Ph.D. Thesis

ANJU.T.R

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GABA, SEROTONIN, MUSCARINIC RECEPTORS - THEIR SECOND MESSENGERS AND TRANSCRIPTION FACTORS FUNCTIONAL REGULATION IN THE BRAIN REGIONS OF HYPOXIC NEONATAL RATS: RESUSCITATION WITH GLUCOSE, OXYGEN AND EPINEPHRINE

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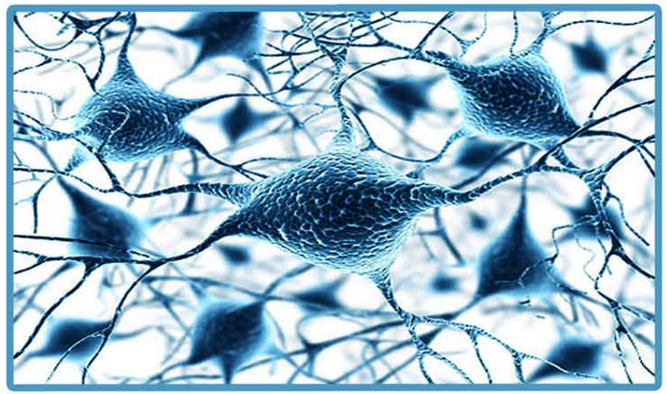

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BY

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CERTIFICATE

This is to certify that the thesis entitled **"GABA, serotonin, muscarinic receptors - their second messengers and transcription factors functional regulation in the brain regions of hypoxic neonatal rats: Resuscitation with glucose, oxygen and epinephrine"** is a bonafide record of the research work carried out by **Mrs. Anju T R,** 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

(C. S. Paulose)

September 15, 2010

DECLARATION

I hereby declare that the thesis entitled **"GABA, serotonin, muscarinic receptors - their second messengers and transcription factors functional regulation in the brain regions of hypoxic neonatal rats: Resuscitation with glucose, oxygen and epinephrine"** is the authentic record of research work carried out by me for my doctoral degree, under the supervision and guidance of Dr. C. S. Paulose, Professor, Department of Biotechnology, Director, Centre for Neuroscience, Cochin University of Science and Technology and that no part thereof has previously formed the basis for the award of any degree or diploma, associateship or other similar titles or recognition.

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DEDICATED TO MY BELOVED

HUSBAND, PARENTS

& SON.

ABBREVIATIONS

5-HIAA	5-hydroxy indole - 3 acetic acid
5-HT	5-Hydroxy tryptamine
ACh	Acetylcholine
AChE	Acetylcholine esterase
ATP	Adenosine triphosphate
B_{max}	Maximal binding
BSA	Bovine serum albumin
cAMP	Cylic adenosine monophosphate
CAT	catalase
ChAT	Choline acetyltransferase
cDNA	Complementary deoxy ribonucleic acid
CNS	Central Nervous System
CREB	cAMP regulatory element binding protein
Ct	Crossing threshold
DEPC	Di ethyl pyro carbonate
DNA	Deoxyribonucleic acid
ECD	Electrochemical detector
EDTA	Ethylene diamine tetra acetic acid
EPI	Epinephrine
FITC	Florescent isothiocyanate

GABA	Gamma amino butyric acid
$GABA_AR$	$GABA_A$ receptor
$GABA_BR$	$GABA_B$ receptor
GAD	Glutamate Decarboxylase
GOD	Glucose oxidase
GPCR	G protein-coupled receptor
GPx	Gluthathione peroxidase
GTP	Guanosine triphosphate
HBSS	Hank's balanced salt solution
HPLC	High performance liquid chromatography
HIF	Hypoxia Inducible Factor
INS	Insulin
i.p.	Intraperitoneally
K_d	Dissociation constant
K_m	Michaelis constant
mRNA	Messenger ribonucleic acid
NADH	Nicotinamide adenine dinucleotide, reduced form
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NE	Norepinephrine
P	Level of significance
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction

PFC	Prefrontal cortex
PLC	Phospholipase C
QNB	Quinuclidinylbenzilate
RIA	Radioimmunoassay
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT-PCR	Reverse-transcription-polymerase chain reaction
SEM	Standard error of mean
Ser	Serine
SOD	Superoxide dismutase
T3	Triiodothyronine
T4	Thyroxine
Thr	Threonine
Tyr	Tyrosine

CONTENTS

Introduction

Hypoxia is one of the major causes of damage to the foetal and neonatal brain. Newborn babies are frequently exposed to hypoxia and ischemia during the perinatal period as a result of stroke, problems with delivery or respiratory management after delivery (William *et al*., 2005). Hypoxic–ischemic (HI) injury in term neonates, whether resulting from birth asphyxia, cardiac arrest or respiratory failure, is known to produce reduced brain growth and associated cognitive, motor and behavioural deficits later in life. The ventilatory response to hypoxia represents the net result of two counteracting stimuli acting upon the central nervous system: 1) an augmentation of neuronal activities through afferent influences from the peripheral chemoreceptors and 2) a reduction of these activities through influences of hypoxia upon the central nervous system (Neubauer *et al*., 1990; St. John, 1981; Rigatto *et al*., 1988). If a hypoxic insult occurs during a critical cellular or tissue differentiation process, that episode might have a serious impact on brain maturation. For this reason alone the perinatal age is of great importance, but the process of delivery and the sudden adaptation to postnatal life are in their own right stressful and demanding events for the metabolic homeostasis of the newborn organism. Add on the event of an HI episode to this already stressful time and the results can be catastrophic at best or fatal at worst. In mild cases, hypoxia causes inattentiveness, poor judgment and lack of motor coordination. The varying levels of functional damage can be reversed depending on the extent of injury. Cerebral hypoxia refers to a condition in which there is decrease in oxygen supply to the brain in spite of adequate blood flow. Hypoxia affects the central nervous system (CNS) both functionally and morphologically (Flynn *et al*., 1977; Nelson & Lynch, 2004).

Foetuses, that experience injuries in the womb, premature births and birth complications, live rest of their lives in fear of growth and development (Mark,

1993). The acute interruption or reduction of cerebral blood flow, induced by several factors and clinical pathologies, reduces available oxygen to the nervous system. As the placenta stops growing during the final months of pregnancy, it becomes tough and fibrous, causing degeneration of blood vessels making the foetus more susceptible to hypoxia (Heinz, 1970; Hein & Kobilka, 1995). Furthermore, the weight of the foetus pressing down into the pelvis can compress blood vessels supplying the placenta, producing additional placental failure (Briend, 1979). Practice contractions near birth give the foetus periodic "squeezes," decreasing oxygen level even further (Joseph, 1947). Birth itself is so hypoxic that "hypoxia of a certain degree and duration is a normal phenomenon in every delivery," and not just in severe cases. The effects on the foetus due to extreme hypoxia are dramatic: normal foetal breathing stops, foetal heart rate accelerates, then decelerates and the foetus thrashes about frantically in a life and death struggle to liberate itself from its terrifying asphyxiation (Peter & Peth, 1980). Sometimes, continuous seizures occur as a result of hypoxia (Lucas, 2002). This causes either focal or global brain damage, with characteristic biochemical and molecular alterations that can result in permanent or transitory neurological sequelae or even death (Rodrigo *et al*., 2005).

The ventilatory response to acute hypoxia (hypoxic ventilatory response; HVR) in humans and some other mammalian species is biphasic (Neubauer *et al*., 1990; Weil & Zwillich, 1976). The initial rise in ventilation (early phase of the HVR) is followed by a marked decline after several minutes to values above the prehypoxic level. This decline in ventilation has been termed "ventilatory roll-off" or "hypoxic ventilatory decline" (HVD). Several neurotransmitters and neuromodulators, such as γ-aminobutyric acid (GABA), (Kazemi & Hoop, 1991; Kneussl *et al*., 1986; Richter *et al*., 1999; Taveira da Silva *et al*., 1987) serotonin (5-HT), (Di Pasquale *et al*., 1992) adenosine, (Elnazir *et al*., 1996; Neylon & Marshall, 1991) and platelet-derived growth factor (PDGF-β) (Gozal *et al*., 2000; Simakajornboon & Kuptanon, 2005) play important roles in HVD. Endogenous

GABA acting on $GABA_A$ or $GABA_B$ receptors modulates ventilation during room air breathing as well that the ventilatory response to acute and sustained hypoxia (Zhang *et al*., 2002). Huang *et al* (1994) reported that the decrease in ventilation during the biphasic ventilatory response to hypoxia in the neonatal piglet is in part mediated through the effect of GABA on the central nervous system. Long-term hypoxia produces a significant but reversible reduction on GABA binding to $GABA_A$ receptor sites in cerebral cortex, which reflect an adaptive response to this sustained pathophysiological state (Viapiano *et al.*, 2001). Hypoxia has been a selective pressure in conserving GABA and glutamate as major inhibitory and excitatory neurotransmitters in vertebrates as well as invertebrates (Nilsson & Lutz, 1993). $GABA_B$ receptors contribute essentially to the modulation of respiratory rhythm in adult mammals and may be involved in the control of respiratory neuronal discharge (Ai-Lun Yang *et al*., 2007).

Serotonin (5-hydroxytryptamine; 5-HT) is one of neurotransmitters participating in the development of hypoxia-induced pulmonary hypertension. Pulmonary vasoactive responses to hypoxia are intensified by 5-HT (Eddahibi, 1997). Hypoxia-induced long-term facilitation requires 5-HT receptors and is characteristic of both hypoglossal and phrenic motor output (Bach & Mitchell, 1996; Jacono *et al.*, 2005). 5HT modulates the dynamics of the hypoxic sensory response through its action on $5\text{-}HT_2$ receptors (Serrano *et al.*, 2003). The neurotransmitter acetylcholine (ACh) acting through muscarinic receptors is involved in many aspects of respiratory neuromodulation (Haji *et al*., 2000), notably central chemosensitivity in brainstem structures (Ballantyne & Scheid, 2000; Burton & Kazemi, 2000) and peripheral chemosensory mechanisms originating in the carotid bodies (Shirahata *et al*., 2007). Muscarinic receptors are also present on rhythm-generating neurons in the pre-B¨otzinger complex (Shao & Feldman, 2000). The muscarinic receptor stimulation by acetylcholine and $5HT_{2A}$ stimulation leads to activation of phospholipase C (PLC), which, in turn, hydrolyses phosphatidylinositol 4, 5-bisphosphate (PIP2) to produce inositol

triphosphate (IP3) and diacylglycerol (DAG) (Best & Malaisse, 1983; Zawalich *et al*., 1989). G protein coupled receptors like $GABA_{B,}$ 5- HT_2 and muscarinic receptors act through second messengers like IP3, cAMP or cGMP. Studying the level of these messengers reveals the control of signaling cascades in the cell.

cAMP responsive element binding protein (CREB) is a protein that is a transcription factor. It binds to certain DNA sequences called cAMP response elements and thereby increases or decreases the transcription of the downstream genes (Lauren, 2005). In neuronal tissue, CREB regulation by nerve growth factor and insulin-like growth factor-1 is essential for neuronal plasticity, full axonal development, memory consolidation and neuroprotection (Spaulding, 1993; Shimomura *et al.,* 1998). The PLC activity decline in the brain is expected to affect DAG which is the principal molecular species of phosphoinositides in the nervous tissue (Whiting *et al*., 1979). Alterations in glucose utilisation are known to occur in the important regions of brain connected with learning and memory (Auer & Siesjo, 1993).

Investigations on the CNS responses to oxygen deprivation are of obvious importance in revealing mechanisms that participate in coordinated behaviour of respiratory and vasomotor responses to hypoxia. Adaptation to continued moderate hypoxia in the rat brain includes structural and metabolic changes. As a result of deprivation of oxygen (hypoxia) and nutrients, the growth and viability of cells is reduced. Hypoxia-inducible factor 1 alpha (HIF-1A) helps to restore oxygen homeostasis by inducing glycolysis, erythropoiesis and angiogenesis (Paul *et al.*, 2004). HIF-1α protein level is an indicator for hypoxic regions undergoing apoptotic cell death. After exposed to hypoxia, many kinds of cells increase their synthesis of HIF protein, which in turn binds to and activates many genes (Wang *et al.*, 2007)

Perinatal hypoxic ischemic injury is accompanied by neurodegeneration, including features of both necrotic and apoptotic neuronal death as well as destruction of neurites connecting different neuronal populations (Ferriero, 2004).

Apoptosis is regulated and executed by many proteins, which are expressed *de novo* or activated in response to apoptotic signals. The mitochondrial pathway has frequently been implicated in neuronal apoptosis, along with the pro-apoptotic BAX protein, a major component of this pathway. BAX plays a role in cell death following hypoxia and ischemia. The expression of BAX in brain regions can be taken as an index of the brain damage caused by hypoxic stress. The occurrence of hypoxic brain injury during foetal or neonatal development leads to damaged immature neurons and result in behavioural and/or cognitive dysfunction, including motor or learning disabilities, cerebral palsy, epilepsy or even death (Delivoria-Papadopoulos & Mishra, 2000; Levison *et al.*, 2001; Saikumar *et al.*, 1998). Mild hypoxia–ischemia induces significant cerebral injury in neonates and is frequently accompanied by motor and cognitive impairments throughout life (Lindstrom *et al.*, 2006; van Handel *et al.*, 2007). Neither the mechanisms determining the severity of long-term consequences, nor treatment options are sufficiently understood. Perinatal hypoxic–ischemic injury is a serious problem in both full-term and premature human neonates, with a high risk of future behavioural and neurological deficits. In spite of improvements in obstetric and neonatal intensive care, hypoxic–ischemic brain damage with severe neurological disability remains a clinical problem and studies in animal models continue to be of high demand.

Brain cells are extremely sensitive to oxygen deprivation and begin to die within five minutes after oxygen supply has been blocked. Brain damage due to an episode of cerebral hypoxia remains a major problem in the human infant (Tuor *et al.*, 1996). Every year thousands of newborn infants require some form of resuscitation immediately after birth. It is a standard practice to resuscitate newborn infants, both term and premature, who are asphyxiated at birth, with 100% oxygen. In addition, a small number of these newborns will require the administration of epinephrine (10μg/kg) and intravenous fluids, which include 10% glucose (500mg/ kg body wt) as part of their initial resuscitation. Over the

past decades, neonatal resuscitation programmes have been well developed, but some of the procedures employed in these programmes are not based on scientific evidence (Nong *et al*., 2000). Glucose acts directly on the brain to regulate neural processing, a function that seems incompatible with the traditional view that brain glucose levels are high and invariant except under extreme conditions. However, recent data suggest that the glucose levels of the brain extracellular fluid are lower and more variable than previously supposed (Oltmanns *et al*., 2004). Hypoxia in newborn infants is becoming much easier to prevent, detect and treat. Nevertheless the successful management of potentially hypoxic fetuses and newborn infants remains the major challenge to all physicians concerned with perinatal care. What is at stake is not only that sick infants should survive, but equally or more importantly that the survivors should be normal children.

The present study was designed to investigate the protective effect of glucose, oxygen and epinephrine resuscitation on impairment in the functional role of GABAergic, serotonergic, muscarinic receptors, PLC, BAX, SOD, CAT and GPx expression in the brain regions of hypoxia induced neonatal rats. Also, the role of hormones - Triiodothyronine (T3) and insulin, second messengers – cAMP, cGMP and IP3 and transcription factors – HIF and CREB in the regulation of neonatal hypoxia and its resuscitation methods were studied. Behavioural studies were conducted to evaluate the motor function and cognitive deficit in one month old control and experimental rats. The efficient and timely supplementation of glucose plays a crucial role in correcting the molecular changes due to hypoxia, oxygen and epinephrine. The sequence of glucose, epinephrine and oxygen administration at the molecular level is an important aspect of the study. The additive neuronal damage effect due to oxygen and epinephrine treatment is another important observation. The corrective measures by initial supply of glucose to hypoxic neonatal rats showed from the molecular study when brought to practice will lead to healthy intellectual capacity during the later developmental stages, which has immense clinical significance in neonatal care.

OBJECTIVES OF THE PRESENT STUDY

1. To induce hypoxia in Wistar neonatal rats and resuscitate with glucose, oxygen and epinephrine.

2. To measure the free radical scavenging capability in the heart and cerebral cortex of control, hypoxic and resuscitated groups of neonatal rats by assaying SOD and catalase activity and gene expression of SOD and GPx.

3. To measure the circulating triiodothyronine and insulin level and triiodothyronine receptor binding parameters and insulin receptor expression in control, hypoxic and resuscitated groups of neonatal rats.

4. To measure 5-HT and 5-hydroxy indole acetic acid (5-HIAA) content in the brain regions - BS and CB of control, hypoxic and resuscitated groups of neonatal rats using HPLC.

5. To measure GABA content in cerebral cortex, cerebellum, brain stem and corpus striatum of control, hypoxic and resuscitated groups of neonatal rats using displacement method.

6. To study the total GABA, $GABA_A$ and $GABA_B$ receptor subtypes binding parameters in cerebral cortex, cerebellum, brain stem and corpus striatum of control, hypoxic and resuscitated groups of neonatal rats.

7. To study the total 5-HT and 5-HT_{2A} receptor subtype binding parameters in cerebral cortex, cerebellum, brain stem and corpus striatum of control, hypoxic and resuscitated groups of neonatal rats.

