson. H. & Kiessling. K. H. (1973). The subcellular distribution and properties of aldehyde dehydrogenases in rat liver. *Biochemical Journal*, **135**, 577–586

Tuomainen. P., Patsenka. A., Hyytia. P., Grinevich. V. & Kiianmaa. K. (2003). Extra-cellular levels of dopamine in the nucleus accumbens in AA and ANA rats after reverse microdialysis of ethanol into the nucleus accumbens or ventral tegmental area. *Alcohol*, **29**, 117- 124.

Unis. A. S., Micheal. D. R., Rene. R., James. H. A. & Daniel. M. D. (1997). Ontogeny of human brain dopamine receptors I.Differential expression of [3H]-SCH23390 and [3H]-YM-09151-2 specific binding .*Developmental Brain Research*, **106**, 109-117.

Unwin. N. (1993). Neurotransmitter action: opening of ligand-gated ion channels. *Cell*, **72**, 31-41.

Ursula Bailer. F., Julie Price. C., Carolyn Meltzer. C., Chester Mathis. A., Guido Frank. K., Lisa Weissfeld., Claire McConaha. W., Shannan Henry. E., Sarah Brooks-Achenbach.,Uyama. N., Geerts. A. & Reynaert. H. (2004). Neural connections between the hypothalamus and the liver. *Anat Rec A Discov Mol Cell Evol Biol.*, **280**, 808-820.

Uzbay. I. T., Usanmaz. S. E., Tapanyigit. E. E., Aynacioglu. S. & Akarsu. E. S. (1998). Dopaminergic and serotonergic alterations in the rat brain during ethanol

withdrawal: association with behavioural signs. *Drug and Alcohol Dependence*, **53**, 39-47.

Vallar. L. & Meldolesi. J. (1989). Mechanisms of signal transduction at the dopamine D_2 receptor. *Trends Pharmacol Sci.*, **10**, 74-7.

Van Tol H. H. M., Bunzow. J. R., Guan. H. C., Sunahara. R. K., Seeman. P.& Niznik. H. B. (1991).Cloning of a human dopamine D_4 receptor gene with high affinity for the antipsychotic clozapine. *Nature*, **350**, 614-619.

Vasconcelos. S. M., Cavalcante. R. A., Aguiar. L. M., Sousa. F. C., Fonteles. M. M. & Viana. G. S. (2004). Effects of chronic ethanol treatment on monoamine levels in rat hippocampus and striatum. *Braz J Med Biol Res.*, **37,** 1839-1846.

Vasiliou. V. & Nebert. D. W. (2005). Analysis and update of the human aldehyde dehydrogenase (ALDH) gene family. *Hum Genomics.*, **2**, 138-143.

Volkow. N. D., Mullani. N. & Gould. (1988). Effects of acute alcohol intoxication on cerebral blood flow measured with PET. *Psychiatry Research,* **24**, 201-209.

Volkow. N. D., Wang. G. J. & Fowler. J. S. (1996).Decreases in dopamine receptors but not in dopamine transporters in alcoholics. *Alcohol Clin Exp Res.*, **20**, 1594-1598.

Vom Dahl. S., Stoll. B., Gerok. W. & Haussinger. D. (1995). Inhibition of proteolysis by cell welling in liver requires intact micromolecular structures. *Biochem. J.*, **308**, 529-536.

von Knorring. A. L., Bohman. M., von Knorring. L., & Oreland. L. (1985). *Acta Psychiatr. Scand.,* **72**, 51-58 .

Wall. T. L. & Ehlers. C. L. (1995). Genetic influences affecting alcohol use among Asians. *Alcohol Health and Research World*, **19**, 184-189.

Wallis. C., Rezazadeh. M. & Lal. H. (1993). Role of serotonin in ethanol abuse. *Drug Development Research*, **30**, 178-188.

Wang. Y. L., Wei. J. W. & Sun. A. Y.(1993). Effects of ethanol on brain monoamine content of spontaneously hypertensive rats (SHR). *Neurochem Res.*, **18**, 1293-7.

Wang. G. J., Volkow. N. D. & Franceschi. D. (2000). Regional brain metabolism during alcohol intoxication. *Alcoholism: Clinical and Experimental Research,* **24**, 822-829.

Ward. R. J., Colantuoni. C., Dahchour. A., Quertemont. E. & De Witte. P. (1997). Acetaldehyde-induced changes in monoamine and amino acid extracellular microdialysis content of the nucleus accumbens. *Neuropharmacology,* **36**, 225–232.

Wei. V. L. & Singh. S. M. (1988).Genetically determined response of hepatic aldehyde dehydrogenase activity to ethanol exposures may be associated with alcohol sensitivity in mouse genotypes. *Alcohol Clin Exp Res.*, **12**, 39-45.

Weissenborn. R. & Duka. T. (2003). Acute alcohol effects on cognitive function in social drinkers: Their relationship to drinking habits. *Psychopharmacology,* **165**, 306-312.

Wickens. J. R., Begg. A. J. & Arbuthnott. G. W. (1996). Dopamine reverses the depression of rat corticostriatal synapses which normally follows high-frequency stimulation of cortex in vitro. *Neuroscience*, **70**, 1-5.

Wilde. M. I. & Markham Ondansetron. A. (1996). A review of its pharmacology and preliminary clinical findings in novel applications. *Drugs,* **52**, 773-794

Wing Ming Keung (1998). Daidzin and its antidipsotropic analogs inhibit serotonin and dopamine metabolism in isolated mitochondria, *Proc. Natl. Acad. Sci.*, **95**, 2198-2203.

Wisden.W., Parker. E. M., Mahle. K. D. A. G., Nowak. H. P., Yocca. F. C. C. F.,, Seeburg. P. & Voigt. M. M. (1993). Cloning and characterization of the rat 5-HT_{5B} receptor. Evidence that the 5-HT_{5B} receptor couples to a G protein in mammalian cell membranes. *FEBS Lett.*, **133**, 25-31.

Wonnacott. S., Kaiser. S., Mogg. A., Soliakov. L. & Jones. I. W. (2000). Presynaptic nicotinic receptors modulating dopamine release in the rat striatum. *Eur J Pharmacol.*, **393**, 51-58.

Woodward. J. J. (2000). Ethanol and NMDA receptor signalling. *Crit Rev Neurobiol.*, **14**, 69- 89.

Yamashita. H., Kaneyuki T. & Tagawa. K. (2001). Production of acetate in the liver and its utilization in peripheral tissues. *Biochim. Biophys. Acta,* **1532**, 79–87

Yamauchi. T., Iwai. M., Kobayashi. N. & Shimazu. T. (1998). Noradrenaline and ATP decrease the secretion of triglyceride and apoprotein B from perfused rat liver. *Pflugers Arch.*, **435**, 368-374.

Yamazaki. H., Nishiguchi. K., Miyamoto. R. & Nakanishi. S. (1984). Activity and electrophoretic profiles of brain aldehyde dehydrogenases in mice genetically selected for their ethanol preference. *Int J Biochem.*, **16**, 247-252.

Yan. Q. S. (1999). Extracellular dopamine and serotonin after ethanol monitored with 5-minute microdialysis. *Alcohol*, **19**, 1-7.

Yoneda. M., Nakamura. K., Nakade. Y., Tamano. M., Kono. T., Watanobe. H., Shimada. T., Hiraishi. H. & Terano. A. (2005). Effect of central corticotropin releasing factor on hepatic circulation in rats: the role of the CRF2 receptor in the brain. *Gut*, **54**, 282-8.

Yoneda[a]. M., Kono. T., Watanobe. H., Tamano. M., Shimada. T., Hiraishi. H. & Nakamura. K. (2005).Central thyrotropin-releasing hormone increases hepatic cyclic AMP through vagal-cholinergic and prostaglandin-dependent pathways in rats. *Peptides*, **26**, 1573-1579.

Yoneda. M., Watanobe. H. & Terano. A. (2001).Central regulation of hepatic function by neuropeptides.*J.Gastroenterol.*, **36**, 361-367.

Yoshihara. E., Nakamura. K., Itoh. M., Ameno. K., Takeuchi. Y., Ijiri. I. & Iwahashi. K. (2000).The Human Serotonin Receptor Gene (HTR2) MspI Polymorphism in Japanese Schizophrenic and Alcoholic Patients. *Neuropsychobiology*, **41**, 124-126.

Yoshimoto. K., Ueda. S., Kato. B., Takeuchi. Y., Kawai. Y., Noritake. K. & Yasuhara. M. (2000). Alcohol enhances characteristic releases of dopamine and serotonin in the central nucleus of the amygdala. *Neurochemistry International*, **37**: 369-376.

Youssef Sari., Teresa Powrozek. & Feng Zhou. C. (2001). Alcohol Deters the Outgrowth of Serotonergic Neurons at Midgestation. *Journal of Biomedical Science*, **8**, 119-125.

Yue. M., Ni. Q., Yu. C. H., Ren. K. M. & Chen. W. X., Li. Y. M. (2006). Transient elevation of hepatic enzymes in volunteers after intake of alcohol. *Hepatobiliary Pancreat Dis Int.*, **5**, 52-55.

Yuki. T., Hashimoto. T., Ohkuma. S., Tamura. J. & Kuriyama. K. (1984). Alteration of acetaldehyde metabolism in carbon tetrachloride-intoxicated rat liver: analysis using liver perfusion system. *Alcohol Alcohol,* **19**, 101-107.