8. To study the total muscarinic receptor binding parameters in cerebral cortex, cerebellum, brain stem and corpus striatum of control, hypoxic and resuscitated groups of neonatal rats.

9. To study the gene expression of $GABA_{A\alpha1,}$ $GABA_{A\alpha5,}$ $GABA_{A\gamma3,}$ $GABA_{A\delta,}$ $GABA_{B}$, $5\text{-}HT_{2A,}$, muscarinic M1, muscarinic M2, muscarinic M3 receptors, GAD, 5-HTT, acetylcholine esterase, choline acetyltransferase, transcription factors - HIF1A and CREB, pro- apoptotic protein BAX and enzyme PLC in the cerebral cortex, cerebellum, brain stem and corpus striatum of control, hypoxic and resuscitated groups of neonatal rats using Real Time PCR.

10. To study localisation and expression status of $GABA_{A\alpha1}$, 5-HT and 5-HTT in the brain slices of cerebral cortex, cerebellum and brain stem of control, hypoxic and resuscitated groups of neonatal rats using specific antibodies in confocal microscope.

11. To study the second messengers – cAMP, cGMP and IP3 content in the corpus striatum of control, hypoxic and resuscitated groups of neonatal rats.

12. To study the behavioural changes in control and experimental neonatal rats using Y-maze, radial arm maze, water maze, wire maneuver test and righting reflex.

Literature Review

Hypoxia-ischemia (HI) occurring before or shortly after birth is a major cause of life-threatening injury and lifelong disability (du Plessis, 2002; Schubert *et al.*, 2005). HI results in multi-organ failure and structural / functional damage especially devastating to the cardiovascular, renal, gastrointestinal and central nervous systems (Shah *et al.*, 2004; Vento *et al.*, 2005). The two most important causes of infant's death were "intrapartum asphyxia and birth trauma" (intrapartum hypoxia) which resulted in neonatal "hypoxia" and "spontaneous preterm labour" leading to "immaturity related" births. Death rates in pre-term infants were particularly high in cities, towns and rural areas where neonatal care facilities are very limited. The large number of deaths associated with perinatal hypoxia suggested problems and inadequacies in care of women in labour and the resuscitation of newborn infants. There is probably poor insight into the deficiencies in the basic management of newborn infants as well as a lack of knowledge on neonatal resuscitation and care compared to intrapartum care (Robert *et al*., 2005).

Impact of Hypoxia

HI brain injury is very complex and has different neuropathological manifestations depending on the maturity of the newborn. As such, HI is etiologically linked to cerebral palsy, hearing and vision loss, mental retardation, learning disabilities, attention deficit hyperactivity disorder, schizophrenia and neuronal migration disorders (Johnston *et al*., 2002; Schubert *et al*., 2005). Brain is of special interest for hypoxia studies as it is extremely sensitive to reductions in oxygen supply. The brain damage occurs within a few minutes of hypoxia and result in severe and complex disabilities or death (Slavin, 1994). The reason for this vulnerability is that the brain has committed high energy costs that cannot be compromised. 50–60% of

the brain cells energy expenditure is devoted to transporting ions across the cell membranes in order to maintain cellular ion homeostasis (Lipton, 1999). As a result, the brain suffers energy failure even after few minutes' interruption in oxygen supply. The anatomical consequences of neonatal hypoxia on the developing CNS vary from neuronal death (Dell'Anna *et al.*, 1995) to altered neuronal differentiation. After acute hypoxia surviving immature neurons have a compromised neurite outgrowth and synapse formation. These minimal anatomical abnormalities underlie both behavioural-psychological dysfunction and neuroendocrine deficits (Nyakas *et al.*, 1996).

Cerebral hypoxic ischemia appears to stimulate massive extracellular catecholamine release in the cortex, striatum and hippocampus. *In vitro* studies have also demonstrated elevated catecholamine concentrations and reduced uptake in gerbil synaptosomes during ischemia (Weinberger & Neives-Rosa, 1988). Central norepinephrine release during brain ischemia increases neuronal metabolism and exaggerates the discrepancy between impaired blood flow to ischemic tissue and an increase in the metabolic demand. Further, metabolism of excessive catecholamines leads to the formation of neurotoxic free radicals, whereas prevention of oxidative deamination of catecholamines reduces hydrogen peroxide production during reperfusion (Simonson *et al*., 1993). In addition to their direct detrimental effects, catecholamines also sensitize neurons to the excitatory amino acid glutamate, thus exacerbating the damage caused by glutamate during ischemia.

Thus, understanding the diagnosis, pathogenesis, resuscitation and treatment of those infants suffering hypoxic brain injury is paramount to reducing disability, improving survival and enhancing quality of life. Upon delivery, 5-10% of all newborns require some degree of resuscitation and assistance to begin breathing (Davis *et al*., 2004; Tan *et al*., 2006). The aim of resuscitation is to prevent neonatal death and adverse long-term neurodevelopment sequelae associated with a hypoxic event and rapidly reverse fetal hypoxemia and acidosis.

Hypoxia and neuronal death

Perinatal HI injury is accompanied by neurodegeneration, including features of both necrotic and apoptotic neuronal death as well as destruction of neurites connecting different neuronal populations (Ferriero, 2004). In studies with experimental animals, reducing neuronal death during and/or immediately after HI injury has been shown to markedly decrease long-term disability (Almli *et al*., 2000). Hypoxia is the key regulating factor that triggers inflammation as well as apoptosis in the human atherosclerotic plaque (Bitto *et al*., 2010). Hypoxia-reoxygenation injury is reported to induce apoptosis in neonatal rat cardiomyocytes (Zhang *et al*., 2010). Multiple cell death mediators have been reported to be activated in *in vivo* and *in vitro* models of neonatal HI injury, including various Bcl-2 family members, death receptors and caspases (Calvert & Zhang., 2005). The occurrence of hypoxic brain injury during fetal or neonatal development leads to damaged immature neurons and result in behavioral and/or cognitive dysfunction, including motor or learning disabilities, cerebral palsy, epilepsy or even death (Delivoria-Papadopoulos & Mishra, 2000; Levison *et al*., 2001; Saikumar *et al*., 1998).

The Bcl-2 family of proteins is an important determinant of apoptotic cell death. It consists of pro-apoptotic (Bax, Bcl-Xs, Bak and Bad) and anti-apoptotic (Bcl-2, Bcl-XL and Bcl-w) proteins (Adams & Cory, 1998). Bcl-2 family members determine cell death and survival by controlling mitochondrial membrane ion permeability, cytochrome *c* release and the subsequent activation of caspase (caspase 3, caspase 9) executor functions (Allen *et al*., 1998; Banasiak *et al*., 2000; Glasgow & Perez-Polo., 2000). Bax is one of the key proteins that turn on the apoptotic cascade. The Bax protein was increased as a function of increase in degree of cerebral tissue hypoxia (Ravishankar *et al*., 2001). Hypoxia results in increased caspase-9 and caspase-3 activity in the cytosolic fraction of the cerebral cortex (Khurana *et al*., 2002; Mishra & Delivoria-Papadopoulos, 2006). Hypoxia results in increased fragmentation of nuclear DNA and the degree of DNA fragmentation increases as a function of

cerebral hypoxia (Akhter *et al*., 2001). Furthermore, it was shown that hypoxia results in generation of nitric oxide (NO) free radicals in the cerebral cortical tissue (Mishra *et al*., 2000) and administration of a nitric oxide synthase (NOS) inhibitor prevented the hypoxia -induced increased expression of Bax, caspase-9 activation, lipid peroxidation and increased fragmentation of nuclear DNA (Mishra & Delivoria-Papadopoulos, 2006; Numagami *et al*., 1997; Zubrow *et al*., 2002[b]). The neuronal death caused by neonatal hypoxic insult was mediated by the proteins involved in apoptotic pathways like Bax. Regulating the expression of these proteins can control the neuronal death due to hypoxic stress.

Hypoxia and Medications

Drugs are rarely indicated in resuscitation of the newly born infant (Burchfield, 1999). Bradycardia in the newly born infant is usually the result of inadequate lung inflation or profound hypoxia. Adequate ventilation is the most important step in correcting bradycardia. Administration of medications is required if, despite adequate ventilation with 100% oxygen and chest compressions, the heart rate remains <60 bpm. Epinephrine administration is indicated when the heart rate remains <60 bpm after a minimum of 30 seconds of adequate ventilation and chest compressions. Epinephrine is particularly indicated in the presence of asystole.

The standard approach of resuscitation for neonatal hypoxia is to use 100% O_2 (American Heart Association, American Academy of Pediatrics, 2005). Further, resuscitation with 100% is recommended as a beneficial short-term therapy that is generally thought to be non-toxic (Kuisma *et al*., 2006; Martin *et al*., 2005). Although the use of 100% O_2 appears intuitive to maximize the gradient required to drive O_2 into hypoxic cells, (Corff & McCann., 2005) a building body of evidence derived from animal models, has demonstrated that although resuscitation with 100% O_2 improves restoration of cerebral and cortical perfusion, it occurs at the price of greater biochemical oxidative stress (Martin *et al*., 2005). Further, results from investigations

by Munkeby *et al* (2004) suggest that resuscitation of asphyxiated piglets with 100% O_2 is detrimental to the brain. However in mice, resuscitation with 100% O_2 restores cerebral blood flow significantly faster than resuscitation with 21% O_2 and improves late neurofunctional outcome (Presti *et al*., 2006). Studies performed on asphyxiated human infants have shown that room air rather than 100% O_2, favors clinical recovery (Vento *et al*., 2001[a]; Vento *et al*., 2001[b]).

Epinephrine has both α- and β-adrenergic stimulating properties; however, in cardiac arrest, α-adrenergic mediated vasoconstriction is the important mode of action (Zaritsky & Chernow., 1984). Vasoconstriction elevates the perfusion pressure during chest compression, enhancing delivery of oxygen to the heart and brain (Berkowitz *et al*., 1991). Epinephrine also enhances the contractile state of the heart, stimulates spontaneous contractions and increases heart rate. The recommended intravenous or endotracheal dose is 0.1 to 0.3 ml/Kg of a 1:10,000 solution (0.01 to 0.03 mg/Kg), repeated every 3 to 5 minutes as indicated. The data regarding effects of high dose epinephrine for resuscitation of newly born infants is inadequate to support routine use of higher doses of epinephrine. Higher doses have been associated with exaggerated hypertension but lower cardiac output in animals (Berg *et al*., 1996; Burchfield *et al*.,1993). The sequence of hypotension followed by hypertension likely increases the risk of intracranial hemorrhage, especially in preterm infants (Pasternak *et al*., 1983).

Epinephrine at a dose of 1 mg after every cycle of three unsuccessful shocks or after every three minutes of cardio pulmonary resuscitation during a non shock able arrest improves cerebral and coronary blood flow. In experimental animals, it increases peripheral resistance by adrenergic stimulation, thereby preventing arterial collapse during the release phase of cardiac compression. It also increases myocardial contractility and rate by β-adrenergic stimulation after restoration of an effective heart beat, or if in apparent PEA cardiac contraction is present but impalpable. Perhaps surprisingly, its benefit for survival in man is still debatable; no randomized controlled

trial has been attempted to support its use. High dose adrenaline has no clear advantage and is suggested to be deleterious (Vandycke & Martens, 2000).

Neonatal Resuscitation during Hypoxia

Neonatology, perinatology and neonatal resuscitation developed to a great extent during the 1970's in response to an epidemic of litigation involving birth brain injury; foetal monitoring was detecting foetal distress *in utero* and specialized perinatal intensive care promised great improvement in neonatal morbidity and mortality. One third of all neonates receive some form of resuscitation treatment. About 6% to 10% of all neonates are "morbid" and need NICU care - many of these are premature. NICU mortality is extremely rare; however, in terms of neurological and mental disability, especially in NICU babies, long-term morbidity is anything but rare (Hack *et al*., 2002). The life saving procedures of neonatal resuscitation and NICU care are much less successful in preserving brains. Apgar score is a quantitative rating test with a maximum of ten used to measure the vital signs of a newborn a minute or so after birth: a score greater than seven signifies good health. Neurological impairment is likely if, resuscitation does not result in a five minute Apgar of 7 or more (Thorngren-Jerneck & Herbst, 2001).

The term "resuscitation" implies restoration of deficient life support systems, especially respiration; in the depressed newborn, that deficiency is in the placenta and cord, as the lungs have not yet begun to function. The rationale on which current resuscitation is based is that early detection of foetal asphyxia combined with rapid delivery and rapid establishment of pulmonary respiration (reversal of asphyxia) will prevent brain injury. If brain damage by neuron necrosis has occurred *in utero*, resuscitation will not heal it; however, overt brain damage seldom is evident at birth and it often appears after resuscitation. Hypoxic ischemic encephalopathy usually is diagnosed hours after birth when the child convulses; germinal matrix hemorrhage in

preemies (preterm babies) develop a day or two after birth; mental and behavioral problems will surface for years.

The general consensus is that birth "asphyxia" is the cause of the brain damage; hypoxia is a more precise term, although asphyxia implies arrest of respiration - respiration includes oxygen supply and removal of carbon dioxide. Iatrogenic resuscitation usually corrects this asphyxia promptly by initiating pulmonary ventilation; most organs survive superbly, except the brain. This strongly implies that there are other factors active in neonatal "depression" besides hypoxia and acidosis that must be corrected during "resuscitation". The placenta is much more than a respiratory organ. Correction of the placental/ cord deficiency that caused the depression and support of placental function are thus rational priorities in revival of a depressed neonate, just as they are in the "resuscitation" of the "distressed" foetus *in utero*.

In utero, the normal blood supply of the foetal brain is relatively hypoxic. Umbilical vein blood is fairly well oxygenated, but it is mixed in the inferior vena cava and in the heart with deoxygenated blood from the venae cavae; this is then circulated systemically. The color of a normal newborn is purple – it has been purple for nine months – circulating a mixture of haemoglobin (blue) and oxyhaemoglobin (red). It turns pink only after the foetal circulation is changed to the adult circulation and is combined with aeration of the lungs. The foetal brain thus grows and develops with a copious blood supply that is only partially oxygenated, but which readily removes products of aerobic and anaerobic respiration and excretes them through the placenta. The foetal kidneys and gut thrive on blood with the same oxygen partial pressure as the blood flowing to the placenta to be oxygenated. The newborn brain and other organs are therefore relatively immune to pure hypoxic injury (Kirks & Thorne, 1998) as long as organ and placental perfusion are copious.

The same basic principles apply to the adult brain; five minutes or more of cardiac arrest will produce some brain damage or brain death; occlusion of a cerebral

artery rapidly results in infarction (death) of the supplied tissue. On the other hand, five minutes or more of pure anoxia (*e.g.* breathing pure nitrogen) will produce unconsciousness that is fully reversible without brain damage provided that brain perfusion is not impaired. The integrity of the newborn brain is maintained (by perfusion and oxygenation) at normal (physiological) birth; therefore the physiological mechanisms that ensure these functions (perfusion and oxygenation) should be supported and/or duplicated during resuscitation if brain damage is to be avoided.

The severely depressed/asphyxiated newborn typically shows not only sign of breathing but also lack of muscle tone and reflexes needed to initiate breathing as well as signs of hypoxia such as cyanosis; in the most severe cases, pallor indicates vasomotor collapse. Such a child has obviously suffered a major respiratory insult prior to or during birth; the cause of that insult and its specific effects are factors that must be corrected, if possible, in the resuscitation process. In any and every case of newborn depression, if a child is born alive – with a heart beat and a pulsating cord – the placental life support system has not failed completely; utilization of this system in resuscitation and transition to "adult" life support systems in the depressed newborn is essential in restoring the physiological state – health – without the incursion of organ damage, primary or secondary, from "birth asphyxia." With early detection of foetal distress and with rapid delivery, the neonate's CNS should be undamaged at birth; the objective of therapy should be that it remains so.

The switch from placental to lung "breathing" is only a portion of the whole; the switch from placental alimentation and placental excretion to the newborn's alimentary and excretory organs is also part of "natural" resuscitation. To initiate and establish the newborn functions of the lungs, gut, kidneys and other systems, including the brain, continuous copious perfusion of these organs is required; a large transfusion of placental blood during natural childbirth "resuscitates," or more correctly "activates" all these organ systems as the massive flow of blood through the

placenta (40% of the foetal cardiac output) is diverted to these organs during physiological closure of the cord vessels.

Cord closure abruptly halts the placental supply of glucose to the brain (used in aerobic and anaerobic respiration); the neonatal liver (glycogen stores) must begin to maintain blood glucose levels. A major portion of the liver's blood supply is from the hepatic portal vein that derives its blood from the mesenteric arteries. If the gut (and hence the liver) is not "copiously perfused," hypoglycemia result in a neonatal convulsion. Deficient perfusion of the liver is also a factor in bilirubin excretion and "physiological" jaundice. Copious perfusion of the neonatal kidneys with adequate blood pressure is required for solute excretion, fluid, electrolyte and acid-base regulation after the placenta ceases to function. During the third stage of labor while the cord is pulsating, warm blood from the placenta courses through the newborn. After cord closure, temperature regulation is suddenly required of the neonate; switch of blood flow to and from the epidermis requires a copious amount of blood to regulate heat loss and heat retention.

In the foetus, pulmonary circulation is minimal; after the adult circulation is established, the entire cardiac output flows through the lungs. A major portion of the placental transfusion is utilized in establishing pulmonary blood flow after birth. Jaykka (1965) demonstrated that perfusion of the foetal lung "erected" the alveoli and actually initiated aeration; the high colloid osmotic pressure of the circulating blood rapidly absorbs amniotic fluid from the erected alveoli. Thus adequate "copious perfusion" of the lungs result in pulmonary oxygenation before any muscular respiratory effort occurs. Respiratory effort is reflexively controlled through the CNS; hypoxia and increased concentration of carbon dioxide are strong stimulants for receptors. For the reflex to function, copious perfusion of the reflex circuit is required, as is copious perfusion of the respiratory muscles (Jaykka, 1957).

Free Radical Release and Toxicity

Free radicals and reactive oxygen species (ROS) cause tissue damage only when the radicals exceed the brain's endogenous antioxidant defences. Newborns and particularly pre-term infants are at high risk of oxidative stress and they are very susceptible to free radical oxidative damage. Free radicals are produced as a result of mitochondrial oxi-reductive processes and also produced by the action of enzymes such as xanthine/urate oxidase at extra-mitochondrial sites. These free radicals cause lipid peroxidations, especially in the cell membranes, inactivate cellular enzymes, inhibit nucleic acids and protein synthesis.

Free radicals or ROS are formed under hypoxic conditions. Antioxidants such as vitamin E can attenuate the effects of cerebral ischemia (Yamagata *et al*., 2010). Treatment of hypoxic pulmonary hypertension of rats includes stimulation of vasodilation of pulmonary artery and inhibition of oxidative stress (Fan *et al*., 2010). Cells have an enzymatic antioxidant pathway against ROS which are generated during oxidative metabolism: firstly, superoxide dismutase (SOD) catalyzes the formation of hydrogen peroxide from superoxide radicals, which is removed by a reaction catalyzed by catalase (CAT) and glutathione peroxidase (GPx) (Michel *et al*., 1994). The neonatal brain is especially at risk of free radical mediated injury because neuronal membranes are rich in polyunsaturated fatty acids and the human newborn has a relative deficiency of brain superoxide dismutase and glutathione peroxidase (Buonocore *et al*., 2001). Normally, various antioxidant enzymes protect the body from these free radicals, but in hyperoxic situations, there is explosive free radical production leading to swamping of the enzyme systems and as a result free radicals escape inactivation (Chawla & Lavaniya, 2001). Roberto *et al*., (2005) reported that hyperoxia with 100% oxygen after hypoxia-ischemia cause more damage in the cerebral cortex than room air in newborn rats.

Flamm *et al*., (1978) correlated the generation of free radicals with cell damage in cerebral ischemia. Free radicals are highly reactive molecules that initiate

radical chain reactions and damage cellular macromolecules, including proteins, DNA and lipids, ultimately leading to cell death. Free radicals have been implicated in neuronal cell death in acute CNS injury and in chronic neurodegenerative diseases (Chan, 1994; Coyle & Puttfarcken, 1993). There are a number of potential sources for free radicals generation in the ischemic brain. This comprises leaks from mitochondrial respiratory chain; sequences catalyzed by cyclo-oxygenase and lipooxygenase, peroxidation of lipid membrane, auto-oxidation of various small molecules, including catecholamines, by the microsomal cytochrome P450 reductase system (Freeman & Crapo, 1982) and xanthine oxidase reactions. The brain and nervous system is especially prone to oxidative damage for a number of reasons (Ozben, 1998): the membrane lipids are especially rich in polyunsaturated fatty acid side-chains, which are prime targets for free radicals attack; the brain has only moderate amounts of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Also, it is relatively lacking in vitamin E; some areas of the brain are rich in iron ions which are released from injured cells or from bleeding in the reperfused area and enhancing lipid peroxidation. One particular role of oxygen free radicals in brain injury appears to involve reperfusion after cerebral ischemia (Chan, 1996). Reoxygenation during reperfusion provides oxygen to sustain neuronal viability and also provides oxygen as a substrate for numerous enzymatic oxidation reactions that produce reactive oxidants. In addition, reflow after occlusion often causes an increase in oxygen to levels that cannot be utilized by mitochondria under normal physiological flow conditions.

Hormonal changes during Hypoxia.

The endocrine system has an important role in the adaptation to hypoxia and stress and is reciprocally affected by these situations (Health & Williams, 1981). Hypoxia influences secretion of insulin and glucagon by regulating production of lactic acid which causes hyperinsulinemia and either up regulates or down regulates

secretion of glucagon depending on degree of hypoxia and glucose level. Adipocyte is highly expressed of insulin receptor, at the same magnitude as hepatocyte. Therefore growing plasma insulin by hypoxia is in favour for building-up of fat at adipocyte. Hypoxia imposes constant oxidative stress to islet beta cells which is by design in favour of oxidative phosphorylation. Insulin and muscle contraction stimulate glucose transport into muscle cells by separate signaling pathways, and hypoxia has been shown to operate via the contraction signaling pathway (Azevedo *et al*., 1995). Long term exposure to hypoxia plays a role in failure of beta cells which leads to diabetes mellitus (Linda & Nils, 2002). Hypoxia in adipose tissue contributes to obesity-related chronic inflammation, insulin resistance and metabolic dysfunction (Zhang *et al*., 2010). HIF-1alpha, the major transcription factor activated under hypoxia, is required for normal beta cell function and that dysregulation contribute to the pathogenesis of type 2 diabetes (Cheng *et al*., 2010)

Thyroid hormones are the most significant factors in regulating energy transformations in mammals by both short term (say few hours) and long term (say few days) effects (Hoek, 1992; Oppenheimer *et al*., 1985; Soboll, 1993). The calorigenic-thermogenic activity of thyroid hormone (T3) has been ascribed to uncoupling of mitochondrial oxidative phosphorylation (Yehuda-Shnaidman *et al*., 2010). *In vivo*, thyroid hormones are involved in setting the basal metabolic rate in many target tissues, such as liver, heart, kidney and brain. Many of the physiological actions of T3, the intracellular form of the hormone and its analogs are mediated through chromatin-associated nuclear thyroid hormone receptors (TRs), which are encoded by the a- and b- c-erbA protooncogene family (Apriletti *et al*., 1998; Lazar, 1993). TRa1 is a functional receptor that binds T3 with high affinity. TRa2 is a non hormone binding receptor that may act as a negative regulator. TRb1 and TRb2 differ in their N2- terminal sequences but both bind thyroid hormone and mediate its actions. TRa1 and TRb1 are expressed in virtually all tissues and are involved in regulating such diverse physiologic functions as metabolic rate, thermogenesis,

glucose utilization and organ development. TRb2 is restricted to the brain and anterior pituitary gland where it plays a key role in mediating negative feedback by thyroid hormone on the hypothalmic-pituitary–thyroid axis. More than 100 gene products are required for assembly of the mitochondrial respiratory apparatus. These products are encoded primarily in the nucleus with only thirteen being coded on the mammalian mitochondrial genome (Attardi & Schatz, 1988).

Although T3 is considered to be a major regulator of mitochondrial respiration only nine of the nuclear-encoded genes have been shown to respond directly to thyroid hormone (Pillar & Seitz, 1997). This paradox has been resolved, at least in part, by the identification of nuclear-encoded transcription factors that are sensitive to stimulation by T3 and activate expression of respiratory genes (Weitzel *et al*., 2003). In addition, T3-binding sites directly on mitochondria have been identified consisting of truncated TRa1 isoforms (Bigler *et al*., 1992). The smaller version, p28, binds to the inner mitochondrial membrane. The larger version, p43, is localized to the mitochondrial matrix where it stimulates the mitochondrial transcriptional machinery in the presence of T3 (Casa *et al*., 1999). There have been no detailed studies on the effects of TH analogs on mitochondrial respiration or other non genomic actions, but they could have the potential to act through one or more of the mechanisms used by T3.

Second Messenger changes during hypoxia

Second messengers relay signals received at receptors on the cell surface to target molecules in the cytosol and/or nucleus. Three major classes of second messengers are (1) cyclic nucleotides (e.g., cAMP and cGMP), (2) inositol trisphosphate (IP_3) and diacylglycerol (DAG), (3) calcium ions (Ca^{2+}). The signal transduction in metabotrophic neurotransmitters occur through activation of second messengers, whereas ionotrophic neurotransmitters act through ligand gated ion

channels. The changes in neurotransmitter level and its receptor should agree with a concomitant change in second messenger for effective signal transduction.

Inositol 1,4,5-trisphosphate

Inositol 1,4,5-trisphosphate receptors are the IP3 gated intracellular Ca^{2+} channels that are mainly present in the endoplasmic reticulum (ER) membrane. Many biological stimuli, such as neurotransmitters and hormones, activate the hydrolysis of phosphatidyl inositol 4,5-bisphosphate, generating the second messenger IP3. The IP3 mediates Ca^{2+} release from intracellular Ca^{2+} stores by binding to IP3 receptors (IP3R). The IP3 induced Ca^{2+} signaling plays a crucial role in the control of diverse physiological processes such as contraction, secretion, gene expression and synaptic plasticity (Berridge, 1993).

In mammalian cells, there are three IP3R subtypes, type 1 (IP3R1), type 2 (IP3R2), and type 3 (IP3R3), which are expressed to varying degrees in individual cell types (Taylor *et al.*, 1999; Wojcikiewicz, 1995) and form homotetrameric or heterotetrameric channels (Monkawa *et al.*, 1995). The confocal images of plasmid vector containing full-length rat IP3R3 linked to green fluorescent protein (GFP-IP3R3) provided strong evidence that IP3Rs are distributed preferentially on the ER network (Morita *et al.*, 2002; Morita *et al.*, 2004). Furthermore, Morita *et al.*, (2004) demonstrated that the expressed GFP-IP3R3 acts as a functional IP3-induced Ca^{2+} channel. Frequently, IP3Rs are not uniformly distributed over the membrane but rather form discrete clusters (Bootman *et al.*, 1997). The clustered distribution of IP3Rs has been predicted to be important in controlling elementary Ca^{2+} release events, such as Ca^{2+} puffs and blips, which act as triggers to induce the spatiotemporal patterns of global Ca^{2+} signals, such as waves and oscillations (Shuai & Jung, 2003; Swillens *et al.*, 1999; Thomas *et al.*, 1998). Tateishi *et al.*, (2005) reported that GFP-IP3R1 expressed in COS-7 cells aggregates into clusters on the ER network after agonist stimulation. They concluded that IP3R clustering is induced by its IP3-

induced conformational change to the open state, not by Ca^{2+} release itself, because IP3R1 mutants that do not undergo an IP3 induced conformational change failed to form clusters. However, their results are inconsistent with studies by other groups (Chalmers *et al*., 2006; Wilson *et al*., 1998), which suggested that IP3R clustering is dependent on the continuous elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). Thus, the precise mechanism underlying IP3R clustering remains controversial. Studies by Tojyo *et al.* (2008) have shown that IP3 binding to IP3R, not the increase in $[Ca^{2+}]$, is absolutely critical for IP3R clustering.

Hypoxia results in a modification of the binding characteristics of the neuronal nuclear membrane inositol tetrabisphosphate (IP4) and inositol triphosphate (IP3) receptors. Mishra and Delivoria-Papadopoulos (2004) observed an IP4- as well as IP3-dependent increase in nuclear Ca^{2+} influx with increasing cerebral tissue hypoxia, suggesting a hypoxia-induced modification of the nuclear membrane IP4 and IP3 receptors.

Cyclic Guanosine Monophospahte (cGMP)

cGMP generation has been associated with neurotransmission (Hofmann *et al.*, 2000), vascular smooth muscle relaxation (Fiscus *et al.*, 1985) and inhibition of aldosterone release from adrenal glomerulosa cell suspension (Matsuoka *et al.*, 1985). The most extensively studied cGMP signal transduction pathway is that triggered by nitric oxide (NO) (Bredt & Snyder, 1990). One of the second messenger pathways, which have a role in learning and memory, is the NO–cGMP signaling cascade. NO–cGMP signaling is mechanistically involved in a number of animal models for learning and behavior, e.g. object recognition and passive avoidance (Baratti & Boccia, 1999; Bernabeu *et al*., 1996, 1997; Prickaerts *et al*., 1997; Prickaerts *et al*., 2002[a, b]; Rubin *et al*., 1997). cGMP effects are primarily mediated by the activation of cGMP-dependent protein kinases (PKGs). Two distinct mammalian PKGs, PKG-I and PKG-II, have been identified, as well as two splice variants of PKG-I (PKG-Iα and -

Iβ). In the brain, PKG-I is highly expressed in cerebellar Purkinje cells and, to a lesser extent, in striatal medium spiny neurons (De Camilli *et al.*, 1984). PKG-II is a membrane-associated protein that is expressed throughout the brain (de Vente *et al*., 2001). The effects produced by the cGMP signaling pathway modulate drug-induced neural plasticity leading to behavioural alterations (Jouvert *et al.*, 2004).

Activation of the NMDA receptor increases cAMP in the CA1 region of the hippocampus; this increase is mediated through Ca^{2+} calmodulin-dependent adenylyl cyclase (Chetkovich & Sweatt, 1993). The influx of Ca^{2+} also stimulates Ca^{2+} calmodulin-dependent nitric-oxide synthase (NOS) type to produce NO, which stimulates guanylyl cyclase to produce cGMP (Garthwaite, 1991).

Cyclic nucleotide pathways cross talk to modulate each other's synthesis, degradation and actions. Increased cGMP increase the activity of cGMP stimulated PDE2 to enhance hydrolysis of cAMP, or it inhibit the PDE3 family and decrease the hydrolysis of cAMP (Pelligrino & Wang, 1998). cAMP and cGMP are involved in NMDA receptor-mediated signaling in cerebral cortical and hippocampal neuronal cultures. The influx of Ca^{2+} *via* the NMDA receptor stimulates calcium/calmodulin dependent adenylyl cyclase, leading to production of cAMP. This increase in cAMP seems to be tightly regulated by PDE4. The Ca^{2+} influx also stimulates the production of NO and subsequent activation of guanylyl cyclase, leading to cGMP production (Suvarna & O'Donnell, 2002).

Cyclic Adenosine Monophosphate (cAMP)

The second messenger concept of signalling was born with the discovery of cyclic AMP and its ability to influence metabolism, cell shape and gene transcription (Sutherland, 1972) *via* reversible protein phosphorylations. cAMP is produced from ATP in response to a variety of extracellular signals such as hormones, growth factors and neurotransmitters. Elevated levels of cAMP in the cell lead to activation of different cAMP targets. It was long thought that the only target of cAMP was the

cAMP-dependent protein kinase (cAPK), which has become a model of protein kinase structure and regulation (Canaves & Taylor, 2002; Doskeland *et al*., 1993; Francis & Corbin, 1999). In recent years it has become clear that not all effects of cAMP are mediated by a general activation of cAPK (Dremier *et al*., 1997). Several cAMP binding proteins have been described: cAPK (Walsh *et al.*, 1968), the cAMP receptor of *Dictyostelium discoideum*, which participates in the regulation of development (Klein *et al*., 1998), cyclic nucleotide gated channels involved in transduction of olfactory and visual signals (Goulding *et al*., 1992; ; Kaupp *et al*., 1989) and the cAMP-activated guanine exchange factors Epac 1,2, which specifically activate the monomeric G protein Rap (Kawasaki,*et al*., 1998; Rooij *et al*., 1998).

Transcription Factor changes during hypoxia

Hypoxia selectively activates various transcription factors and proteins to overcome the ATP depletion induced brain damage and its consequences. Activation of cAMP responsive element binding protein (CREB) plays a key role in the lung-specific responses to hypoxia and that lung microvascular endothelial cells are important, proximal effector cells in the specific responses of the pulmonary circulation to hypoxia (Leonard *et al*, 2008). Hypoxia inducible factor (HIF) is another transcription factor important in the homeostatic regulation of hypoxia.

Hypoxia inducible factor (HIF)

The ability of cells to adapt to hypoxia is important for cell survival in both physiological and pathophysiological states (Bun & Poyton, 1996). Hypoxia inducible factor-1 (HIF-1) is a heterodimeric transcription factor which consists of HIF-1α, a subunit that is tightly regulated by cellular oxygen concentration and HIF-1, a constitutively expressed subunit (Wenger, 2002). HIF-1α plays an essential role in cellular oxygen homeostasis by regulating the expression of genes involved in glycolysis, erythropoiesis and angiogenesis (Semenza, 2000[a, b]). A critical cell-

autonomous adaptive response to chronic hypoxia controlled by HIF-1 is reduced mitochondrial mass and/or metabolism. HIF-1 reduces ROS production under hypoxic conditions by multiple mechanisms (Semenza, 2010). Induction of HIF-1alpha expression increases the release of several cytokines, including proangiogenic mediators (Hatfield *et al*., 2010). Although HIF-1α is essential for adaptation to low oxygen levels, it has also been shown *in vitro* to mediate hypoxia-induced growth arrest and apoptosis (Goda *et al*., 2003). HIF-1α is also a key component of the cellular response to hypoxia and ischemia under pathophysiological conditions. Hypoxia-ischemia often results in severe brain injury in neonates (Ferriero, 2004). In neonatal brains, hypoxia-ischemia brain damage usually causes cell death through either necrosis or apoptosis. How the cell dies depends on the severity of the injury. Several studies have shown that apoptosis was more frequent in hypoxia-ischemia brain damage (Beilharz *et al*., 1995; Greijir & van der Wall, 2004). Apoptosis resulting from hypoxia is co-regulated by HIF-1α, as well as many other factors (Greijir & van der Wall, 2004). In adult mice lacking HIF-1α in brain cells, investigators found that the main role of HIF-1α in acute hypoxia is proapoptotic (Helton *et al*., 2005). On the other hand, in a rat neonatal stroke model with moderate ischemia–reperfusion, HIF-1α plays a neuroprotective role (Mu *et al*., 2003, 2005). Therefore, HIF-1α functions differently in regulating apoptosis in different animal models, species, or stimulus intensity.