Zeiner. A. R., Paredes. A. & Christianson. H. D. (1979). The role of acetaldehyde in mediating reactivity to an acute dose of ethanol among different racial groups. *Alcoholism: Clinical and Experimental Research*, **3**, 11-18.

Zifa. E. & Fillion. G. (1992). 5-Hydroxytryptamine receptors. *Pharmacol. Rev.*, **44**, 401-458.

Zimatkin. S. M. & Pronko. P. S. (1997). Grinevich V. P. Alcohol action on liver: dose dependence and morpho-biochemical correlations,*Cas Lek Cesk.* **136**, 598-602.

Papers published/presented

1. Akash. K. George and C. S. Paulose (**2004**). Decreased dopamine, serotonin contents and enhanced ALDH activity in the liver of alcoholic rats. Proceedings of International conference of Biotechnology and Neuroscience, 295-298.

2. Akash. K. George, Kuruvilla Thomas, M. Chandrasekharan and C. S. Paulose (**2004**). Altered serotonin and dopamine contents in the platelets of alcoholic patients. Proceedings of International Conference of Biotechnology and Neuroscience, 299-303.

3. Akash. K. George. (**2006**). Decreased dopamine D_2 receptor binding with [^{3}H]YM-09151-2 in cerebral cortex and brain stem of ethanol induced adult male Wistar rats. Extended abstracts of XVIIIth Kerala Science Congress, 508-509.

4. Santhosh. V., Akash. K. George, Kuruvilla Thomas and C. S. Paulose. (**2003**). Down regulation of $\alpha 2$ adrenergic receptors in the cerebral cortex of alcoholic rats. International Medical Science Academy - Annual Conference IMSACON

5. Akash. K. George, Remya Robinson, Savitha Balakrishnan and C. S. Paulose (**2004**). Decreased 5-HT content increased ALDH activity in the liver of alcoholic rats. International Neuroscience Conference.

Figure-1

Ethanol consumption of experimental rats during the period of experiment

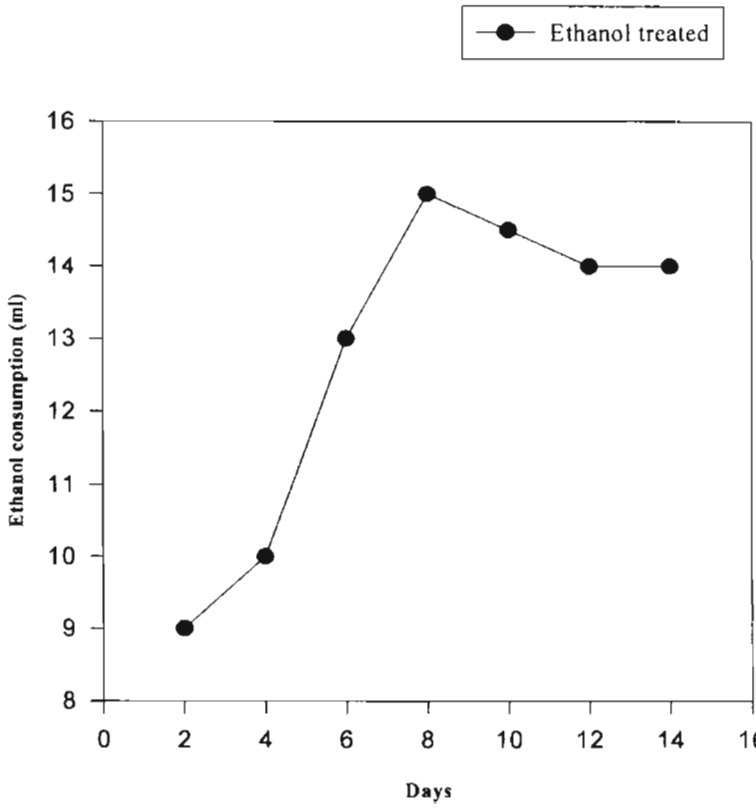


Figure-2

Body weight of control and ethanol treated rats during the period of experiment

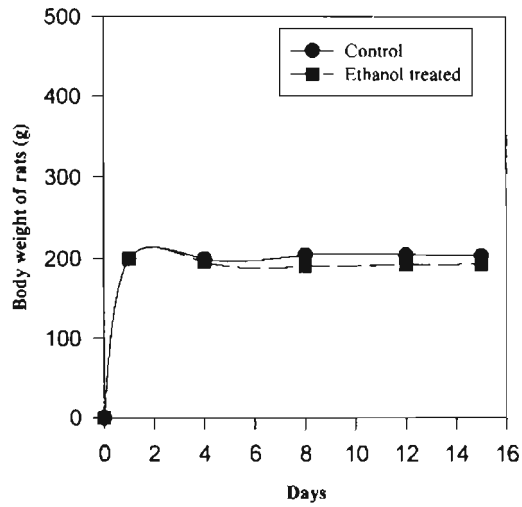


Table-1

Body weight of control and ethanol treated rats during the period of experiment

Experimental status	Wt (g)
Control	203 ± 1.2
Ethanol treated	194 ± 1.6*

Values are mean ± S.E.M. of 4-6 separate experiments
*$p<0.05$ when compared with control

Figure-3

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in cerebral cortex of control and ethanol treated rats

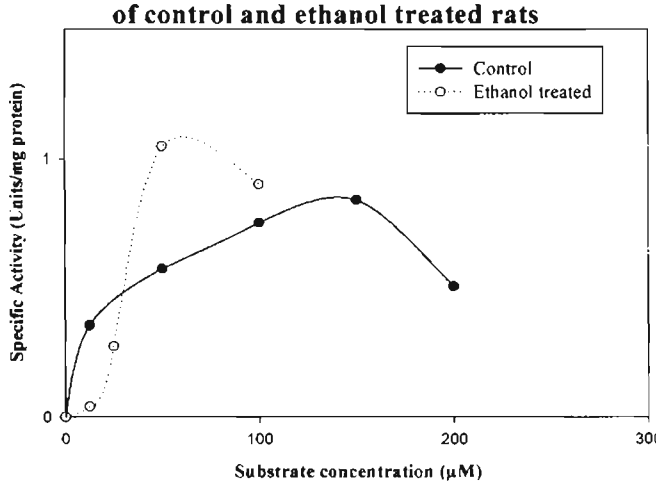


Table-2

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in the cerebral cortex of control and ethanol treated rats

Experimental status	V_{max} (Units/mg protein)	K_m (μM)
Control	0.76 ± 0.03	25.00 ± 0.05
Ethanol treated	1.00 ± 0.06*	40.00 ± 1.59***

Values are mean ± S.E.M. of 4-6 separate experiments
*$p<0.05$ when compared with control
***$p<0.001$ when compared with control

Figure-4

Real -Time PCR amplification of the ALDH mRNA from the cerebral cortex of control and ethanol treated rats

Table -3

No.	Experimental status	Ct value
1	Control	29.94
2	Ethanol treated	28.01

1. Graph representing the crossing threshold (Ct) of sample, 2. Melt curve of the sample of the amplification obtained after the reaction, 3. Graph representing the crossing threshold of the house keeping gene (β-actin), 4. Melt curve of the house keeping gene obtained after the reaction.

Ct value represents the cycle number at which the fluorescence crosses the set threshold

Figure-5

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenas in the brainstem of control and ethanol treated rats

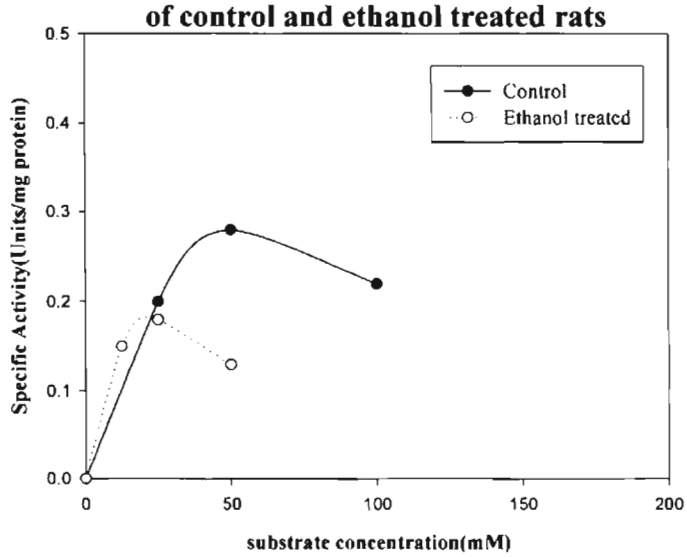


Table-4

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in the brainstem of control and ethanol treated rats

Experimental status	V_{max} (Units/mg protein)	K_m (μM)
Control	0.27 ± 0.02	11.17 ± 0.73
Ethanol treated	0.21 ± 0.03	5.75 ± 0.38***

Values are mean ± S.E.M. of 4-6 separate experiments
***p<0.001 when compared with control

Figure-6

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in the cerebellum of control and ethanol treated rats