Cyclic AMP responsive element binding protein (CREB)

The cAMP responsive element binding protein is a nuclear protein that modulates the transcription of genes with cAMP responsive elements in their promoters. Increases in the concentration of either Ca^{2+} or cAMP trigger the phosphorylation and activation of CREB. This transcription factor is a component of intracellular signaling events that regulate a wide range of biological functions, from spermatogenesis to circadian rhythms and memory. Evidence from *Aplysia*,

Drosophila, mice and rats show that CREB-dependent transcription is required for the cellular events underlying long-term but not short-term memory (Byrne, 1993). While the work in *Aplysia* and *Drosophila* only involved CREB function in very simple forms of conditioning, genetic and pharmacological studies in mice and rats demonstrate that CREB is required for a variety of complex forms of memory, including spatial and social learning, thus indicating that CREB is a universal modulator of processes required for memory formation (Silva, 1998). Beitner-Johnson and Millhorn (1998) reported that physiological reduction in O_2 levels induces a functional phosphorylation of CREB at Ser133 via a novel signaling pathway.

Neurotransmitter Receptors and their Role in Hypoxia

The brain neurotransmitter receptor activity and hormonal pathways control many physiological functions in the body. The pharmacological challenge strategy involves administering a test agent under controlled conditions to elucidate some aspect of biological or behavioural function in the organism being studied. It is based on the assumption that true functional abnormalities will not be evident in the basal state because of the action of compensatory mechanisms. Under such circumstances, pharmacological perturbation of a specific target system will reveal information about the functional integrity of both that system and systems that modulate it (Lawrence *et al*., 2000). Basing a treatment on symptoms alone (traditional medicine) will not provide the information needed to address the underlying brain imbalance. New sophisticated equipment and tests are now available to evaluate neurotransmitter imbalances using a urine or blood sample. This provides a neurotransmitter baseline assessment and is useful in determining the root causes for many diseases and illnesses. Laboratory analyses provide precise information on brain neurotransmitter deficiencies or overloads as well as detect hormonal and nutrient cofactor imbalances which influence neurotransmitter production. Testing helps to determine exactly which neurotransmitters are out of balance and helps to determine which therapies are

needed for an individualized treatment plan. It also helps in monitoring the effectiveness of an individual's treatment.

Adrenergic Receptors

Adrenergic receptors belong to the large family of G-protein coupled receptors. These receptors form the interface between the sympathetic nervous system as well as many endocrine and parenchymal tissues (Hein & Kobilka, 1995). The adrenergic receptors contain seven stretches of 20-28 hydrophobic amino acids that represent membrane spanning regions. Adrenergic receptors are classified into α- and β- adrenergic receptors.

α_1-adrenergic receptors are activated by epinephrine (EPI) and norepinephrine (NE). α_2-adrenergic receptors mediate many physiological actions of the endogenous catecholamines, EPI and NE are targets of several therapeutic agents. EPI and NE are endogenous amines that are secreted in response to stress; they do not cross the blood-brain barrier. EPI is more potent agonist of β-adrenoceptors than α-adrenoceptors whereas NE is primarily an α-adrenoceptor agonist with some β-adrenergic activity. β-adrenergic receptor stimulation normally results in signaling by the heterotrimeric G_s protein, leading to the activation of adenylate cyclase, production of cAMP and activation of cAMP dependent protein kinase A (PKA).

If hypoxia is not too severe, coronary vasodilation enables myocardial O_2 delivery to balance increased myocardial O_2 demand in spite of reduced arterial O_2 content (Hermann & Feigl, 1992; Martinez *et al*., 2005). The role of sympathetic activation in controlling coronary blood flow during hypoxia is complex, since sympathetic activation directly initiates both vasodilatory and vasoconstrictory mechanisms and indirectly initiates vasodilatory mechanisms. Central α -adrenergic receptor activity is important for fetal adaptation to hypoxia before birth. Endogenous inhibitory α-adrenergic receptor activation after severe hypoxia appears to significantly limit evolving hippocampal damage in the immature brain (Dean *et al*.,

2006). Earlier studies reported an up regulation of β-adrenergic receptors during neonatal hypoxia while α_2-adrenergic receptors were down regulated. The up regulation was through the activation of cAMP pathway (Finla, 2007).

Glutamate Receptors

The majority of excitatory synapses are glutamergic, in which glutamate transmits the signal through postsynaptic ionotropic N-methyl-D-aspartic acid (NMDA), amino-3-hydroxy-5-methysoxazole-4-propionic acid (AMPA) and kainate (KA)] and metabotropic receptors (Bettler & Mulle, 1995). Glu is a fast excitatory transmitter in the CNS and has been shown, with GABA, to interact primarily with receptors in the synaptic cleft (Dingledine *et al*., 1999). The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA–KA (Choi, 1988). The presence of G-protein coupled glutamate receptors (metabotropic Glu receptors) has been described. The metabotropic glutamate (mGlu) receptors are a family of eight G protein-coupled receptors that modulate cell excitability and synaptic transmission in the nervous system. Group I mGlu receptors stimulate release of Ca^{2+} from intracellular stores, which then modulates many signaling pathways, including those coupled to multiple receptor systems. Another type of interaction occurs at the second messenger level, where synergistic signaling is stimulated with simultaneous activation of receptors.

Glutamate functions as a fast excitatory transmitter in the mammalian brain. Recent experiments in a variety of preparations have shown that either blockade of synaptic transmission or the specific antagonism of postsynaptic glutamate receptors greatly diminishes the sensitivity of central neurons to hypoxia (Rothman & Olney, 1986). Glutamate triggers neuronal death when released in excessive concentrations by over excitation of its receptors (Vizi, 2000). Cell death due to excitotoxicity occurs in many types of cells in the newborn brain and the initial trigger will be impairment

of the uptake of glutamate by glia, resulting in over activation of the receptors (McDonald & Johnston, 1990). It is reported that any sort of disturbances in the metabolic pathway of glutamate causes physiological and cognitive disorders (Preetha *et al*., 1996). Neurodegeneration by the glutamate receptor activation was reported to mediate motor dysfunction under hypoglycemia (Anu *et al*., 2010).

Hypoxia increases GABA levels in neurons by ATP depletion-induced activation of glutamate decarboxylase and by inhibiting GABA transaminase. Hypoglycemia, which also depletes ATP, reduces neuronal levels of GABA and its precursor Glu (Madl & Royer, 2000). Under hypoxia or ischemia, the release of aspartate, glutamate, glycine, alanine, taurine and GABA increased mainly by a Ca^{2+}-independent mechanism. However, ischemia highly potentiated the reduction of the energy charge, as compared with hypoglycemia or hypoxia alone. Addition of glucose metabolites, pyruvate and malate, attenuated neuronal death after exposure to glutamate or H_2O_2 (Desagher *et al*., 1997; Ruiz *et al*., 1998).

GABA Receptors

Gamma- aminobutyric acid (GABA) was discovered over 40 years ago as a key inhibitory neurotransmitter in the brain (Bazemore *et al.*, 1957; Krnjevic & Phillis, 1963). Since then, evidence has accumulated that this amino acid function as a neurotransmitter not only in the CNS but also in the peripheral nervous system, including the mesenteric plexus (Amenta, 1986), major pelvic ganglia (Akasu *et al*., 1999), sympathetic ganglia, encompassing the rat superior cervical ganglion (Kasa *et al*., 1988; Wolff *et al*., 1986) and abdominal prevertebral ganglia (Parkman & Stapelfeldt, 1993). In the mammalian central nervous system, GABA is the most important inhibitory neurotransmitter occurring in 30-40% of all synapses. Three types of GABA receptors have been identified: $GABA_A$ and $GABA_C$ receptors are ligand-gated Cl^- channels, while $GABA_B$ receptors are G-protein coupled (Chebib & Johnston, 1999). $GABA_A$ receptors are ligand gated Cl^- channels that consist of a

heteromeric mixture of protein subunits forming a pentameric structure and $GABA_B$ receptors couple to Ca^{2+} and K^+ channels *via* G-proteins and second messengers (Johnston, 1996). In the CNS, application of GABA reduces excitability by a combination of $GABA_A$ and $GABA_B$ receptor activation, leading to membrane re-polarization, reduced Ca^{2+} influx and suppression of neurotransmitter release. $GABA_A$ receptors are composed of five subunits from seven different subunit families with multiple subtypes (α1–6, β1–3, γ1–3, δ, ε, θ, π) that form a ligand-gated chloride ion channel.

The ventilatory response to hypoxia is influenced by the balance between inhibitory (GABA, glycine and taurine) and excitatory (glutamate and aspartate) amino acid neurotransmitters. GABA and glutamate are the two important neurotransmitters involved in Hypoxic Ventilatory Response. Decrease in ventilation during hypoxia in neonates is mediated through the effects of GABA on central nervous system. Decreased GABAergic function was reported under various stressful conditions like hypoglycemia and hyperglycemia (Antony *et al*., 2010) and CNS dysfunction like epilepsy (Mathew *et al*., 2010) which account for the increased vulnerability of brain to neuronal damage and motor learning deficits. GABA in the nucleus tractus solitarii has a pivotal role in the hypoxic ventilatory decline (HVD) and this mechanism is not activated without chemoreceptor stimulation (Tabata *et al*., 2001). Tissue, perfused with artificial cerebrospinal fluid at 37°C with zero glucose and gassed with 95% nitrogen and 5% carbon dioxide, showed a five fold increase in glutamate release with little effect on GABA release. Pre-conditioning with three 5min periods of hypoxia/hypoglycemia preceding continuous hypoxia/hypoglycemia, significantly decreased glutamate release while significantly elevating GABA release. These results suggest that GABA reduce the release of glutamate and consequently decrease the neurotoxic effects of glutamate (Johns *et al*., 2000). Accumulation of GABA in hypoxic conditions results from sustained activity of glutamic acid decarboxylases (GAD), the enzymes that convert glutamate to GABA, but there is

concurrent inhibition of GABA breakdown by GABA transaminase A (GABA-T) (Hoop *et al*., 1999; Xia & Haddad, 1992).

$GABA_A$ Receptor:

$GABA_A$ receptors are pentameric in structure, with the five subunits arranged like spokes of a wheel around a central Cl^- selective pore (Barnard, 2001). Nineteen GABA receptor subunits have been cloned from rats, which include α1–6, β1–3, γ1–3, ρ1–3, δ, θ, ε, and π (Whiting *et al*., 1999). The 19 subunits are encoded by 19 distinct genes. Each subunit has four transmembrane segments, with both the amino and carboxy termini located extracellularly. These extracellular segments form the recognition sites, two per channel, for GABA and also, in some channel types, the recognition site, one per channel, for benzodiazepine-like allosteric modulators. The genetic diversity of multiple $GABA_A$ receptor subunits permits the assembly of a vast number of receptor heteromeric isoforms. Apparently, the subunit composition determines the pharmacological profile of the resulting receptor subtypes (Barnard *et al*., 1998). Mechanisms that modulate the stability and function of postsynaptic $GABA_A$ receptor subtypes and that are implicated in functional plasticity of inhibitory transmission in the brain are of special interest (Luscher & Keller, 2004). Modification of $GABA_A$ receptor function has been implicated in a range of hypoxia-related pathologies, including encephalopathy (Low *et al*., 1985), seizures (Bergamasco *et al*., 1984) and myoclonus (Hallett, 2000).

$GABA_AR$ binding in gerbil hippocampus was reduced after hypoxia, which was thought to result from receptor internalization (Alicke & Schwartz-Bloom, 1995). It is reported that prolonged exposure to hypobaric hypoxia transiently reduces $GABA_A$ receptor number in mice cerebral cortex (Viapiano *et al.*, 2001). GABA-mediated currents were reduced in CA1 pyramidal neurons in hippocampal slices exposed to hypoxia both *in vivo* and *in vitro* (Xu & Pulsinelli, 1994; Congar *et al*., 1995). Decreased $GABA_AR$ current in cultured hippocampal neurons subjected to

experimental ischemia was attributed to depletion of ATP and increased intracellular Ca^{2+} (Harata *et al*.,1997). Within 1 h after cerebral ischemia, GABA-gated Cl^- flux dramatically decreased in gerbil neurons (Verheul *et al*., 1993). Intracellular Cl^- in rat hippocampal slices was increased early after ischemia, resulting in reduced Cl^- driving force and $GABA_A$ response (Inglefield & Schwartz-Bloom, 1998).

$GABA_B$ Receptor:

The $GABA_B$ receptor is part of the class C of GPCRs that also includes the mGlu, the Ca^{2+}-sensing and the sweet and umami taste receptors among others (Pin *et al.,* 2003). These receptors are dimers, either homodimers linked by a disulphide bond (mGlu and Ca^{2+}-sensing receptors) or heterodimers made of two similar, but distinct subunits (the $GABA_B$ and taste receptors). Indeed, the $GABA_B$ receptor was the first G protein coupled receptor to be identified that requires two distinct subunits to function: the $GABA_{B1}$ and $GABA_{B2}$ subunits (Jones *et al*., 1998; Kaupmann *et al*., 2003; White *et al*., 1998). Although the $GABA_{B1}$ subunit was soon shown to bind all known $GABA_B$ ligands (both agonists and antagonists) this protein did not form a functional $GABA_B$ receptor when expressed alone (Kaupmann *et al*., 1997). Endogenous GABA acting on $GABA_A$ or $GABA_B$ receptors modulates ventilation during room air breathing as well that the ventilatory response to acute and sustained hypoxia (Zhang *et al*., 2002). Rhythm generation in mature respiratory networks is influenced strongly by synaptic inhibition. Zhang *et al* (2002) reported that $GABA_B$-receptor-mediated postsynaptic modulation plays an important role in the respiratory network from post-natal day zero (P_0) on. $GABA_B$-receptor-mediated presynaptic modulation develops with a longer postnatal latency and becomes predominant within the first postnatal week (Suzuki *et al*., 1999). $GABA_B$ receptors may contribute essentially to the modulation of respiratory rhythm in adult mammals and may be involved in the control of respiratory neuronal discharge (Ai-Lun Yang *et al*., 2007). In the elevated plus maze, the agonist of GABA-B receptor was reported to improve

consolidation of passive avoidance in rats undergoing hypoxia (Car *et al*., 2001). $GABA_B$ receptor-mediated activation of TASK-1 or a related channel provides a presynaptic autoregulatory feedback mechanism that modulates fast synaptic transmission in the rat carotid body (Ian *et al*, 2003).

$GABA_C$ Receptors:

$GABA_C$ receptors, which are a subfamily of $GABA_A$ receptors, are members of the Cys-loop superfamily of ligand-gated ion channels (LGICs), an important group of receptors involved in rapid synaptic transmission and whose malfunction results in a variety of neurological disorders; hence, understanding their mechanism of action is of considerable pharmacological interest. $GABA_C$ receptors are mostly located in retinal neurons where they play a role in retinal signaling involved in diseases such as macromolecular degeneration (Bormann, 2000). The receptors are activated by the binding of GABA, the main inhibitory neurotransmitter in the central nervous system. $GABA_C$ receptors have distinct pharmacological properties from $GABA_A$ receptors, e.g., they are not inhibited by bicuculline, the classic $GABA_A$ receptor antagonist (Barnard *et al*., 1998; Chebib *et al*., 2000). Like all the LGICs belonging to the Cys-loop superfamily, $GABA_C$ receptors are composed of five subunits arranged in a pentagonal array around a central ion-permeant pore. Each subunit has an extracellular N-terminal domain (ECD), a transmembrane domain composed of four α-helices and an intracellular domain. Three subunits (ρ_{1-3}) have been identified; these all form functional homomeric or heteromeric receptors (Enz, 2001).

Glutamic Acid Decarboxylase

GABA the main inhibitory neurotransmitter in the brain is synthesized by glutamic acid decarboxylase (GAD). GAD exists in two isoforms termed GAD65 and GAD67 due to their molecular weights of 65 and 67 kDa, respectively. These enzymes are the products of two independently regulated genes sharing 65% sequence

homology in rats (Erlander *et al*., 1991[a]; 1991[b]). Most GABAergic interneurons express both subtypes of GAD (Esclapez *et al*., 1994; Houser & Esclapez 1994) which are simultaneously detectable in the rat brain as early as embryonic day 17 (Dupuy & Houser, 1996). GAD67 is found in axonal regions as well as in neuronal cell bodies, whereas GAD65 is mainly associated with synaptic terminals (Kaufman *et al*., 1991). Therefore it has been suggested that GAD67 mostly provides a pool of GABA for general metabolic activity while GABA synthesized by GAD65 is likely to be more involved in synaptic transmission (Martin & Rimvall, 1993). Mice lacking GAD65 are vital and do not exhibit changes in their brain GABA content though they have an increased susceptibility to seizures (Asada *et al*., 1996; Kash *et al*., 1997).

Adenosine Receptors

Adenosine is a powerful modulator of neuronal function, which mainly decreases the release of excitatory neurotransmitters and neuronal firing through the activation of inhibitory A1 receptors (Dunwiddie & Masino, 2001). Since adenosine is released in particularly high amounts in noxious situations, adenosine is conceived as an important endogenous neuroprotective agent against different noxious insults to the brain (de Mendonça *et al*., 2000). Adenosine, a purine nucleoside, is a neuromediator involved with many inhibitory mechanisms and thus regulates brain metabolism (Winn *et al*., 1981). Intracerebral concentrations of adenosine rise during hypoxia and are associated with increase in local cortical blood flow, decrease in whole-body oxygen consumption, reduction in T_b and protection against cerebral damage (Barros & Branco, 2000; Blood *et al*., 2003; Karimi *et al*., 1996; Koos *et al*., 1997; Rudolphi *et al*., 1992). Studies in anesthetized rats and in awake cats and lambs showed that adenosine is critically involved in the hypoxic ventilatory decline (Koos *et al*., 2004; Long & Anthonisen, 1994; Neylon & Marshall, 1991).

Blood *et al*., (2003) reported that in the near term foetal sheep, adenosine mediates a decrease of cerebral metabolic rate during acute moderate hypoxia *via* the adenosine A_1 receptor activation. Furthermore, an inhibition of adenosine A_1 receptors during severe asphyxia resulted in an increased neuronal cell death accompanied by delayed suppression of neural activity and increased cerebral metabolism (Hunter *et al*., 2003). Adenosine inhibits the evoked release of many neurotransmitters, both from peripheral nerves and in the CNS. The inhibitory effect of adenosine on NE (Fredholm & Dunwiddie, 1988) and ACh (Sperlágh *et al*., 1997) release has been particularly well described and proved to be mediated by adenosine A_1 receptors. Adenosine A_1 receptors have the general structure expected of G-protein-linked receptors and there is evidence that G_i proteins are involved in the inhibitory effects of adenosine on neurotransmitter release, inhibiting cAMP production and N-type Ca^{2+} channels and activating K^+ permeability. In addition, there is some evidence that the activation of high-affinity adenosine A_{2A} receptors increases the release of different transmitters (Cunha *et al*., 1994; Gu & MacDermott, 1997; Sebastiao & Ribeiro, 1992) and has an effect on G_s protein and subsequently increases cAMP level. In contrast, its stimulation reduces the release of GABA from the recurrent collaterals of striatopallidal neurons (Kirk & Richardson, 1994).

Serotonin Receptors

5-HT receptors comprise a complex family. On the basis of their pharmacology, signal transduction mechanisms and molecular structure, more than a dozen types of 5-HT receptors have been identified (Hoyer *et al.,* 1994). Most of these receptors are coupled to various G proteins with the exception of the 5-HT3 receptor, which is a ligand gated cation channel (Derkach *et al.,* 1989; Maricq *et al.,* 1991). Multiple 5-HT receptor subtypes are expressed in the cerebral cortex (Mengod *et al.,* 1996). In cerebral cortex, $5\text{-}HT_3$ receptors are only expressed in inhibitory neurons (Morales & Bloom, 1997) whereas $5\text{-}HT_{2A}$ receptors are heavily expressed in

pyramidal cells and to a lesser extent in inhibitory neurons (Hamada *et al.,* 1998; Jakab & Goldman-Rakic 1998; Willins *et al.,* 1997). Since the 1960s, many experiments using *in vivo* microiontophoretic methods have characterized how 5-HT affects neuronal behaviour. The predominant effect of 5-HT on cerebral cortical pyramidal neurons is an inhibition of spontaneous spiking. (Jacobs & Azmitia, 1992; Phillis, 1984; Reader & Jasper, 1984). Intracellular studies in rat cortical slices suggested that 5-HT induces depolarization and action potential firing in pyramidal cells (Araneda & Andrade, 1991; Davies *et al.,* 1987; Tanaka & North 1993). Furthermore, Aghajanian & Marek (1997) reported that 5-HT enhances spontaneous excitatory postsynaptic currents (sEPSCs) without significantly changing spontaneous inhibitory postsynaptic currents (sIPSCs) in frontal pyramidal neurons. These *in vitro* results suggest that 5-HT is mainly excitatory in cortical neuronal circuitry. 5-HT and a-methyl-5-HT had no effect on sEPSCs in layer I neurons. Even though sampling bias might have contributed to this observation, the fact that activation of 5-HT_{2A} receptors induced robust enhancement of sEPSCs in all pyramidal neurons tested suggests that this differential modulation of sEPSCs in the two cell types was real. 5-HT_{2A} receptor expression is high in pyramidal neuron proximal apical dendrites and low in distal parts (Jakab & Goldman-Rakic, 1998; Willins *et al.,* 1997). It is possible that activation of dendritic 5-HT_{2A} receptors induce dendritic transmitter release and/or release of retrograde messenger(s).

During brain development, serotonin provides essential neurotrophic signals (Justin *et al*., 2004). 5-HT is known to play an important role in several physiological functions (Jackson & Paulose, 2000). A root cause of sudden infant death syndrome (SIDS) is due to disturbances of serotonin levels in key pacemaker cells in the brain. In babies, the normal response to hypoxia is to gasp, which wakes the baby and resets the breathing mechanism. That reflex, which kicks in when a baby isn't getting enough oxygen for any reason, is governed by a set of pacemaker neurons in the respiratory neural network (Tryba *et al*., 2006). 5-HT is one of many vasoactive

substances postulated to participate in the development of hypoxia-induced pulmonary hypertension. Pulmonary vasoactive responses to hypoxia are intensified by 5-HT (Eddahibi *et al*., 1997). Several subtypes of signal transducing 5-HT receptors have been characterized pharmacologically and cloned. Depending on their subtype, these receptors act on G-proteins and thereby activate phospholipase C or adenylate cyclase (Fanburg & Lee, 1997). By analogy with other signaling molecules, it is generally assumed that these receptors operate at the cell surface, without necessarily mediating the uptake of 5-HT. In addition, 5-HT is internalized into a variety of cell types, including platelets, neurons, mast cells, endothelial cells and smooth muscle cells, through an active transport mechanism that is powered by a transmembrane Na^+/Cl^- gradient (Junod, 1972).

5-HT$_{2A}$ Receptors:

Phrenic long-term facilitation (LTF) following acute intermittent hypoxia is a form of serotonin-dependent respiratory plasticity that requires 5-HT_{2A} receptor activation, new BDNF synthesis and activation of its high affinity receptor, TrkB (Baker-Herman *et al*., 2004). 5HT modulates the dynamics of the hypoxic sensory response via its action on 5-HT_2 receptors (Serrano *et al*, 2003). Abrea *et a*l (2007) reported that 5-HT_{2A} receptors affect body temperature, ventilation and metabolism in hamsters. The role of $5HT_{2A}$ in glucose homeostasis and insulin secretion was reported from the studies in diabetic rats (Abraham *et al*., 2010[a, b])

Acetylcholine Receptors

Acetylcholine is one of the principal neurotransmitters of the parasympathetic system. Extensive evidence supports the view that cholinergic mechanisms modulate learning and memory formation. Evidence for cholinergic regulation of multiple memory systems, noting that manipulations of cholinergic functions in many neural systems enhance or impair memory for tasks generally associated with those neural

systems. The magnitude of ACh release in different neural systems regulate the relative contributions of these systems to learning. ACh is the neurotransmitter that is released by stimulation of the vagus nerve, which alters heart muscle contractions. It is important for the movement of other muscles as well. ACh induces movement by the locomotion of an impulse across a nerve that causes it to release neurotransmitter molecules onto the surface of the neighbouring cell. ACh is critical for an adequately functioning memory. Studies of ACh release, obtained with *in vivo* microdialysis samples during training, together with direct injections of cholinergic drugs into different neural systems, provide evidence that release of ACh is important in engaging these systems during learning and the extent to which the systems are engaged is associated with individual differences in learning and memory (Paul, 2003).

Hypoxia impairs brain function by incompletely defined mechanisms. Mild hypoxia, which impairs memory and judgment, decreases ACh synthesis, but not the levels of ATP or the adenylate energy charge. The decreases in glucose incorporation into ACh and into the amino acids with hypoxic hypoxia (15% or 10% O_2) or hypoxic hypoxia with 5% CO_2 were very similar to those with the two lowest levels of anaemic hypoxia. Thus, any explanation of the brains' sensitivity to a decrease in oxygen availability must include the alterations in the metabolism of the amino acid neurotransmitters as well as ACh (Gibson & Peterson, 1981).

Muscarinic Receptors

There are five subtypes of muscarinic ACh receptors (M1–M5) which belong to the superfamily of G-protein-coupled receptors. M1, M3, M5 receptors are mainly coupled to the Gq/11 protein which activates phospholipase C. Muscarinic M2 and M4 receptors are mainly coupled to the Gi/o protein, which inhibits adenylate cyclase.

There are at least three muscarinic receptor subtypes, M_1, M_2 and M_3 involved in the modulation of transmitter release (Caulfield, 1993; Caulfield & Birdsall, 1998).

This receptor diversity to some extent explain the diverse range of signal transduction mechanisms; these include inhibition of Ca^{2+} influx (Allen & Brown, 1993, 1996), adenylyl cyclase, stimulation of guanylyl cyclase, activation of phospholipase C, direct inhibition of Ca^{2+} channels and activation of K^+ channels (Felder, 1995). There is reasonably good evidence that the muscarinic M_2 receptors expressed on cholinergic (Allen & Brown, 1996; Aubert *et al*., 1995) and noradrenergic varicosities play a physiologically important role in the modulation of neurotransmitter release. The muscarinic receptors that inhibit NE release appear to be of the muscarinic M_2 subtype in the periphery and CNS. In contrast, there are muscarinic receptors, apparently of the muscarinic M_1 subtype, that increase the release of NE (Raiteri *et al*., 1990[a, b]) expressed on noradrenergic axon terminals in the periphery. The muscarinic M_1 receptor is generally coupled to PTX-insensitive G-protein. Its activation results in formation of inositol trisphosphate and diacylglycerol. In contrast, the muscarinic M_2 receptor is coupled *via* PTX-sensitive G-protein to the N-type Ca^{2+} channel (Hille, 1992). The relative importance of these inhibitory and stimulatory muscarinic receptors vary in noradrenergic neurons from different locations. Cholinergic regulation of glucose utilization and cognitive functions was established in diabetic (Peeyush *et al*., 2010) and hypoglycemic (Anthony *et al*., 2010) conditions.

Muscarinic M1 Receptors:

Muscarinic M1 receptors are predominantly expressed in the forebrain, including the cerebral cortex, hippocampus and corpus striatum, where this sub-type contributes by 50-60% to the total of the muscarinic receptors (Gerber *et al*., 2001; Hamilton *et al*., 1997; Miyakawa *et al*., 2001). The muscarinic M1 receptor subtype, which is also expressed in peripheral tissues, has been implicated in stress adaptive cardiovascular reflexes and central blood pressure control. Studies have shown that central administration of the muscarinic M1 specific antagonist pirenzepine lowered the blood pressure (Brezenoff & Xiao, 1986; Buccafusco, 1996). A putative

overexpression of the muscarinic M1 subtype in selected brain areas of spontaneously hypertensive rats has been reported (Scheucher *et al*., 1991). Muscarinic agonist depolarisation of rat isolated superior cervical ganglion is mediated through muscarinic M1 receptors (Brown *et al*., 1980). Signaling through muscarinic M1 and M3 AChRs promote accumulation and transcriptional activation of HIF-1α. Muscarinic acetylcholine signals activate HIF-1 by both stabilization and synthesis of HIF-1α and by inducing the transcriptional activity of HIF-1α (Hirota *et al*., 2004). Perinatal hypoxia leads to an altered pulmonary circulation in adulthood with vascular dysfunction characterized by impaired endothelium-dependent relaxation and M1 AChR plays a predominant role. This implies that muscarinic receptors are key determinants in pulmonary vascular diseases in relation to perinatal imprinting (Peyter *et al*., 2008).

Muscarinic M2 Receptors:

Muscarinic M2 receptors mediate both negative and positive ionotropic responses in the left atrium of the reserpinized rat, latter effect being insensitive to pertusis toxin (Kenakin & Boselli, 1990). Central cholinergic transmission is activated by inhibition of the presynaptic M2 acetylcholine autoreceptor using selective antagonists. The presynaptic M2 autoreceptor negatively influences the release of acetylcholine in several brain regions, including the striatum, hippocampus and cerebral cortex (Billard *et al*., 1995; Kitaichi *et al*., 1999; Zhank *et al*., 2002). A direct consequence of brain muscarinic M2 autoreceptor inhibition is an elevation of acetylcholine release in the synaptic cleft. Methoctramine and other M2 receptor antagonists have been shown to enhance the release of acetylcholine in different brain structures (Stillman *et al*., 1993; Stillman *et al.*, 1996). Muscarinic receptor activation in guinea pig heart produces a reduction in force of contraction and a decrease in the rate of beating. These effects are probably the consequence of inhibition of voltage-gated Ca^{2+} channels and activation of inwardly rectifying K^{+} channels, respectively.

Extensive studies with many antagonists have defined this response as being mediated by the muscarinic M2 receptors (Caulfield, 1993).

Muscarinic M3 Receptors:

Muscarinic M3 receptors are broadly expressed in the brain, although the expression level is not high, compared to those of the muscarinic M1 and M2 receptors (Levey, 1993). Muscarinic M3 receptor also triggers direct contractions of smooth muscle, however, it only represents a minor fraction of total muscarinic receptor population in smooth muscle. It is expressed in relatively low density throughout the brain. Studies using knock out mice for muscarinic M3 receptors gave evidences for the primary importance of these receptors in the peripheral cholinergic system. In urinary bladder, pupillary muscles and intestinal smooth muscles the cholinergic contractions are mediated predominately through muscarinic M3 receptors (Matsui *et al*., 2000).

Muscarinic M4 Receptors:

Muscarinic M4 receptor is known to be abundantly expressed in the striatum (Levey, 1993). Muscarinic M4 receptors act as inhibitory muscarinic autoreceptors in the mouse (Zhang *et al*., 2002). Inhibition of adenylyl cyclase activity by muscarinic agonists in rat corpus striatum is mediated by muscarinic M4 receptors (Caulfield, 1993; Olianas *et al*., 1996).

Muscarinic M5 Receptors:

Muscarinic M5 receptor was the last muscarinic acetylcholine receptor cloned. Localisation studies have revealed that the M5R is abundantly expressed in dopamine-containing neurons of the substantia nigra par compacta, an area of the midbrain providing dopaminergic innervation to the striatum. Concordantly, oxotremorine-mediated dopamine release in the striatum was markedly decreased in

M5R-deficient mice. More intriguingly, in M5R-deficient mice, acetylcholine induced dilation of cerebral arteries and arterioles was greatly attenuated (Yamada *et al*., 2001), suggesting that the muscarinic M5 receptor is suitable target for the treatment of cerebrovascular ischemia. Muscarinic M5 receptor subtype is expressed at low levels in the brain (Hosey, 1992; Hulme *et al*., 1990).

Developmental Changes due to Hypoxia

Hypoxia occurs when oxygen availability drops below the levels necessary to maintain normal rates of metabolism. Because of its high metabolic activity, the brain is highly sensitive to hypoxia. Severe or prolonged oxygen deprivation in the brain contributes to the damage associated with stroke and a variety of other neuronal disorders. Conversely, the extreme hypoxic environment found in the core of many brain tumours supports the growth of the tumour and the survival of tumour cells. Normal cells exposed to transient or moderate hypoxia are generally able to adapt to the hypoxic conditions largely through activation of the HIF. HIF-regulated genes encode proteins involved in energy metabolism, cell survival, erythropoiesis, angiogenesis and vasomotor regulation. In many instances of hypoxia and ischemia, the induction of HIF target genes is beneficial. When these same insults occur in tissues that are normally poorly vascularized, such as the retina and the core of solid tumours, induction of the same HIF target genes promote disease. Major new insights into the molecular mechanisms that regulate the oxygen-sensitivity of HIF and in the development of compounds with which to manipulate HIF activity are forcing serious consideration of HIF as a therapeutic target for diverse CNS disorders associated with hypoxia (Freeman & Barone, 2005).

From birth, exposure to a single hypoxic stimulus of 10/15 min duration induces an early peak in minute ventilation, followed by a reduction (roll-off), or hypoxic ventilatory depression. During early development, ventilatory responses to hypoxia tend to have a lower initial peak and more rapid decline compared to older

animals of the same species (Bureau *et al*., 1986; Carroll & Bureau, 1987; Mortola, 1999). The initial cardiac and ventilatory responses are stimulated by the peripheral, carotid body (CB) chemoreceptors (Carroll & Bureau, 1988). The time course of this response is short, with the peak response occurring within 1/4 min in most studies.