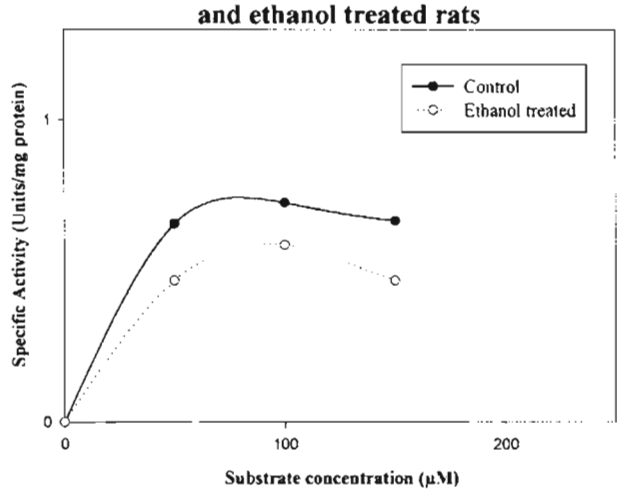


Table -5

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in the cerebellum of control and ethanol treated rats

Experimental status	V_{max} (Units/mg protein)	K_m (μM)
Control	0.76 ± 0.02	11.66 ± 0.83
Ethanol treated	0.56 ± 0.01 **	27.66 ± 1.76***

Values are mean ± S.E.M. of 4-6 separate experiments
**p<0.01 when compared with control
***p<0.001 when compared with control

Figure-7

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in the plasma of control and ethanol treated rats

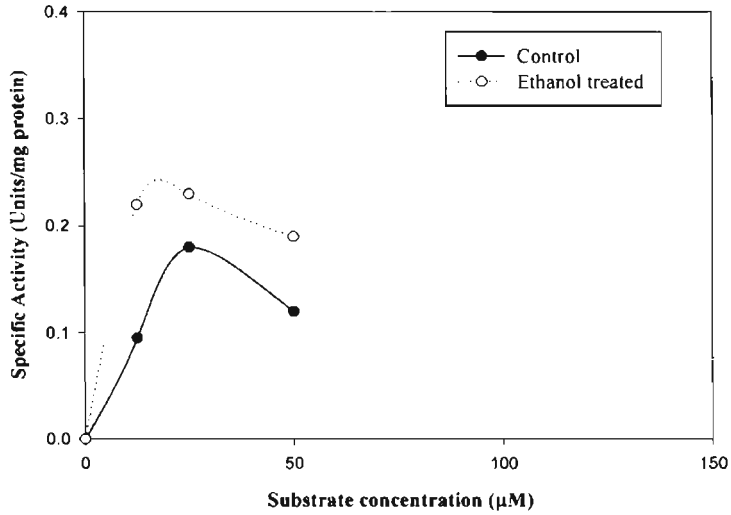


Table -6

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in the plasma of control and ethanol treated rats

Experimental status	V_{max} (Units/mg protein)	K_m (μM)
Control	0.18 ± 0.01	11.00 ± 0.02
Ethanol treated	0.23 ± 0.01*	3.75 ± 0.75**

Values are mean ± S.E.M. of 4-6 separate experiments
*$p<0.05$ when compared with control
**$p<0.01$ when compared with control

Figure-8

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in the liver of control and ethanol treated rats

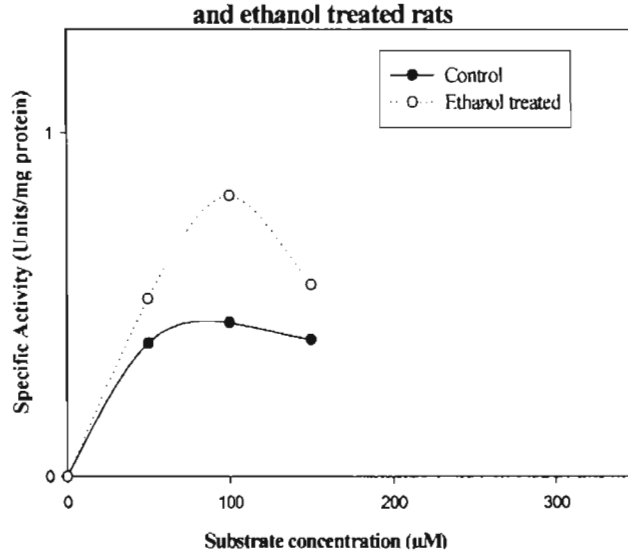


Table-7

Kinetic parameters, V_{max} and K_m, of aldehyde dehydrogenase in the liver of control and ethanol treated rats

Experimental status	V_{max} (Units/mg protein)	K_m (µM)
Control	0.45 ± 0.02	23.00 ± 0.01
Ethanol treated	0.69 ± 0.01*	10.42 ± 0.08**

Values are mean ± S.E.M. of 4-6 separate experiments
*$p<0.05$ when compared with control
**$p<0.01$ when compared with control

Figure-9

Real -Time PCR amplification of the ALDH mRNA from the liver of control and ethanol treated rats

Table - 8

No.	Experimental status	Ct value
1	Control	29.86
2	Ethanol treated	27.29

1.Graph representing the crossing threshold (Ct) of sample, 2. Melt curve of the sample of the amplification obtained after the reaction, 3. Graph representing the crossing threshold of the house keeping gene (β-actin), 4. Melt curve of the house keeping gene obtained after the reaction.

Ct value represents the cycle number at which the fluorescence crosses the set threshold.

Table -9

Dopamine, 5-HT contents and their metabolites in the cerebral cortex of control and ethanol treated rats
(nmoles/g wet weight of the tissue)

Experimental status	5-HT	5-HIAA	5-HIAA/5-HT	DA	HVA	HVA/DA
Control	0.16 ± 0.02	0.79 ± 0.11	4.78 ± 0.14	3.03 ± 0.87	0.11 ± 0.01	0.04 ± 0.01
Ethanol treated	0.06 ± 0.01**	0.81±0.10	13.19 ± 0.31***	1.48 ± 0.44**	0.10 ± 0.01	0.07 ± 0.02*

Values are mean ± SEM of 4-6 separate experiments.
*$p<0.05$ compared with control
**$p<0.01$ compared with control
***$p<0.001$ compared with control

Table -10

Dopamine, 5-HT contents and their metabolites in the brainstem of control and ethanol treated rats
(nmoles/g wet weight of the tissue)

Experimental status	5-HT	5-HIAA	5-HIAA/5-HT	DA	HVA	HVA/DA
Control	0.56 ± 0.14	0.50 ± 0.08	0.88 ± 0.09	0. 31 ± 0.02	0.11 ± 0.03	0.34 ± 0.01
Ethanol treated	0.22 ± 0.04**	0.47 ± 0.09	2.10 ± 0.02***	0.22 ± 0.04*	0.10 ± 0.01	0. 47 ± 0.03**

Values are mean ± SEM of 4-6 separate experiments.
*$p<0.05$ compared with control
**$p<0.01$ compared with control
***$p<0.001$ compared with control

Table -11

Dopamine, 5-HT contents and their metabolites in the hypothalamus of control and ethanol treated rats
(nmoles/g wet weight of the tissue)

Experimental status	5-HT	5-HIAA	5-HIAA/5-HT	DA	HVA	HVA/DA
Control	0.25 ± 0.05	0.44 ± 0.22	2.20 ± 0.02	0.48 ± 0.03	0.04 ± 0.22	0.07 ± 0.01
Ethanol treated	0.61 ± 0.08**	0.35 ± 0.05	0.61±0.07***	0.27± 0.04**	0.04 ± 0.004	0.17 ± 0.04***

Values are ± SEM of 4-6 separate experiments.
**p<0.01 compared with control
***p<0.001 compared with control

Table -12

Dopamine, 5-HT contents and their metabolites in the corpus striatum of control and ethanol treated rats
(nmoles/g wet weight of the tissue)

Experimental status	5-HT	5-HIAA	5-HIAA/5-HT	DA	HVA	HVA/DA
Control	0.52 ± 0.07	1.57 ± 0.18	5.51± 1.02	4.22 ± 0.62	1.37± 0.21	0.33 ± 0.04
Ethanol treated	0.14 ± 0.02**	1.74 ± 0.13	16.72 ± 2.35**	0.35 ± 0.07***	2.43 ± 0.17	5.21± 2.51***

Values are mean ± SEM of 4-6 separate experiments.
**p<0.01 compared with control
***p<0.001 compared with control

Table -13

Dopamine, 5-HT contents and their metabolites in the liver of control and ethanol treated rats
(nmoles/g wet weight of the tissue)

Experimental status	5-HT	5-HIAA	5-HIAA/5-HT	DA	HVA	HVA/DA
Control	1.13 ± 0.24	0.49 ± 0.09	0.44 ± 0.04	0.30 ± 0.01	0.07 ± 0.02	0.24 ± 0.04
Ethanol treated	0.14 ± 0.03***	0.47 ± 0.09	3.37 ± 0.29***	0.13 ± 0.02***	0.13 ± 0.02	1.80 ± 0.01***

Values are mean ± SEM of 4-6 separate experiments.
***p<0.001 compared with control

Figure-10

Scatchard analysis of [^{3}H] YM-09151-2 binding against sulpiride in the cerebral cortex of control and ethanol treated rats.