The increase in minute ventilation (˙V/E) is initially due to an increase in tidal volume (VT) and respiratory frequency (f). The time course of this response is short, with the fall-off in respiratory frequency occurring within approximately 5 min. The subsequent decrease is due to a decrease in VT despite increased f in conscious animals, but a decrease in f contributes to the decrease in ˙V/E in anaesthetised animals (Bonora *et al*., 1994). Tidal volume changes little in response to hypoxia in the neonate and early maturational changes primarily result in an improved ability to sustain f during hypoxia. Indeed, in pre-term infants there is a rapid fall in f and poorly sustained increases in f have also been observed in pre-term infants at 4/8 weeks of age (Carroll *et al*., 1993; Martin *et al*., 1998). Other species, such as piglets, show sustained or progressive increase in f during sustained hypoxia (Mortola *et al*., 1989; Scott *et al*., 1990). In the early postnatal period, ˙V/E fall below the baseline values that preceded the stimulus, when moderate hypoxia is sustained, so the respiratory response is termed biphasic.

Role of Glucose in Regulating Energy Demand

Glucose is the major source of energy for organ function. In the human foetus, oxidation of glucose accounts for approximately 80% of foetal oxygen consumption, demonstrating that glucose is the major substrate for foetal oxidative metabolism (Jane & McGowan, 1999). Reports say that there occurs a close association between hypoxia and the emergence of glucose intolerance, but the experimental evidence of a causative role for hypoxia in this metabolic dysfunction is lacking (Oltmanns *et al*., 2004). Hypoxic respiratory diseases are frequently accompanied by glucose intolerance. One of the factors mediating this effect could be an elevated release of

epinephrine (Kerstin *et al*., 2004). The cerebral metabolic rate for glucose (CMRGlu) increased 70-80% after 2 min of hypoxia but then returned to nearly the normal rate by the end of the 30-min period of hypoxia. Glycolytic flux appeared to be facilitated in both groups initially but was inhibited as the hypoxic period continued. This slowing of glycolysis after 15 or 30 min of hypoxia appears to be modulated by the regulatory enzyme phosphofructokinase. A significant amount of the glucose entering the brain during the posthypoxic period appears to be used for metabolite synthesis rather than energy production (Kintner *et al*., 1983). Hypoxic-ischemic insult in the perinatal period in humans is a significant risk factor for the development of epilepsy later in life. Hypoxia is a leading cause of neonatal encephalopathy and is frequently associated with seizures (Jensen *et al*., 1991).

Role of ATP in Regulating Energy Demand

As a major consumer of energy, the brain is very susceptible to the effects of hypoxia, especially those parts of the brain – such as the hippocampus – that are crucial for cognitive function. There is no irreversible loss of neuronal/synaptic function, as long as nerve cells have an adequate supply of glucose and ATP (from anaerobic glycolysis) to maintain the minimal Na^+ -K^+ pump activity and protein synthesis essential for cell survival. These conditions are not met when both oxygen and glucose are deficient, as in strokes. Then the cell's protective mechanisms cannot cope with massive Ca^{2+} influx and it succumbs to the deleterious effects of Ca^{2+} overload (Krešimir, 1999). Of the approximately 130 million infants born worldwide each year, it is estimated that four million infants die during the first month of life. In animals, hypoxia is signalled at three levels: an immediate systemic response which involves central and peripheral chemoreceptors, an immediate/chronic gene response initiated by cellular oxygen signals and an immediate emergency or crisis response signalled by changes in energy metabolite concentrations (Peter & Howard, 2002).

ATP is a fast transmitter in sympathetic ganglia and at the sympatho-effector junction. In primary cultures of dissociated rat superior cervical ganglion neurons, ATP elicits noradrenaline release in an entirely Ca^{2+}-dependent manner. Nevertheless, ATP-evoked noradrenaline release was only partially reduced (by ~50%) when either Na^{+} or Ca^{2+} channels were blocked, which indicates that ATP receptors themselves mediated transmembrane Ca^{2+} entry (Stefan, 1999).

All kinds of biochemical reactions are linked to energy transfer, therefore each physiological function, as well as each pathological disorder or therapy, must have a consequence for biological energy. The adaptive changes related to hypoxia or energy deficit have been divided into defense and rescue phases. The defense phase occurs immediately after a decline in oxygen and consists of channel arrest, decreased Na^{+}/K^{+}-ATPase activity, urea synthesis, gluconeogenesis, protein synthesis and proteolysis (a highly ATP-consuming process), in such a way that ATP demand equals ATP production. Then the rescue phase involves transcriptional effects, HIF-mediated activation of genes for sustained survival at low ATP turnover (increased glycolytic enzymes, decreased enzymes involved in aerobic-linked metabolism) and finally production of tertiary cell signalling messengers - fos and jun. The consequences of cellular deficit and the mechanisms underlying adaptation to this situation can be understood from the results of numerous studies, both in hypoxia and in ischemia. Such adaptations must rely on a permanent adjustment between energy demand and ATP synthesis (Stefan, 1999).

Effect of Hyper Oxygenation

Oxygen availability plays a pivotal role in many cellular processes and therefore it is not surprising that most biological systems elaborate a variety of mechanisms for sensing oxygen and maintaining pO_2 homeostasis (Lopez-Barneo *et al*., 2001; Semenza, 1999). In neuronal cells, responses to a decrease in oxygen availability or hypoxia include both facilitation and inhibition of neurotransmitter

release (Gibson & Peterson, 1981; Gibson *et al*., 1991). For example, hypoxia increase catecholamine releases (Hirsch & Gibson, 1984) or inhibits acetylcholine release (Freeman *et al*., 1987; Gibson & Peterson, 1981) from brain cells. In a peripheral chemosensory organ, the mammalian carotid body, hypoxia stimulates catecholamine release from specialized O_2-chemoreceptor (glomus) cells, whether present in the intact organ (Donnelly, 1993; Fidone *et al*., 1982), in tissue slices (Pardal *et al*., 2000) or as isolated cells or cell clusters *in vitro* (Jackson & Nurse, 1997; Montoro *et al*., 1996; Urena *et al*., 1994). Hypoxia also stimulates catecholamine release from neonatal adrenal chromaffin cells (Mojet *et al*., 1997; Thompson *et al*., 1997) and from PC-12 cells, an O_2-sensitive cell line derived from the adrenal medulla (Kumar *et al*., 1998; Taylor *et al*., 2000). In particular, hypoxia causes inhibition of K^+ channels, leading to increased membrane depolarization or action potential frequency, entry of extracellular calcium and amine secretion (Lopez-Barneo *et al*., 2001).

When blood supply and oxygen become compromised, local neurons die or become damaged in a pattern consistent with the injury. In this immediate area where blood and oxygen loss has occurred, the neurons die quickly. The surrounding neurons also react to the decreased oxygen levels by shutting down to conserve energy in an attempt to survive. This often results in an exaggeration of the symptoms experienced by brain-damaged patients. Presently, there is little information available on whether resuscitation using room air is equal to or even better than that using 100% oxygen (Nong *et al*., 2000). Newborns and particularly pre-term infants are at high risk of oxidative stress and they are easily susceptible to free radical oxidative damage. While no known treatments are yet able to resuscitate dead neurons, hyperbaric oxygen therapy (HBOT) serves to re-oxygenate the dormant neurons and restore a portion of their previous activity (Satoskar *et al*., 1997). The clinical settings in which oxygen toxicity occurs are broadly divided into two groups; one is in which the patient is exposed to very high concentrations of oxygen for short duration, like in

HBOT and the second is in which lower concentrations of the oxygen are used but for longer duration. These two can result in the so called ‘acute’ and ‘chronic’ oxygen toxicity, respectively (Edmonds *et al*., 1992). The acute toxicity has predominant CNS effects, while chronic toxicity has predominant pulmonary effects (Clark, 1982). Hyperbaric medicine is considered extremely safe under appropriate supervision and utility. Toxic effects of oxygen are observed at extremely high doses over prolonged periods. Hyperbaric oxygen treatment increases the relative dose of oxygen; thus susceptible patients need to be recognized and modifications made to prevent the manifestations of oxygen toxicity. Oxygen derived free radicals had been suggested by Gerschman *et al*., (1954) as being the probable aetiological factor in the development of these toxic effects. Oxygen free radicals are reactive species that although crucial to normal biological processes can lead to injury and cell death. They are implicated in the pathogenesis of many neonatal diseases such as perinatal asphyxia, bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis, intracranial haemorrhage, pulmonary hypertension and persistence of ductus arteriosus. Birth is associated with transition to a hyperoxic environment in comparison with uterine environment which leads to increased generation of free radicals. The newborn has undeveloped antioxidant systems and therefore at increased risk of free radical oxidative injury. The understanding of neonatal factors involved in the pathogenesis of "oxygen free radical diseases" will lead to the development of new therapies for prevention and treatment of these neonatal diseases (Rodrigues, 1998).

The key to successful neonatal resuscitation is establishment of adequate ventilation. Reversal of hypoxia, acidosis and bradycardia depends on adequate inflation of fluid-filled lungs with air or oxygen (de Burgh Daly, 1979, 1986). Although 100% oxygen has been used traditionally for rapid reversal of hypoxia, there is biochemical evidence and preliminary clinical evidence to argue for resuscitation with lower oxygen concentrations (Ramji *et al*., 1993; Rootwelt *et al*., 1993). Current clinical data, however, is insufficient to justify adopting this as routine practice. If

assisted ventilation is required, deliver 100% oxygen by positive-pressure ventilation. If supplemental oxygen is unavailable, initiate resuscitation of the newly born infant with positive-pressure ventilation and room air (Saugstad *et al*., 1998).

Behavioural Changes Associated with Hypoxia

Chronic hypoxia in advanced chronic obstructive pulmonary disease (COPD) result in altered and reduced neuropsychological functioning, which, in turn, leads to memory impairment even when other mental faculties remain unaffected (Sandhu, 1986). Multiple neuropsychological tests with these patients have revealed neuropsychological dysfunction, which is largely due to brain hypoxia. Reversal of cognitive dysfunction has been reported after oxygen therapy (Heaton *et al*., 1993; Krop *et al*., 1973) and even abnormalities in electroencephalograms have been shown to improve (Brezinova *et al*., 1979).

The temporal lobes and the hesh gyrus receive auditory information, modulate memory and language skills and relay information to the cortex where cognitive judgments are made and motor responses are integrated (Davidson & Irwin., 1999). The thalamus and basal ganglia act as relay stations between lower centres and the cortex (Kropotov & Etlinger, 1999). The brainstem enables endurance and survival capabilities, modulating heart rate, respiratory function and autonomic actions (Reid & Milsom, 1998). The pineal gland is thought to modulate sleep-wake cycles (Barrera-Mera & Barrera-Calva, 1998). The hippocampal area including the mammillary bodies modulates spatial memory formation, declarative memory, working memory, memory indexing/storage, relating expectancy to reality and internal inhibition. Memory is recorded in several parts of the brain at same time as 'memory molecules' for storage. These molecules are modulated by limbic system, especially the mammillary bodies. Bilateral hippocampal resection results in short term anterograde amnesia (Wise & Murray, 1999). The hippocampus has receptors for neurosteroids, both mineralocorticoid and glucocorticoid. The high affinity

mineralocorticoid receptors are agonized by aldosterone and antagonized by spironolactone. The low affinity glucocorticoid receptors are agonized by dexamethasone. There are no known antagonists to glucocorticoid receptors. The locus coeruleus is a small structure on the upper brainstem under the fourth ventricle and is involved in the regulation of wakefulness, attention and orientation (Smythies, 1997).

Some parts of the brain that are especially involved in higher cognitive functions (including consciousness) must be very dependent on a rich supply of energy – presumably because they are extremely active. In the first place, as their neurons continually generate many synaptic and action potentials, resulting in large inward and outward fluxes of ions, cellular and ionic homeostasis can be preserved only by the ATP-consuming Na^+ -K^+ pump, which maintains the trans-membrane Na^+ and K^+ gradients and thus indirectly supports such vital transport processes as uptake of sugars and amino acids. Even more than for the pump, 60% of ATP consumption is utilized for protein synthesis (Hochachka, 1996), presumably required to maintain the cell's structure, as well as the rapid turnover of enzymes, receptors and other proteins involved in neurotransmitter release, action and transport. These processes are crucial for synaptic transmission and plasticity and the closely related cognitive processes of memory, learning and selective attention. Hypoxia or hypoglycemia has almost immediate effects on behaviour and brain function. Though dramatic, they are fully reversible if the hypoxia or hypoglycemia is not sustained. But longer or more severe energy deprivation leads to irreversible functional and indeed cellular damage (cell death) – which develop only after a delay of some days (Pulsinelli *et al*., 1982). High incidence of minor neurological deficits, mainly regarding the fields of language and behaviour was reported in persistent pulmonary hypertension of the newborn (Berti *et al*., 2010). Extensive evidence indicates that peripheral or direct central glucose administration enhances cognitive processes in rodents and humans. These behavioural findings suggest that glucose acts directly on the brain to regulate neural

processing, a function that seems incompatible with the traditional view that brain glucose levels are high and invariant except under extreme conditions. However, recent data suggest that the glucose levels of the brain extracellular fluid are lower and more variable than previously supposed. In particular, the level of glucose in the extracellular fluid of a given brain area decreases substantially when a rat is performing a memory task for which the brain area is necessary. Together with results identifying downstream effects of such variance in glucose availability, the evidence leads to new thinking about glucose regulation of brain functions including memory (Ewan *et al*., 2002).

Brain Wave Activity and Seizures as a Result of Hypoxia

In severe encephalopathy there is an initial period of irritability or high arousal, often accompanied by seizures and apnoeic spells, for the first 24 hours. The earlier the seizure, the more severe is the insult. This stage is followed by increasing coma with extreme hypotonia and progressive decline in brainstem function. Brainstem involvement is the best indicator of severe encephalopathy and the signs include abnormal eye movements and interference with sucking, swallowing which often persists as the bulbar and pseudo-bulbar palsy of the severe quadriplegic. Ongoing apnoea and cardio-respiratory arrest bring death at 2 to 3 days of age. When such cases “recover” the incidence of severe neuro-developmental abnormality is 100%. In a variety of clinical settings, an EEG-based monitoring system is considered to be optimal for the detection of an impending failure of cerebral oxygen supply (Prior & Brierley, 1980). In addition, there are other neurological structures implicated in cerebral palsy. The frontal lobe is in charge of voluntary motion. The left lobe controls the motor movements involved in language (speech and writing). The right lobe is usually involved in non-verbal activities. Damage to one frontal lobe usually results in a person's inability to move the opposite side of his body. Moreover,

damage to the frontal lobes can also cause the inability to initiate or respond to speech even though language can still be understood.

The parietal lobe is a structure where sensory information, such as touch, pressure, muscles, temperature and pain, is processed. Damage to one parietal lobe usually results in a loss of sensation in the opposite side of the body as well as being unable to feel touch, temperature and pain. The most frequent clinical syndrome, caused by lesions in the cerebral cortex and underlying white matter, is spastic paralysis (spastic cerebral palsy), which accounts for approximately 50% of all cerebral palsy cases (Miyahara & Mobs, 1995).

Seizures occur commonly in neonatal intensive care units (NICUs). They are an important clinical consequence of CNS diseases in the newborn including brain haemorrhage, stroke, meningitis and hypoxic-ischemic encephalopathy (Stephen *et al*., 2005). Seizures in the newborn are often clinically unsuspected. Consequently, the extent of the electrographic seizures burden in the sick baby can be greatly underestimated (McBride *et al*., 2000). A seizure affects the entire brain (generalized seizure), or it will be confined to one neural region (partial seizure). Autonomic changes are the most common symptoms of simple partial seizures but they go unrecognized. As effective seizure control in the neonate requires abolition of both clinical and electrographic seizures, EEG monitoring is necessary.

Neonatal seizures are paroxysmal alterations in neurological function. This can be behavioural, motor or autonomic (Volpe, 2000). Early pioneering work of 1970s by Wasterlain & Plum (1973) and Meldrum (1978) suggested that prolonged seizures and status epilepticus in mature and immature animals produced an energy failure leading to severe brain cell injury. Later experiments in the last 2 decades, however, seem to have disproved that theory by showing that 10 day old rats (equivalent to human newborns) maintain energy production in the brain by virtue of increased glycolysis and high adenosine triphosphate (ATP) release if there are no systemic complications such as hypoxia or hypotension (Ingvar & Siesjo, 1990). Most

of the literature about neonatal seizures concludes that the prognosis of a particular baby depends upon the etiology of the seizures. It is reported that certain etiologies, such as hypoxic-ischemic encephalopathy (HIE), meningitis, congenital brain abnormalities and inborn errors of metabolism, almost uniformly have severe neurological sequelae (Richard, 1999).

From infancy to adulthood, tonic–clonic seizures and complex partial seizures of temporal or extratemporal origin often lead to sympathetic activation. Because the memory circuits originate in the temporal lobe, repeated seizure activity which involves these structures cause difficulties with memory and intellectual function. Seizures typically activate sympathetic nerve activity, increasing the heart rate and blood pressure, although parasympathetic activation or sympathetic inhibition predominates during partial seizures (Orrin, 2004). Brain requires continuous supply of oxygen for energy utilization and efficient functioning. Hypoxia leads to disruption of this energy utilization, resulting in neuronal functional failure, cerebral palsy and neuro-developmental delay with characteristic biochemical and molecular alterations that can result in permanent or transitory neurological sequelae or even death. Structural and functional integrity of brain depends on regular oxygen and glucose supply.

In the present study, we investigated the role of glucose, epinephrine and oxygen resuscitation in GABA, serotonin, muscarinic receptors – their second messengers and transcription factors regulation in the brain regions of hypoxic neonatal rats. Gene expression studies using Real-Time PCR were done to analyse the changes in receptor, transporters, and transcription factor expression. Immunohistochemical studies using specific antibodies for the receptors were done in confocal microscopy to confirm the receptor data. The receptor pathway was studied by assaying second messenger levels and the proteins and enzymes involved in its pathway. The behavioural studies were done after one month in all experimental groups of neonatal rats to confirm the behavioural changes in later stages of life in

these rats. This study on hypoxic neonates shows that glucose supplementation has significant impact in controlling hypoxia induced functional damage to the neurotransmitter receptors and its signalling cascade, which has immense therapeutic application in the neonatal care.

Materials and Methods

CHEMICALS USED AND THEIR SOURCES

Biochemicals

5-hydroxytrptophan, 5-hydroxy indole acetic acid, ketanserin, bicuculline, baclofen, atropine, sodium octyl sulfonic acid, ethylene diamine tetra acetic acid-EDTA, D-glucose, calcium chloride, bovine serum albumin fraction V, Superoxide dismutase from bovine erythrocytes were purchased from Sigma Chemical Co., St. Louis, USA. All other reagents were of analytical grade purchased locally. HPLC solvents were of HPLC grade obtained from SRL and MERCK, India. Tissue freezing medium Jung was purchased from Leica Microsystems Nussloch GmbH, Germany.

Radiochemicals

4-amino-*n*-[2,3- ^{3}H]butyric acid (Specific activity- 84.0 Ci/mmol) was purchased from Amersham Bioscience, USA, Baclofen, (-)-[butyl-4-3H(N)] (Specific activity- 42.9 Ci/mmol), Bicuculline methyl chloride, (-)-[methyl-^{3}H] (Specific activity- 82.9 Ci/mmol), Quinuclidinylbenzilate, L-[Benzilic-4,4'-3H]-[4-3H] (Sp. Activity 42 Ci/mmol) were purchased from NEN Life Sciences Products, Inc., Boston USA. 5-Hydroxy [G-^{3}H] tryptamine creatine sulphate ([^{3}H]5-HT, 18.4Ci/mmol) and [Ethylene-^{3}H]-Ketanserin Hydrochloride (Sp. Activity 63.3Ci/mmol) were purchased from Perkin Elmer Nen Life and Analytical Sciences, Boston, MA, USA.

Radioimmunoassay kit for insulin and triiodothyronine was purchased from Baba Atomic Research Centre (BARC), Mumbai, India. The [^{3}H] IP3, [^{3}H]cGMP and [^{3}H]cAMP Biotrak Assay Systems were purchased from G.E Healthcare UK Limited, UK.

Molecular Biology Chemicals

Tri-reagent kit was purchased from Sigma Chemical Co., St. Louis, USA. ABI PRISM High Capacity cDNA Archive kit, Primers and TaqMan probes for Real-Time PCR were purchased from Applied Biosystems, Foster City, CA, USA. $GABA_{A\alpha1}$ (Rn 00788315_m1), $GABA_{A\alpha5}$ (Rn 00568803_m1), $GABA_{A\delta}$ (Rn 00568740_m1), $GABA_{A\gamma3}$ (Rn 00577639_m1), $GABA_B$ (Rn 00578911_m1), GAD1 (Rn 00690304_g1), Hif1A (Rn 00577560_m1), $5HT_{2A}$ (Rn01468302_m1), 5HTT (Rn00564737_m1), insulin receptor (Rn 00567070_m1), muscarinic M_1 (Rn 00589936_s1), muscarinic M_2 (Rn 02532311_s1), muscarinic M_3 (Rn 00560986_s1), choline acetyl transferase (Rn 01453446_m1), acetyl choline esterase (Rn 00596883_ m1), SOD (Rn 01477289), GPx (Rn00577994), BAX (Rn 01480160_g1), Phospholipase C (Rn 01647142), CREB (Rn 00578826_m1) primers were used for the study.

Confocal Dyes

Primary antibody for $GABA_{A\alpha1}$ (No-31775), $5\text{-}HT_{2A}$ (No: RA24288 BD PharmenginTM), 5-HTT (No: AB9726 Chemicon) and rhodamine coated secondary antibody (No- AP307R) and FITC (No: AB7130F, Chemicon) were used for immunohistochemistry studies using confocal microscope.

ANIMALS

Pups with dams were purchased from Amrita Institute of Medical Sciences, Kochi. Neonatal rats of four days old were weighed and used for experiments. All groups of neonatal rats were maintained with their mothers under optimal conditions 12 hour light and 12 hour dark periods and were fed standard food and water *ad libitum*.

INDUCTION OF ACUTE HYPOXIA IN NEONATAL RATS

Wistar neonatal rats of 4-days old (body weight, 6.06 ± 0.45g) were used for the experiments and were grouped into seven as follows: (i) Control neonatal rats were given atmospheric air (20.9% oxygen) for 30 minutes (C); (ii) Hypoxia was induced by placing the neonatal rats in a hypoxic chamber provided with 2.6% oxygen for 30 minutes (Hx); (iii) Hypoxic neonatal rats were injected 10% dextrose (500mg/ Kg body wt) intra-peritoneally (i.p.) (Hx+G). (iv) Hypoxic neonatal rats were supplied with 100% oxygen for 30 minutes (Hx+O); (v) Hypoxic neonatal rats were injected 10% dextrose (500mg/ Kg body wt. i.p.) and treated with 100% oxygen for 30 minutes (Hx+G+O). (vi) Hypoxic neonatal rats were injected 10% dextrose (500mg/ Kg body wt), epinephrine (0.1µg/Kg body wt. i.p.) and treated with 100% oxygen for 30 minutes (Hx+G+E+O) (vii) Hypoxic neonatal rats were injected with epinephrine (0.10g/Kg body wt) i.p. (Hx + E). The experimental animals were maintained in the room temperature for one week.

Neonatal experimental rats were kept for one month for behavioural studies. Body weights were measured before the experiment.

TISSUE PREPARATION

Control and experimental neonatal rats were sacrificed by decapitation on post natal day 14. The brain regions and body parts were dissected out quickly over ice according to the procedure of Glowinski and Iversen (1966) and the tissues were stored at $^{-}$80°C for various experiments.

All animal care and procedures were in accordance with Institutional and National Institute of Health guidelines.

ESTIMATION OF BLOOD GLUCOSE

Blood glucose was estimated by GOD-POD glucose estimation kit from Biolab Diagnostics Pvt. Ltd. The glucose was estimated on post natal day 14. The

spectrophotometric method using glucose oxidase-peroxidase reactions was as follows:

Principle: Glucose oxidase (GOD) catalysed the oxidation of glucose in accordance with the following equation:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{(GOD)}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol+ 4- aminoantipyrene} \xrightarrow{\text{(PEROXIDASE)}} \text{Coloured complex} + H_2O$$

The hydrogen peroxide formed in this reaction reacted with 4-aminoantipyrine and 4-hydroxybenzoic acid in the presence of peroxidase (POD) to form N-(-4-antipyryl)-p-benzo quinoneimine. The addition of mutarotase accelerated the reactions. The amount of dye formed was proportional to the glucose concentration. The absorbance was read at 500nm in (Shimadzu UV-1700 pharmaSPEC) spectrophotometer.

KINETICS OF SUPEROXIDE DISMUTASE (SOD)

The ability of the flavonoid to inhibit the reduction of nitro blue tetrazolium (NBT) by superoxide generated by the reduction of photoreduced riboflavin and oxygen was assayed. SOD assay was done in heart and cerebral cortex homogenate as described previously (Winterbourn *et al*., 1975).

The SOD concentration (U/mg) that established IC_{50} (50% inhibition of the reaction) was determined using a standard SOD (2000-10000 U/mg protein). Then, the dilution rate of heart and cerebral cortex homogenates that established IC_{50} was determined and the unit (U/mg) of the extract was calculated by the SOD concentration that established IC_{50} determined using standard SOD. One unit of SOD was defined as the amount of protein that inhibited the rate of NBT reduction by 50%. Kinetic parameters V_{max} and K_m, were calculated from the data of SOD assay measured at substrate concentrations of 0.03 mM, 0.06 mM, 0.12 mM, 0.15 mM and 0.2mM.

KINETICS OF CATALASE (CAT)

Catalase assay was done in heart and cerebral cortex homogenate as described previously (Aebi, 1984). The reaction mixture contained 40 mM H_2O_2 in a 50 mM phosphate buffer pH 7.0, and 0.1 ml pure enzyme in a total volume of 3 ml. CAT activity was estimated by decrease in absorbance of H_2O_2 at 240 nm. Kinetic parameters V_{max} and K_m, were calculated from the data of catalase assay measured at substrate concentrations of 200 µM, 400 µM, 600 µM and 800 µM.

ESTIMATION OF TRIIODOTHYRONINE (T3) BY RADIOIMMUNOASSAY

Principle of the assay

The assay was done according to the procedure of BARC radioimmunoassay kit. The radioimmunoassay method was based on the competition of unlabelled endogenous T3 with [^{125}I] T3 for the limited binding sites on the antibody (Ab1) made specially for T3. The antibody was in the form of a complex with second antibody (Ab2). At the end of incubation, the T3 (Ag) bound to the antibody- second antibody complex (Ag-Ab1-Ab2) and free T3 was separated by the addition of PEG. The amount bound to the antibody complex in the assay tubes were compared with values of known T3 standards and the T3 concentration in the samples were calculated.

Assay Protocol

Standards, ranging from 0.15 to 2.5ng, T3 free serum, [^{125}I] T3 and antiserum complex were added together and the volume was made up to 275µl with assay buffer. Samples of appropriate concentration from experiments were used for the assay. They were incubated at 37°C for 45 minutes. The PEG was added to all tubes and they were centrifuged at 1500 x g for 20 minutes. The

supernatant was aspirated out and the radioactivity in the pellet was determined in a gamma counter.

T3 concentrations in the samples were determined from the standard curve plotted using MultiCalc™ software (Wallac, Finland).

A standard curve was plotted with $\%B/B_o$ on the Y-axis and insulin concentration/ml on the X-axis of a log-logit graph. $\%B/B_o$ was calculated as:

$$\frac{\text{Corrected average count of standard or sample}}{\text{Corrected average count of zero standard}} \times 100$$

T3 concentration in the samples was determined from the standard curve plotted using MultiCalc™ software (Wallac, Finland).

ESTIMATION OF CIRCULATING INSULIN BY RADIOIMMUNOASSAY

Principle of the assay

The assay was done according to the procedure of BARC radioimmunoassay kit. The radioimmunoassay method was based on the competition of unlabelled insulin in the standard or samples and [^{125}I] insulin for the limited binding sites on a specific antibody. At the end of incubation, the antibody bound and free insulin were separated by the second antibody-polyethylene glycol (PEG) aided separation method. Measuring the radioactivity associated with bound fraction of sample and standards quantitates insulin concentration of samples.

Assay Protocol

Standards, ranging from 0 to 200µU/ml, insulin free serum and insulin antiserum (50µl each) were added together and the volume was made up to 250µl

with assay buffer. Samples of appropriate concentration from experiments were used for the assay. They were incubated overnight at 2°C. Then 50µl [^{125}I] Insulin was added and incubated at room temperature for 3 hrs. 50µl second antibody was added along with 500µl of PEG. The tubes were then vortexed and incubated for 20 minutes and they were centrifuged at 1500 x g for 20 minutes. The supernatant was aspirated out and the radioactivity in the pellet was determined in a gamma counter.

A standard curve was plotted with $\%B/B_o$ on the Y-axis and insulin concentration/ml on the X-axis of a log-log it graph. $\%B/B_o$ was calculated as:

$$\frac{\text{Corrected average count of standard or sample}}{\text{Corrected average count of zero standard}} \times 100$$

Insulin concentration in the samples was determined from the standard curve plotted using MultiCalc™ software (Wallac, Finland).

QUANTIFICATION OF GABA IN THE EXPERIMENTAL GROUPS OF NEONATAL RATS

GABA content in the brain regions of control and experimental rats were quantified by displacement method using the procedure of Kurioka *et al.*(1981) where the incubation mixture contained 30 nM [^{3}H]GABA with and without GABA at a concentration range of 10^{-9} M to 10^{-3} M. The unknown concentrations were determined from the standard displacement curve using appropriate dilutions and calculated for µmoles/g wt. of the tissue.

QUANTIFICATION OF BRAIN SEROTONIN (5-HT) AND ITS METABOLITE IN THE EXPERIMENTAL GROUPS OF NEONATAL RATS

The monoamines were assayed according to the modified procedure of Dakshinamurti *et al.,* (1988). The brain stem and cerebellum of experimental

groups of rats was homogenised in 0.4N perchloric acid. The homogenate was then centrifuged at 5000xg for 10 minutes at 4°C in a Sigma 3K30 refrigerated centrifuge and the clear supernatant was filtered through 0.22 µm HPLC grade filters and used for HPLC analysis.

5-Hydroxy indole Acetic Acid (5-HIAA) and 5-HT contents were determined in high performance liquid chromatography (HPLC) with electrochemical detector (ECD) (Waters, USA) fitted with CLC-ODS reverse phase column of 5 µm particle size. The mobile phase consisted of 50mM sodium phosphate dibasic, 0.03M citric acid, 0.1mM EDTA**,** 0.6mM sodium octyl sulfonate and 15% methanol. The pH was adjusted to 3.25 with orthophosphoric acid, filtered through 0.22 µm filter (Millipore) and degassed. A Waters model 515, Milford, USA, pump was used to deliver the solvent at a rate of 1 ml/minute. The neurotransmitters and their metabolites were identified by amperometric detection using an electrochemical detector (Waters, model 2465) with a reduction potential of +0.80 V. Twenty microlitre aliquots of the acidified supernatant were injected into the system for quantification. The peaks were identified by relative retention times compared with external standards and quantitatively estimated using an integrator (Empower software) interfaced with the detector. Data from different brain regions of the experimental and control rats were statistically analysed and tabulated.

GABA RECEPTOR BINDING STUDIES USING [^{3}H] RADIOLIGANDS

Total GABA Receptor Binding studies

[^{3}H]GABA binding to the GABA receptor was assayed in Triton X-100 treated synaptic membranes (Kurioka *et al*., 1981). Crude synaptic membranes were prepared using sodium-free 10mM tris sulphate buffer (pH 7.4). Each assay tube contained a protein concentration of 0.3-0.4 mg. In saturation binding

experiments, 5-40 nM of [^{3}H] GABA was incubated with and without excess of unlabelled GABA (100μM) and in competition binding experiments the incubation mixture contained 30 nM of [3H]GABA with and without GABA at a concentration range of 10^{-9}M to 10^{-4}M. . The incubation was continued for 20 min at 0-4^{o}C and terminated by centrifugation at 35,000 x*g* for 20 min. Bound radioactivity was counted with cocktail-T in a liquid scintillation counter. Specific binding was determined by subtracting non-specific binding from the total binding.

$GABA_A$ Receptor Binding studies

[^{3}H]bicuculline binding to the GABA receptor was assayed in Triton X-100 treated synaptic membranes (Kurioka *et al*., 1981). Crude synaptic membranes were prepared using sodium-free 10mM Tris sulphate buffer, pH 7.4. Each assay tube contained a protein concentration of 0.3-0.4 mg. In saturation binding experiments, 5nM to 75nM concentrations of [^{3}H] bicuculline was incubated with and without excess of 100μM unlabelled bicuculline. The incubation was continued for 20 min at 0-4^{o}C and terminated by centrifugation at 35,000x*g* for 20 min. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter. Specific binding was determined by subtracting non-specific binding from the total binding.

$GABA_B$ Receptor Binding studies

[^{3}H]baclofen binding to GABA receptor in the synaptic membrane preparations were assayed as previously described (Hills *et al.*, 1987). Crude synaptic membrane preparation was suspended in 50mM Tris sulphate buffer, pH 7.4 containing 2mM $CaCl_2$ and 0.3-0.4 mg protein. In saturation binding experiments, 10-100nM of [^{3}H] baclofen was incubated with and without excess of 100μM unlabelled baclofen. The incubations were carried out at 20^{o}C for 20

min. The binding reactions were terminated by centrifugation at 14000x*g* for 10 min. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter. Specific binding was determined by subtracting non-specific binding from the total binding.