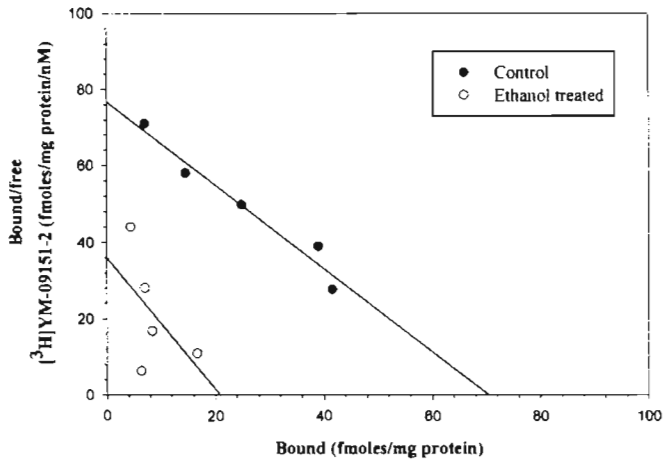


Table -14

[^{3}H]YM-09151-2 binding parameters in the cerebral cortex of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	59.60 ± 1.80	0.92 ± 0.22
Ethanol treated	26.50 ± 3.30 ***	0.69 ± 0.18 *

Values are mean ± SEM of 4-6 separate experiments.
*p<0.05 compared with control
***p<0.001compared with control

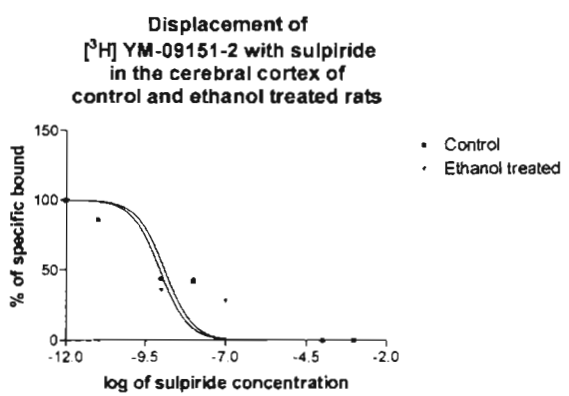
Table-15

Binding parameters of [^{3}H] YM-09151-2 with sulpiride in the cerebral cortex of control and ethanol treated rats

Experimental status	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One -site	-8.894	$1.27x10^{-9}$	-0.93
Ethanol treated	One -site	-9.042	$9.07 x10^{-10}$	-0.89

Values are mean of 4-6 separate experiments.

Figure-11



Data were fitted with interactive nonlinear regression software (Prism, GraphPad, San Diego, CA). K_i - the affinity of the receptor for the Competing drugs. EC_{50} is the concentration of the competitor that competes for half the specific binding and it is same as IC_{50}

Figure-12

Real -Time PCR amplification of the D_2 receptor mRNA from the cerebral cortex of control and ethanol treated rats

Table -16

No.	Experimental status	Ct value
1	Control	29.77
2	Ethanol treated	31.30

1.Graph representing the crossing threshold (Ct) of sample, 2. Melt curve of the sample of the amplification obtained after the reaction, 3. Graph representing the crossing threshold of the house keeping gene (β-actin), 4. Melt curve of the house keeping gene obtained after the reaction.

Ct value represents the cycle number at which the fluorescence crosses the set threshold.

Figure-13

Scatchard analysis of [^{3}H]YM-09151-2 binding against sulpiride in the brainstem of control and ethanol treated rats

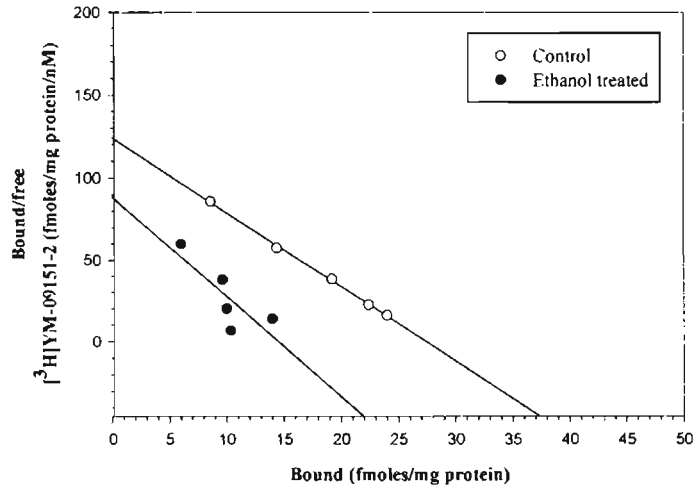


Table -17

[^{3}H]YM-09151-2 binding parameters in the brainstem of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	32 .16 ± 2.19	0.50 ± 0.15
Ethanol treated	17.50 ± 2.5 *	0.28 ± 0 .06*

Values are mean ± SEM of 4-6 separate experiments.
* $p<0.05$compared with control

Table-18

Binding parameters of [^{3}H] YM-09151-2 with sulpiride in the brainstem of control and ethanol treated rats

Experimental status	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One –site	-6.091	$8.1x10^{-7}$	-0.96
Ethanol treated	One –site	-7.434	$3.7 x10^{-8}$	-0.89

Values are mean of 4-6 separate experiments.

Data were fitted with interactive nonlinear regression software (Prism, GraphPad, San Diego, CA). K_i - the affinity of the receptor for the Competing drugs. EC_{50} is the concentration of the competitor that competes for half the specific binding and it is same as IC_{50}

Figure-14

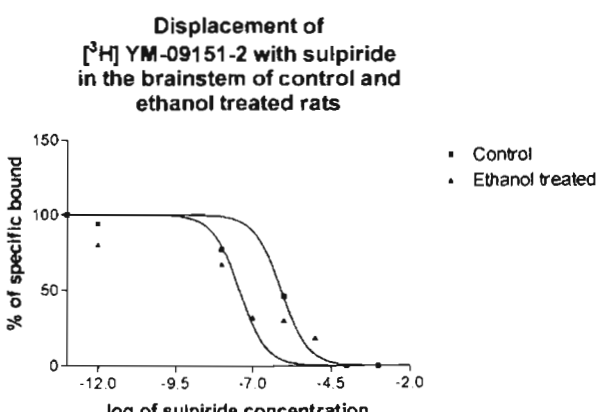


Figure-15

Scatchard analysis of [^{3}H]YM-09151-2 binding against sulpiride in the hypothalamus of control and ethanol treated rats

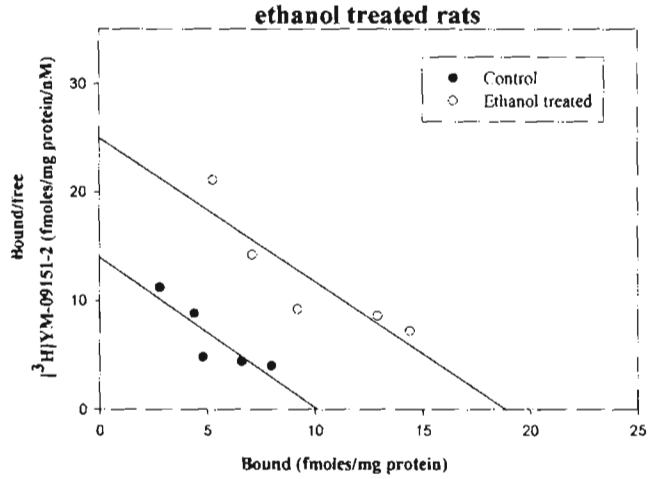


Table -19

[^{3}H]YM-09151-2 binding parameters in the hypothalamus of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	10.40 ± 0.32	0.76 ± 0.01
Ethanol treated	19.07 ± 0.43 ***	0.79 ± 0.06

Values are mean ± SEM of 4-6 separate experiments.
***p<0.001 compared with control

Table-20

Binding parameters of [^{3}H] YM-09151-2 with sulpiride in the hypothalamus of control and ethanol treated rats

Experimental status	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One -site	-7.113	$7.70x10^{-8}$	-0.99
Ethanol treated	One -site	-6.309	$4.90 x10^{-7}$	-0.97

Values are mean of 4-6 separate experiments.

Data were fitted with interactive nonlinear regression software (Prism, GraphPad, San Diego, CA). K_i - the affinity of the receptor for the Competing drugs. EC_{50} is the concentration of the competitor that competes for half the specific binding and it is same as IC_{50}

Figure-16

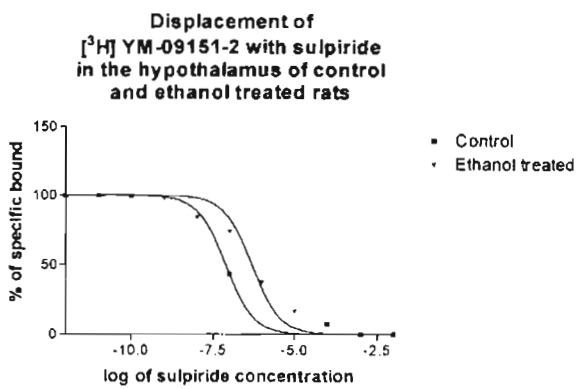


Figure-17

Real -Time PCR amplification of the D_2 receptor mRNA from the hypothalamus of control and ethanol treated rats

1 2

3 4

Table-21

No.	Experimental status	Ct value
1	Control	29.41
2	Ethanol treated	28.39

1.Graph representing the crossing threshold (Ct) of sample, 2. Melt curve of the sample of the amplification obtained after the reaction, 3. Graph representing the crossing threshold of the house keeping gene (β-actin), 4. Melt curve of the house keeping gene obtained after the reaction.

Ct value represents the cycle number at which the fluorescence crosses the set threshold

Figure-18

Scatchard analysis of [^{3}H] YM-09151-2 binding against sulpiride in the corpus striatum of control and ethanol treated rats.

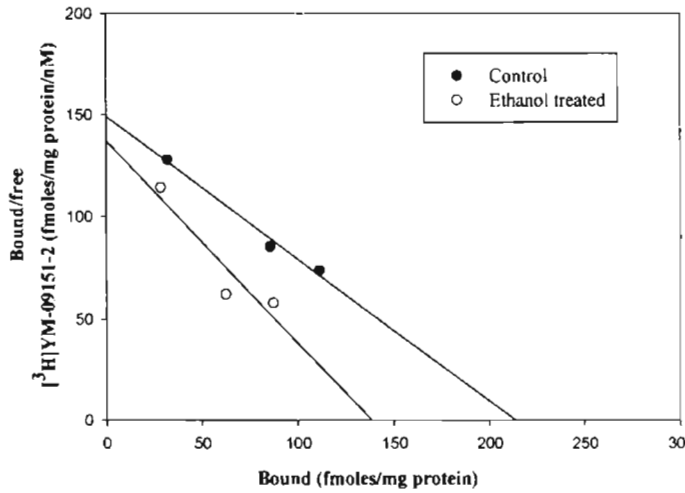


Table -22

[^{3}H]YM-09151-2 binding parameters in the corpus striatum of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	238.33 ± 19.65	1.32 ± 0.20
Ethanol treated	160.00 ± 20.82 *	0.92 ± 0.09*

Values are mean ± SEM of 4-6 separate experiments.
*$p<0.05$ compared with control

Table-23

Binding parameters of [^{3}H] YM-09151-2 with sulpiride in the corpus striatum of control and ethanol treated rats

Experimental status	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One -site	-4.949	$1.12x10^{-5}$	-0.99
Ethanol treated	One -site	-5.126	$7.48 x10^{-6}$	-0.99

Values are mean of 4-6 separate experiments.

Figure-19

Data were fitted with interactive nonlinear regression software (Prism, GraphPad, San Diego, CA). K_i - the affinity of the receptor for the Competing drugs. EC_{50} is the concentration of the competitor that competes for half the specific binding and it is same as IC_{50}

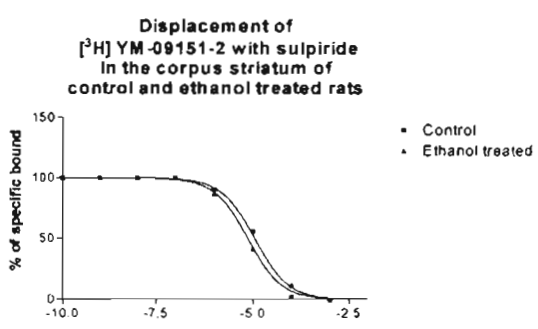


Figure-20

Real -Time PCR amplification of the D_2 receptor mRNA from the corpus striatum of control and ethanol treated rats

1 2

3 4

Table -24

No.	Experimental status	Ct value
1	Control	28.24
2	Ethanol treated	29.45

1.Graph representing the crossing threshold (Ct) of sample, 2. Melt curve of the sample of the amplification obtained after the reaction, 3. Graph representing the crossing threshold of the house keeping gene (β-actin), 4. Melt curve of the house keeping gene obtained after the reaction.

Ct value represents the cycle number at which the fluorescence crosses the set threshold.

Figure-21

Scatchard analysis of [^{3}H]YM-09151-2 binding against sulpiride in the cerebellum of control and ethanol treated rats

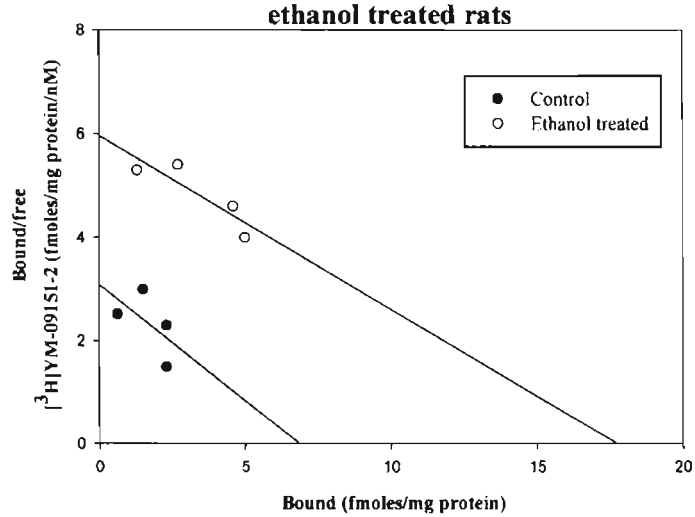


Table -25

[^{3}H]YM-09151-2 binding parameters in the cerebellum of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	8.00 ± 0.01	1.97 ± 0.74
Ethanol treated	13.50 ± 0.83 ***	2.88 ± 0.46 **

Values are mean ± SEM of 4-6 separate experiments.
**p<0.01 compared with control
***p<0.001 compared with control

Table-26

Binding parameters of [^{3}H] YM-09151-2 with sulpiride in the cerebellum of control and ethanol treated rats

Experimental status	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One -site	-5.521	$3x10^{-6}$	-0.95
Ethanol treated	One -site	-5.218	$6 x10^{-6}$	-0.97

Values are mean of 4-6 separate experiments.

Figure-22

Data were fitted with interactive nonlinear regression software (prism, GraphPad, San Diego, CA). K_i - the affinity of the receptor for the Competing drugs. EC_{50} is the concentration of the competitor that competes for half the specific binding and it is same as IC_{50}

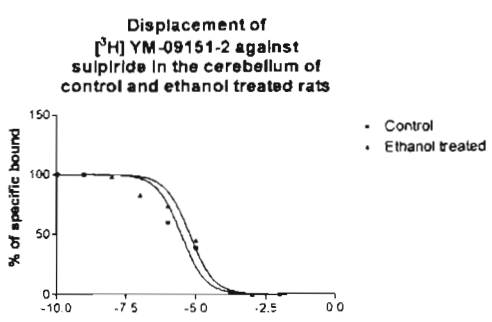


Figure-23

Real -Time PCR amplification of the D_2 receptor mRNA from the cerebellum of control and ethanol treated rats

Table - 27

No.	Experimental status	Ct value
1	Control	30.43
2	Ethanol treated	29.34

1.Graph representing the crossing threshold (Ct) of sample, 2. Melt curve of the sample of the amplification obtained after the reaction, 3. Graph representing the crossing threshold of the house keeping gene (β-actin), 4. Melt curve of the house keeping gene obtained after the reaction.

Ct value represents the cycle number at which the fluorescence crosses the set threshold.

Figure-24

Scatchard analysis of [^{3}H] MDL 100907 binding against ketanserin in the cerebral cortex of control and ethanol treated rats.

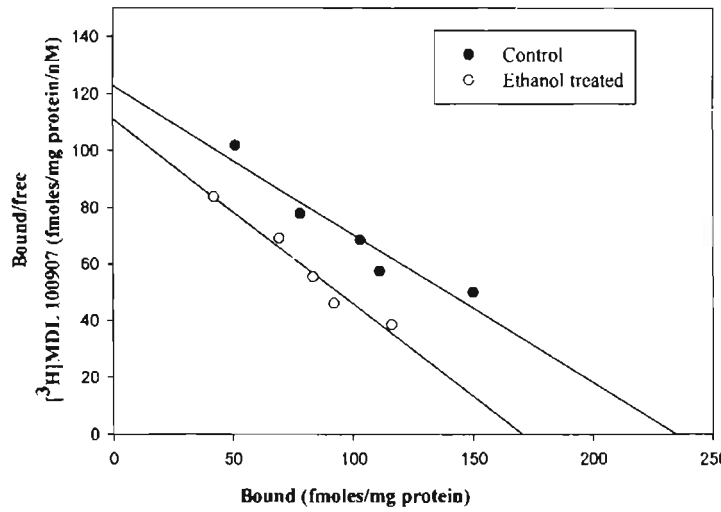


Table – 28

[^{3}H]MDL 100907 binding parameters in the cerebral cortex of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	238.50 ± 6.69	1.57 ± 0.31
Ethanol treated	173.00 ± 5.18 ***	1.72 ± 0.58

Values are mean ± SEM of 4-6 separate experiments.
***p<0.001 compared with control

Table-29

Binding parameters of [^{3}H] MDL 100907 with ketanserin in the cerebral cortex of control and ethanol treated rats

Experimental status	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One -site	-5.542	$2.8x10^{-6}$	-0.98
Ethanol treated	One -site	-6.709	$1.9 x10^{-7}$	-0.91

Values are mean of 4-6 separate experiments.

Figure-25

Data were fitted with interactive nonlinear regression software (prism, GraphPad, San Diego, CA). K_i - the affinity of the receptor for the Competing drugs. EC_{50} is the concentration of the competitor that competes for half the specific binding and it is same as IC_{50}

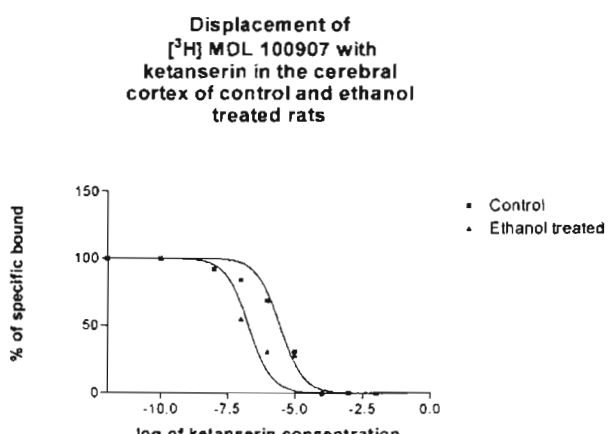


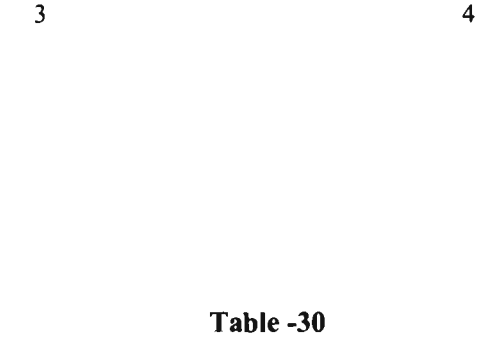
Figure-26

Real -Time PCR amplification of the 5-HT_{2A} receptor mRNA from the cerebral cortex of control and ethanol treated rats

1

2

3



4

Table -30

No.	Experimental status	Ct value
1	Control	30.38
2	Ethanol treated	36.25

1.Graph representing the crossing threshold (Ct) of sample, 2. Melt curve of the sample of the amplification obtained after the reaction, 3. Graph representing the crossing threshold of the house keeping gene (β-actin), 4. Melt curve of the house keeping gene obtained after the reaction.

Ct value represents the cycle number at which the fluorescence crosses the set threshold.

Figure-27

Scatchard analysis of [^{3}H] MDL 100907 binding against ketanserin in the brainstem of control and ethanol treated rats

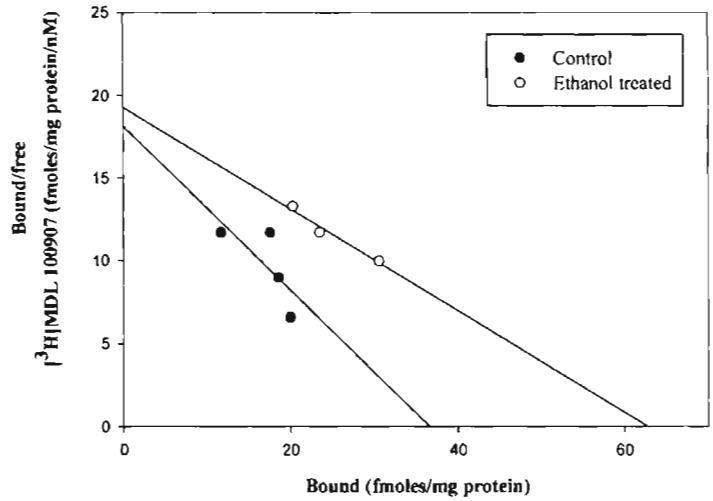


Table -31

[^{3}H]MDL 100907 binding parameters in the brainstem of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	23.00 ± 2.51	1.30 ± 0.10
Ethanol treated	45.67 ± 0.33 ***	1.85 ± 0.07

Values are mean ± SEM of 4-6 separate experiments.
***p<0.001 compared with control

Table-32

Binding parameters of [^{3}H] MDL100907 with ketanserin in the brainstem of control and ethanol treated rats

Experimental status	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One -site	-6.165	$6.8x10^{-7}$	-0.99
Ethanol treated	One -site	-6.483	$3.3 x10^{-7}$	-0.97

Values are mean of 4-6 separate experiments.

Figure-28

Data were fitted with interactive nonlinear regression software (Prism, GraphPad, San Diego, CA). K_i - the affinity of the receptor for the Competing drugs. EC_{50} is the concentration of the competitor that competes for half the specific binding and it is same as IC_{50}

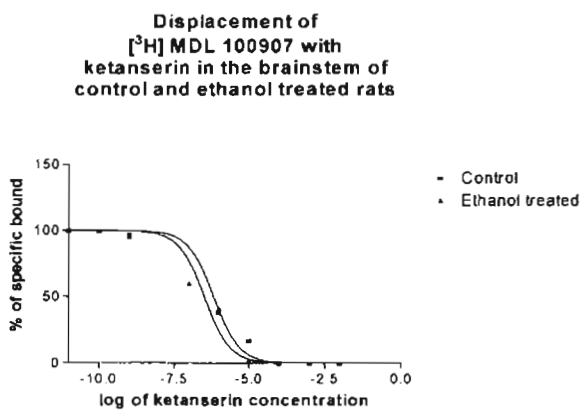


Figure-29

Scatchard analysis of [^{3}H] MDL 100907 binding against ketanserin in the hypothalamus of control and ethanol treated rats.

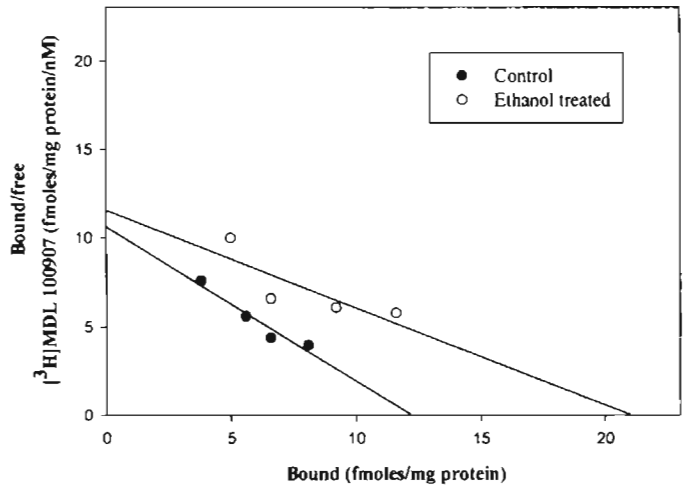


Table -33

[^{3}H]MDL 100907 binding parameters in the hypothalamus of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	14.56 ± 0.87	1.03 ± 0.03
Ethanol treated	27.06 ± 0.81 ***	2.17 ± 0.18***

Values are mean ± SEM of 4-6 separate experiments.
***p<0.001 compared with control

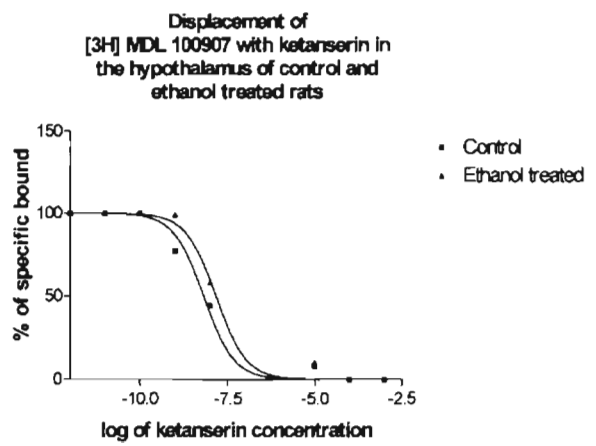
Table-34

Binding parameters of [^{3}H] MDL100907 with ketanserin in the hypothalamus of control and ethanol treated rats

Experimental status	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One -site	-8.186	$6.5x10^{-9}$	-0.98
Ethanol treated	One -site	-7.834	$1.5 x10^{-8}$	-0.99

Values are mean of 4-6 separate experiments.

Figure-30



Data were fitted with interactive nonlinear regression software (Prism, GraphPad, San Diego, CA). K_i - the affinity of the receptor for the Competing drugs. EC_{50} is the concentration of the competitor that competes for half the specific binding and it is same as IC_{50}

Figure-31

Real -Time PCR amplification of the 5-HT_{2A} receptor mRNA from the hypothalamus of control and ethanol treated rats

1 2

3 4

Table- 35

No.	Experimental status	Ct value
1	Control	31.26
2	Ethanol treated	29.37

1.Graph representing the crossing threshold (Ct) of sample, 2. Melt curve of the sample of the amplification obtained after the reaction, 3. Graph representing the crossing threshold of the house keeping gene (β-actin), 4. Melt curve of the house keeping gene obtained after the reaction.

Ct value represents the cycle number at which the fluorescence crosses the set threshold.

Figure-32

Scatchard analysis of [^{3}H] MDL 100907 binding against ketanserin in the corpus striatum of control and ethanol treated rats.

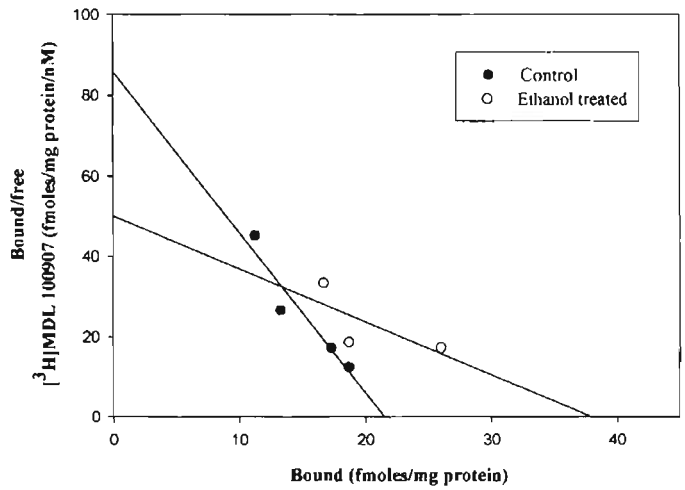


Table -36

[^{3}H] MDL 100907 binding parameters in the corpus striatum of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	23.16 ± 0.92	1.85 ± 0.07
Ethanol treated	37.17 ± 0.17 ***	1.30 ± 0.10**

Values are mean ± SEM of 4-6 separate experiments.
**p<0.01 compared with control
***p<0.001 compared with control

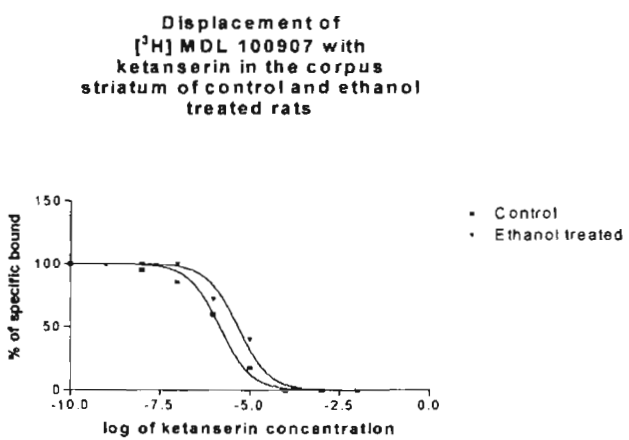
Table-37

Binding parameters of [^{3}H] MDL 100907 with ketanserin in the corpus striatum of control and ethanol treated rats

Experimental staus	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One -site	-5.824	$1.5x10^{-6}$	-0.99
Ethanol treated	One -site	-5.322	$4.8 x10^{-6}$	-0.99

Values are mean of 4-6 separate experiments.

Figure-33



Data were fitted with interactive nonlinear regression software (Prism, GraphPad, San Diego, CA). K_i - the affinity of the receptor for the Competing drugs. EC_{50} is the concentration of the competitor that competes for half the specific binding and it is same as IC_{50}

Figure-34

Real-Time PCR amplification of the 5-HT_{2A} receptor mRNA from the corpus striatum of control and ethanol treated rats

1 2

3 4

Table- 38

No.	Experimental status	Ct value
1	Control	30.31
2	Ethanol treated	29.17

1.Graph representing the crossing threshold (Ct) of sample, 2. Melt curve of the sample of the amplification obtained after the reaction, 3. Graph representing the crossing threshold of the house keeping gene (β-actin), 4. Melt curve of the house keeping gene obtained after the reaction.

Ct value represents the cycle number at which the fluorescence crosses the set threshold.

Figure-35

Scatchard analysis of [^{3}H] MDL 100907 binding against ketanserin in the cerebellum of control and ethanol treated rats.

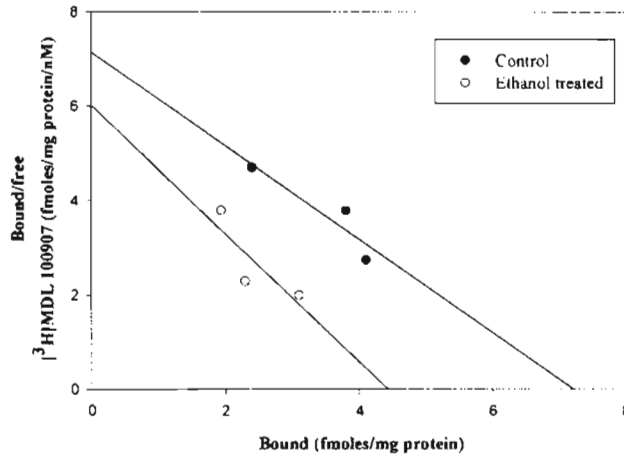


Table -39

[^{3}H]MDL 100907 binding parameters in the cerebellum of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	6.17 ± 0.08	0.61 ± 0.02
Ethanol treated	4.03 ± 0.08**	0.52 ± 0.01

Values are mean ± SEM of 4-6 separate experiments.
**p<0.01 compared with control

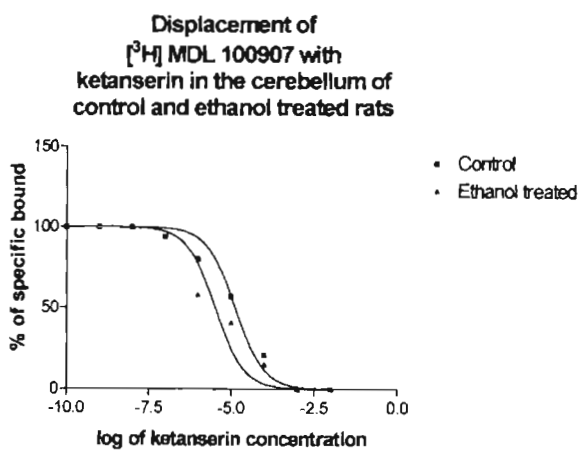
Table-40

Binding parameters of [^{3}H] MDL 100907 with ketanserin in the cerebellum of control and ethanol treated rats

Experimental staus	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One -site	-4.873	$1.3x10^{-5}$	-0.98
Ethanol treated	One -site	-5.473	$3.3\ x10^{-6}$	-0.96

Values are mean of 4-6 separate experiments.

Figure-36



Data were fitted with interactive nonlinear regression software (Prism, Graphpad, San Diego, CA). K_i - the affinity of the receptor for the Competing drugs. EC_{50} is the concentration of the competitor that competes for half the specific binding and it is same as IC_{50}

Figure-37

Real-Time PCR amplification of the 5-HT_{2A} receptor mRNA from the cerebellum of control and ethanol treated rats

Table-41

No.	Experimental status	Ct value
1	Control	30.55
2	Ethanol treated	33.84

1.Graph representing the crossing threshold (Ct) of sample, 2. Melt curve of the sample of the amplification obtained after the reaction, 3. Graph representing the crossing threshold of the house keeping gene (β-actin), 4. Melt curve of the house keeping gene obtained after the reaction.

Ct value represents the cycle number at which the fluorescence crosses the set threshold.

Figure-38

Scatchard analysis of [^{3}H] MDL 100907 binding against ketanserin in the liver of control and ethanol treated rats.

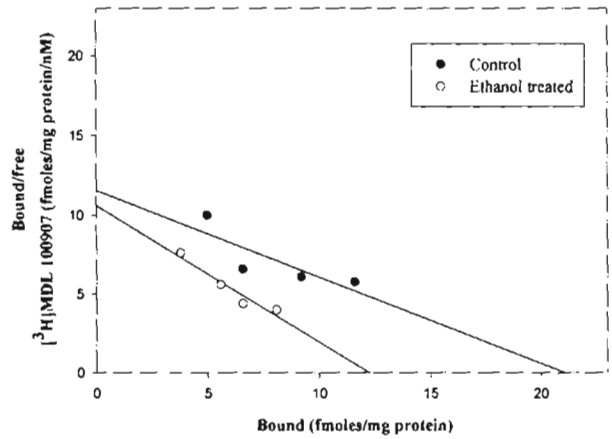


Table – 42

[^{3}H]MDL 100907 binding parameters in the liver of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	21.00 ± 0.99	4.2 ± 0.58
Ethanol treated	9.43 ± 0.99***	2.4 ± 0.35*

Values are mean ± SEM of 4-6 separate experiments.
*p<0.05 compared with control
***p<0.001 compared with control

Table-43

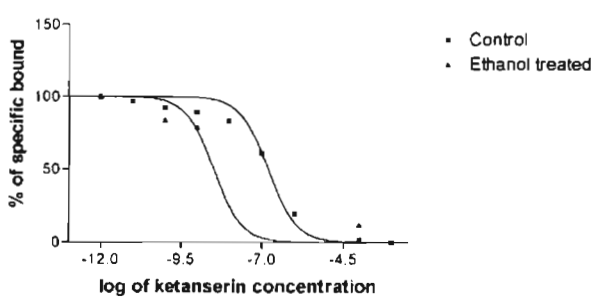
Binding parameters of [^{3}H] MDL100907 with ketanserin in the liver of control and ethanol treated rats

Experimental status	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One -site	-6.802	$1.6x10^{-7}$	-0.98
Ethanol treated	One -site	-8.477	$3.3 x10^{-9}$	-0.96

Values are mean of 4-6 separate experiments.

Figure-39

Displacement of [^{3}H] MDL 100907 with ketanserin in the liver of control and ethanol treated rats



Data were fitted with interactive nonlinear regression software (Prism, GraphPad, San Diego, CA). K_i - the affinity of the receptor for the Competing drugs. EC_{50} is the concentration of the competitor that competes for half the specific binding and it is same as IC_{50}

Figure-40

Real-Time PCR amplification of the 5-HT_{2A} receptor mRNA from the liver of control and ethanol treated rats

1 2

3 4

Table – 44

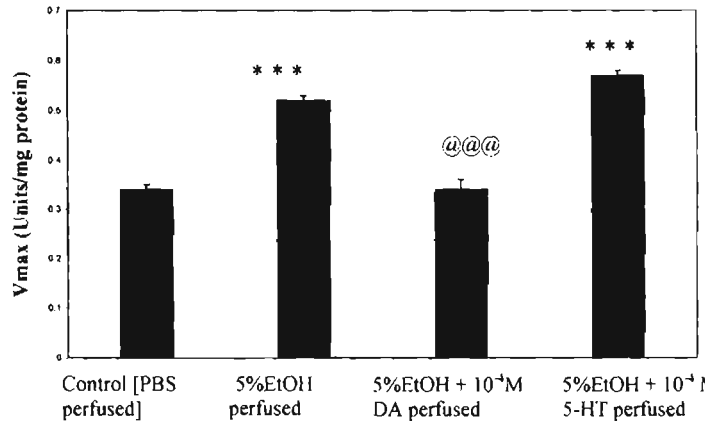
No.	Experimental status	Ct value
1	Control	30.85
2	Ethanol treated	37.31

1.Graph representing the crossing threshold (Ct) of sample, 2. Melt curve of the sample of the amplification obtained after the reaction, 3. Graph representing the crossing threshold of the house keeping gene (β-actin), 4. Melt curve of the house keeping gene obtained after the reaction.

Ct value represents the cycle number at which the fluorescence crosses the set threshold.

Figure-41

ALDH enzyme activity in the perfused liver of experimental rats



Values are mean ± S.E.M. of 4-6 separate experiments
***p<0.01 when compared with control
@@@p<0.001 when compared with 5% EtOH perfused

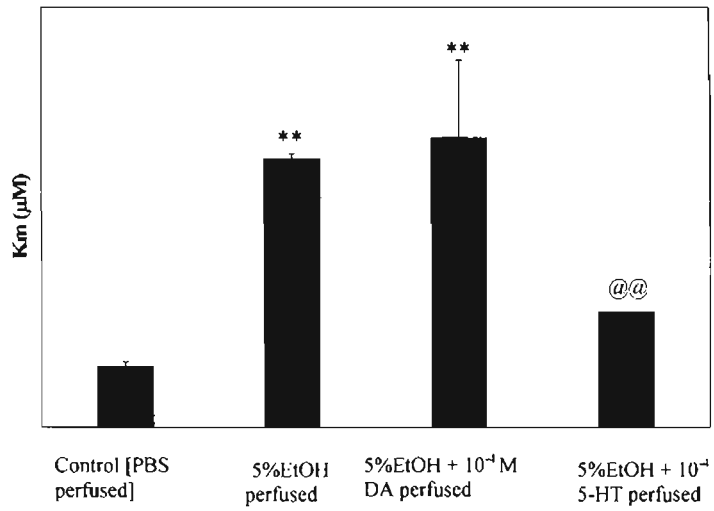
Table-45
ALDH enzyme activity in the perfused liver of experimental rats

Experimental status	V_{max} (Units/mg protein)
Control [PBS perfused]	0.34 ± 0.01
5%EtOH perfused	0.52 ± 0.01***
5%EtOH + 10^{-4} M DA perfused	0.34 ± 0.02 @@@
5%EtOH + 10^{-4} M 5-HT perfused	0.60 ± 0.01***

Values are mean ± S.E.M. of 4-6 separate experiments
***p<0.01 when compared with control
@@@p<0.001 when compared with 5% EtOH perfused

Figure-42

ALDH enzyme activity in the perfused liver of experimental rats



Values are mean ± S.E.M. of 4-6 separate experiments
**p<0.001 when compared with control
@@p<0.01 when compared with 5% EtOH perfused

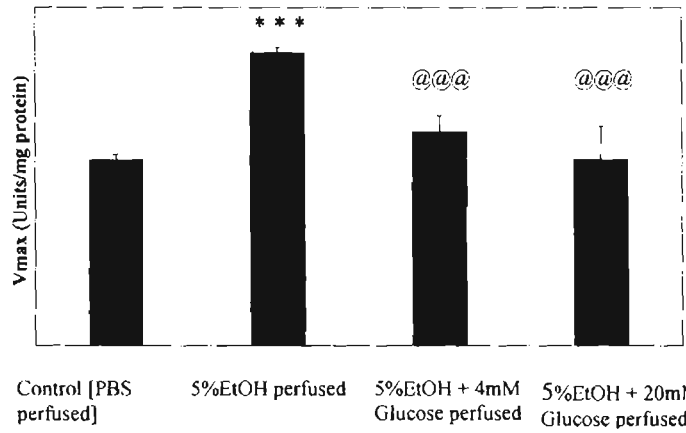
Table-46
ALDH enzyme activity in the perfused liver of experimental rats

Experimental status	K_m (µM)
Control [PBS perfused]	5.88 ± 0.45
5%EtOH perfused	25.50 ± 0.50 **
5%EtOH + 10^{-4} M DA perfused	27.50 ± 7.50 **
5%EtOH + 10^{-4} M 5-HT perfused	11.00 ± 0. 09 @@

Values are mean ± S.E.M. of 4-6 separate experiments
**p<0.001 when compared with control
@@p<0.01 when compared with 5% EtOH perfused

Figure-43

ALDH enzyme activity in the perfused liver of experimental rats



Values are mean ± S.E.M. of 4-6 separate experiments
***p<0.001 when compared with control
@@@ p<0.001 when compared with EtOH treated

Table-47

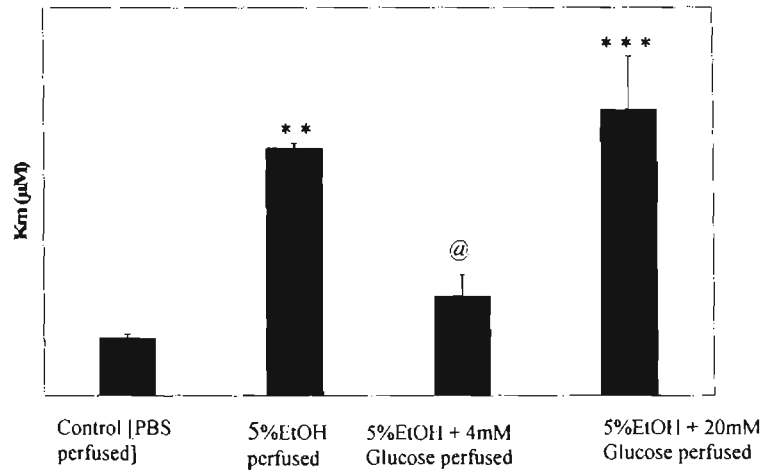
ALDH enzyme activity in the perfused liver of experimental rats

Experimental status	V_{max} (Units/mg protein)
Control [PBS perfused]	0.34 ± 0.01
5%EtOH perfused	0.52 ± 0.01***
5%EtOH + 4mM Glucose	0.38 ± 0.03 @@@
5%EtOH + 20mM Glucose	0.33 ± 0.06 @@@

Values are mean ± S.E.M. of 4-6 separate experiments
***p<0.001 when compared with control
@@@p<0.01 when compared with 5% EtOH perfused

Figure-44

ALDH enzyme activity in the perfused liver of experimental rats



Values are mean ± S.E.M. of 4-6 separate experiments
**p<0.01, ***p<0.001 when compared with control
@ p<0.05 when compared with EtOH treated

Table-48

ALDH enzyme activity in the perfused liver of experimental rats

Experimental status	K_m (μM)
Control [PBS perfused]	5.88 ± 0.45
5%EtOH perfused	25.50 ± 0.50 **
5%EtOH + 4mM Glucose	10.25 ± 2.20 @
5%EtOH + 20mM Glucose	29.50 ± 5.50 ***

Values are mean ± S.E.M. of 4-6 separate experiments
***p<0.001, **p<0.01 when compared with control
@p<0.001 when compared with 5% EtOH perfused

Figure-45

EEG of control rats

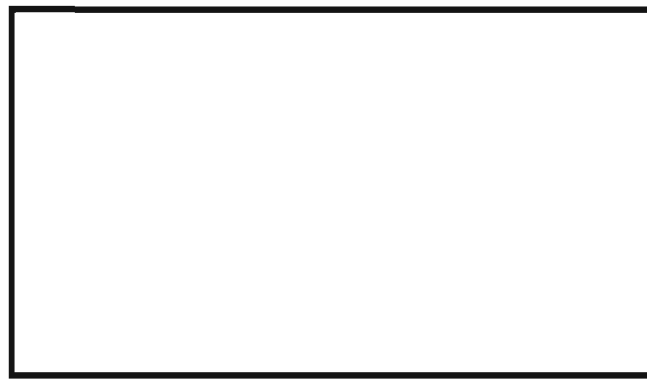
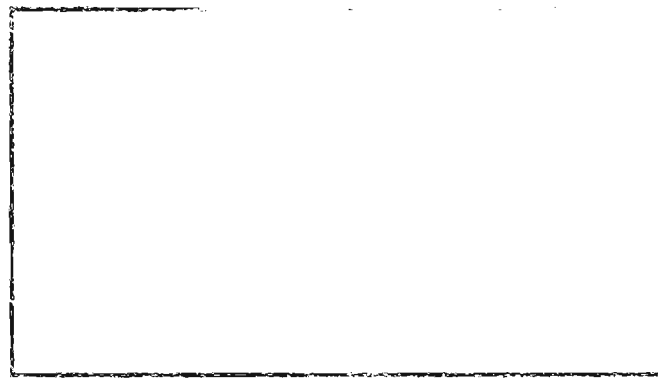


Figure-46

EEG of ethanol treated rats



F3-Frontal lobe - left
P3-Parietal lobe - left
O1-Occipital lobe - left
T3-Temporal lobe- left
A1- Reference - left

F4-Frontal lobe - right
P4-Parietal lobe - right
O2-Occipital lobe- right
T4-Temporal lobe- right
A2-Reference - right