SEROTONIN RECEPTOR BINDING STUDIES USING [^{3}H] RADIOLIGANDS

5-HT Receptor Binding studies

5-HT receptor assay was done using [^{3}H]-5-hydroxytryptamine binding in crude synaptic membrane preparations of cerebral cortex, brain stem, cerebellum and corpus striatum by the modified method of Uzbekov *et al.,* (1979). Crude membrane preparation was suspended in 50 mM Tris-HCl buffer, pH 8.5, containing 1.0 µM paragyline. The incubation mixture contained 0.3-0.4 mg protein. In the saturation binding experiments, assays were done using different concentrations i.e., 1.0nM-30nM of [^{3}H] 5-HT incubated with and without excess of unlabelled 10µM 5-HT. Tubes were incubated at 37^{o}C for 15 minutes and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 5.0 ml of ice cold 50mM Tris buffer, pH 8.5. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

5-HT$_{2A}$ Receptor Binding Studies

5-HT$_{2A}$ receptor assay was done using [^{3}H] Ketanserin binding in crude synaptic membrane preparations of cerebral cortex, brain stem, cerebellum and corpus striatum by the modified method Leysen *et al.,* (1982). Crude membrane preparation was suspended in 50 mM Tris-HCl buffer, pH 7.6. The incubation mixture contained 0.3-0.4 mg protein. In the saturation binding experiments, assays were done using different concentrations i.e., 0.1nM-2.5nM of [^{3}H]

Ketanserin which was incubated with and without excess of unlabelled 10µM Ketanserin. Tubes were incubated at 37°C for 15 minutes and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washings with 5.0 ml of ice cold 50mM Tris buffer, pH 7.6. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

TOTAL MUSCARINIC RECEPTOR BINDING STUDIES USING [^{3}H] RADIOLIGANDS

[^{3}H] QNB binding assay in cerebral cortex, cerebellum, brain stem, and corpus striatum were done according to the modified procedure of Yamamura and Snyder (1981). Brain tissues were homogenised with 20 volumes of cold 50mM Tris-HCl buffer, pH 7.4, containing 1mM EDTA. The supernatant was then centrifuged at 30,000xg for 30 minutes and the pellets were resuspended in appropriate volume of the buffer. Total muscarinic binding parameter assays were done using 0.1-2.5nM of [^{3}H] QNB in the incubation buffer, pH 7.4 in a total incubation volume of 250µl. The non-specific binding was determined using 100µM atropine for total muscarinic receptor. Total incubation volume of 250 µl contains 200-250µg protein concentration. Tubes were incubated at 22°C for 60 minutes and filtered rapidly through GF/C filters (Whatman). The filters were washed quickly by three successive washings with 5.0 ml of ice cold 50mM Tris-HCl buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter. The non-specific binding determined showed 10% in all our experiments.

Protein Determination

Protein was measured by the method of Lowry *et al*., (1951) using bovine serum albumin as standard. The intensity of the purple blue colour formed was

proportional to the amount of protein which was read in Spectrophotometer at 660nm.

ANALYSIS OF THE RECEPTOR BINDING DATA

Linear regression analysis for Scatchard plots

The data was analysed according to Scatchard (1949). The specific binding was determined by subtracting non-specific binding from the total. The binding parameters, 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. The maximal binding is a measure of the total number of receptors present in the tissue and the equilibrium dissociation constant is the measure of the affinity of the receptors for the radioligand. The K_d is inversely related to receptor affinity.

Nonlinear regression analysis for displacement curve

Competitive binding data was analyzed using non-linear regression curve-fitting procedure (GraphPad PRISM™, San Diego, USA). The data of the competitive binding assays were 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 and a two site competitive binding curve has a slope less than 1.0. The concentration of competitor that competes for half the specific binding was defined as EC_{50}, which 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 binds to half the binding sites at equilibrium in the absence of radioligand or other competitors.

GENE EXPRESSION STUDIES IN DIFFERENT BRAIN REGIONS OF CONTROL AND EXPERIMENTAL NEONATAL RATS

Isolation of RNA

RNA was isolated from the different brain regions of control and experimental neonatal rats using the Tri reagent from Sigma Chemicals Co., St. Louis, MO, U.S.A). 25-50mg tissue homogenates were made in 0.5ml Tri Reagent and was centrifuged at 12,000 x g for 10 minutes at 4°C. The clear supernatant was transferred to a fresh tube and it was allowed to stand at room temperature for 5min. 100µl of chloroform was added to it, mixed vigorously for 15sec and allowed to stand at room temperature for 15min. The tubes were then centrifuged at 12,000 x g for 15min at 4°C. Three distinct phases appear after centrifugation. The bottom red organic phase contained protein, interphase contained DNA and a colourless upper aqueous phase contained RNA. The upper aqueous phase was transferred to a fresh tube and 250µl of isopropanol was added and the tubes were allowed to stand at room temperature for 10min. The tubes were centrifuged at 12,000 x g for 10min at 4°C. 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 12,000 x g for 5 min at 4°C. The pellets were semi dried and dissolved in minimum volume of DEPC-treated water. 2µl of RNA was made up to 1ml and absorbance was measured at 260nm and 280nm. For pure RNA preparation the ratio of absorbance at 260/280 was ≥ 1.7. The concentration of RNA was calculated as one absorbance $_{260}$ = 42µg.

cDNA Synthesis

Total cDNA synthesis was performed using ABI PRISM cDNA Archive kit in 0.2ml microfuge tubes. The reaction mixture of 20µl contained 0.2µg total RNA, 10X RT buffer, 25X dNTP mixture, 10X Random primers, MultiScribe RT (50U/µl) and RNase free water. The cDNA synthesis reactions were carried out at

25°C for 10min and 37°C for 2 hours using an Eppendorf Personal Cycler. The primers and probes were purchased from Applied Biosystems, Foster city, CA, USA designed using Primer Express Software Version (3.0).

Real-Time PCR Assay

Real Time PCR assays were performed in 96-well plates in a ABI 7300 Real Time PCR instrument (Applied Biosystems). To detect gene expression, a TaqMan quantitative 2-step reverse transcriptase "polymerase chain reaction (RT-PCR) was used to quantify mRNA levels. First-strand cDNA was synthesized with use of a TaqMan RT reagent kit, as recommended by the manufacturer. PCR analyses were conducted with gene-specific primers and fluorescently labeled TaqMan probe (designed by Applied Biosystems). Endogenous control, β-actin, was labelled with a reporter dye (VIC). All reagents were purchased from Applied Biosystems. The probes for specific gene of interest were labeled with FAM at the 5' end and a quencher (Minor Groove Binding Protein - MGB) at the 3' end. The real-time data were analyzed with Sequence Detection Systems software version 1.7. All reactions were performed in duplicate.

The TaqMan reaction mixture of 20µl contained 25ng of total RNA-derived cDNAs, 200nM each of the forward primer, reverse primer and TaqMan probes, endogenous control (β-actin) and 12.5µl of TaqMan 2X Universal PCR MasterMIX (Applied Biosystems). The volume was made up with RNAse free water. Each run contained both negative (no template) and positive controls.

The thermocycling profile conditions were as follows:

50ºC	-- 2 minutes	---- Activation	
95ºC	-- 10 minutes	---- Initial Denaturation	
95ºC	-- 15 seconds	---- Denaturation	40 cycles
50ºC	-- 30 seconds	--- Annealing	

60ºC -- 1 minutes --- Final Extension

Fluorescence signals measured during amplification were considered positive if the fluorescence intensity was 20-fold greater than the standard deviation of the baseline fluorescence. The ΔΔCT method of relative quantification was used to determine the fold change in expression. This was done by first normalizing the resulting threshold cycle (CT) values of the target mRNAs to the CT values of the internal control β-actin in the same samples ($\Delta CT = CT_{Target} - CT_{\beta\text{-actin}}$). It was further normalized with the control ($\Delta\Delta CT = \Delta CT - CT_{Control}$). The fold change in expression was then obtained ($2^{-\Delta\Delta CT}$).

IMMUNOHISTOCHEMISTRY OF GABAA$_{\alpha 1}$ RECEPTOR, SEROTONIN RECEPTOR SUBTYPE 5-HT$_{2A}$, SEROTONIN TRANSPORTER 5-HTT IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL NEONATAL RATS USING CONFOCAL MICROSCOPE

Control and experimental rats were deeply anesthetized with ether. The rat was transcardially perfused with PBS, pH- 7.4 followed by 4% paraformaldehyde in PBS. After perfusion the brains were dissected out and fixed in 4% paraformaldehyde for 1 hr and then equilibrated with 30% sucrose solution in PBS (0.1 M). 30 µm cerebellum sections were cut using cryostat (Leica, CM1510 S). The sections were treated with PBS. To block unspecific binding the sections were incubated for 1 hour at room temperature with 5% bovine serum albumin in normal goat serum. Brain slices were incubated overnight at 4 °C with rat primary antibody for GABAA$_{\alpha 1}$ (diluted in PBST at 1: 1000 dilution), 5-HT$_{2A}$ (No: RA24288 BD PharmenginTM, diluted in PBST at 1: 500 dilution) and 5HTT (No: AB9726 Chemicon Temecula, diluted in PBST at 1: 500 dilution). After overnight incubation, the brain slices were rinsed with PBS and then incubated with FITC (No: AB7130F, Chemicon, diluted in PBS at 1: 1000 dilution) coated secondary

antibody. The sections were observed and photographed using confocal imaging system (Leica SP 5).

IP3 CONTENT IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS *IN VIVO*

Brain tissue from corpus striatum was homogenised in a polytron homogeniser in 50mM Tris-HCl buffer, pH.7.4, containing 1mM EDTA to obtain a 15% homogenate. The homogenate was then centrifuged at 40,000 x g for 15min. and the supernatant was transferred to fresh tubes for IP3 assay using [^{3}H]IP3 Biotrak Assay System kit.

Principle of the assay

The assay was based on competition between [^{3}H]IP3 and unlabelled IP3 in the standard or samples for binding to a binding protein prepared from bovine adrenal cortex. The bound IP3 was then separated from the free IP3 by centrifugation. The free IP3 in the supernatant was then discarded by simple decantation, leaving the bound fraction adhering to the tube. Measurement of the radioactivity in the tube enables the amount of unlabelled IP3 in the sample to be determined.

Assay Protocol

Standards, ranging from 0.19 to 25pmoles/tube, [^{3}H]IP3 and binding protein were added together and the volume was made up to 100µl with assay buffer. Samples of appropriate concentration from experiments were used for the assay. The tubes were then vortexed and incubated on ice for 15min and they were centrifuged at 2000 x g for 10min at 4°C. The supernatant was aspirated out and the pellet was resuspended in water and incubated at room temperature for 10min. The tubes were then vortexed and decanted immediately into scintillation vials.

The radioactivity in the suspension was determined using liquid scintillation counter.

A standard curve was plotted with %B/Bo on the Y-axis and IP3 concentration (pmoles/tube) on the X-axis of a semi-log graph paper. $\%B/B_o$ was calculated as:

$$\frac{\text{(Standard or sample cpm – NSB cpm)}}{\text{(B}_0\text{ cpm – NSB cpm)}} \times 100$$

NSB- non specific binding and B_0 - zero binding. IP3 concentration in the samples was determined by interpolation from the plotted standard curve.

cGMP CONTENT IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS *IN VIVO*

Brain tissue from corpus striatum was homogenised in a polytron homogeniser with cold 50mM Tris-HCl buffer, pH 7.4, containing 1mM EDTA to obtain a 15% homogenate. The homogenate was then centrifuged at 40,000 x g for 15min and the supernatant was transferred to fresh tubes for cGMP assay using [^{3}H]cGMP Biotrak Assay System kit.

Principle of the assay

The assay is based on the competition between unlabelled cGMP and a fixed quantity of the [^{3}H]cGMP for binding to an antiserum, which has a high specificity and affinity for cGMP. The amount of [^{3}H]cGMP bound to the antiserum is inversely related to the amount of cGMP present in the assay sample. Measurement of the antibody bound radioactivity enables the amount of unlabelled cGMP in the sample to be calculated. Separation of the antibody bound cGMP from the unbound nucleotide was done by ammonium sulphate precipitation, followed by centrifugation. The precipitate which contains the

antibody bound complex was dissolved in water and its activity was determined by liquid scintillation counting. The concentration of unlabelled cGMP in the sample was determined from a linear standard curve.

Assay Protocol

Standards, ranging from 0.5 to 4.0pmoles/tube, and [^{3}H]cGMP were added together and the volume was made up to 100µl with assay buffer. Samples of appropriate concentration from experiments were used for the assay. The antiserum was added to all the assay tubes and then vortexed. The tubes were incubated for 90min at 2 - 8°C. Ammonium sulphate was added to all tubes, mixed and allowed to stand for 5min in ice bath. The tubes were centrifuged at 12000 x g for 2min at room temperature. The supernatant was aspirated out and the pellet was dissolved in water and decanted immediately into scintillation vials. The radioactivity in the suspension was determined using liquid scintillation counter.

A standard curve was plotted with C_o/C_x on the Y-axis and cGMP concentration (pmoles/tube) on the X-axis of a linear graph paper. C_o- the cpm bound in the absence of unlabelled cGMP; C_x- the cpm bound in the presence of standard/unknown cGMP. cGMP concentration in the samples was determined by interpolation from the plotted standard curve.

cAMP CONTENT IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS *IN VIVO*

Brain tissues- BS and CB were homogenised in a polytron homogeniser with cold 50mM Tris-HCl buffer, pH 7.4, containing 1mM EDTA to obtain a 15% homogenate. The homogenate was then centrifuged at 40,000 x g for 15min and the supernatant was transferred to fresh tubes for cAMP assay using [^{3}H]cAMP Biotrak Assay System kit.

Principle of the assay

cAMP assay kit was used. The assay is based on the competition between unlabelled cAMP and a fixed quantity of tritium labeled compound for binding to a protein which has a high specificity and affinity for cAMP. The amount of labeled protein -cAMP complex formed is inversely related to the amount of unlabelled cAMP present in the assay sample. Measurement of the protein-bound radioactivity enables the amount of unlabelled cAMP in the sample to be calculated.

Free [^{3}H] cAMP Bound [^{3}H] cAMP-binding protein

\+ Binding protein = +

cAMP cAMP-binding protein

Separation of the protein bound cAMP from unbound nucleotide is achieved by adsorption of the free nucleotide on to a coated charcoal followed by centrifugation. An aliquot of the supernatant is then removed for liquid scintillation counting. The concentration of unlabelled cAMP in the sample is then determined from a linear standard curve.

Assay Protocol

The tubes were placed on a water bath at 0°C. The assay mixture consisted of different concentrations of standard, [^{3}H]cAMP and binding protein in case of standards; buffer, [^{3}H]cAMP and binding protein for zero blank and unknown samples, [^{3}H]cAMP and binding protein for determination of unknown samples. The mixture was incubated at 2°C for 2h. Cold charcoal reagent was added to the tubes and the tubes were immediately centrifuged at 12,000 x g for 2min at 2°C. Aliquots of the supernatant was immediately transferred to

scintillation vials and mixed with cocktail-T and counted in a liquid scintillation counter (Wallac, 1409).

C_o/C_x was plotted on the Y-axis against picomoles of inactive cAMP on the X- axis of a linear graph paper, where C_o is the counts per minute bound in the absence of unlabelled cAMP and C_x was the counts per minute bound in the presence of standard or unknown unlabelled cAMP. From the C_o/C_x value for the sample, the number of picomoles of unknown cAMP was calculated.

BEHAVIOURAL STUDIES

Wire maneuver test

For wire maneuver test, rat was hung by both forelimbs on the wire 50cm high from the floor. The diameter of the wire was 1mm. The body of the rat was maintained parallel to the floor by hanging the tail by experimenter's hand and the tail was then released. Its falling latency was measured as an index of forelimb strength.

The animal was lifted by its tail and allowed to grasp the horizontal wire with its forepaws and was then rotated partially downward and released. The ability of the animal to grasp to the wire also with its hind limbs was scored.

Righting reflex

For righting reflex, rat was held in the lumbosacral region. When the body was tilted to the left and right, the head was moved in the opposite direction in order to maintain the original position in the normal reflex.

Y-Maze test

The Y-maze was made of grey wood, covered with black paper and consisted of three arms with an angle of 120 degrees between each of the arms. Each arm was 8 cm width ×30 cm length ×15 cm height. The three identical arms were randomly designated: Start arm, in which the rat started to explore (always

open); Novel arm, which was blocked at the 1st trial, but open at the 2nd trial and the other arm (always open). The maze was placed in a separate room with enough light. The floor of the maze was covered with saw dust, which was mixed after each individual trial in order to eliminate olfactory stimuli. Visual cues were placed on the walls of the maze.

The Y-maze test consisted of two trials separated by an inter-trial interval (ITI). The first trial (training) was of 10 minutes duration and allowed the rat to explore only two arms (start arm and the other arm) of the maze, with the third arm (novel arm) blocked. After a 1 hour ITI (Ma *et al*., 2007), the second trial (retention) was conducted, during which all three arms were accessible and novelty *vs* familiarity was analyzed through comparing behaviour in all three arms. For the second trial, the rat was placed back in the maze in the same starting arm, with free access to all three arms for 5 minutes. The time spent in each arm was analyzed. Data was expressed as percentage of performance in all three arms during the five minutes of test (Akwa *et al*., 2001; Jobin, *et al*., 2010).

Radial arm maze Test

Radial maze behavioral testing was conducted under normal room lighting using an eight armed radial maze elevated 100 cm from the floor. Each arm of the maze (11.5 cm wide) extended 68.5cm from an octagonally shaped central platform (40 cm across). Black Plexiglas walls (11.5 cm high) were present for the first 20 cm of each arm to prevent the rat crossing from one arm to another without returning to the central platform. Circular food wells (1.3 cm deep, 3.2 cm diameter) were located 2.5cm from the end of each arm. The maze was centered in an enclosed room where lighting and spatial cues (e.g., posters, door, and boxes) remained constant throughout the course of the experiment. Arms were baited by placing one raisin in each food well.

Rats were placed on the maze 3 days prior to the start of formal acquisition testing in order to habituate them to the apparatus. On the first day of

habituation, 4 food pellets were scattered along the length of each arm. The rats were then systematically confined to each arm for 1 min to ensure their exposure to the entire maze. On the second day of habituation, the previous day's procedure was repeated except that the animals were not confined to each arm following 5 min of exploration. On the third day, one food pellet was placed in the food well at the end of each arm and a second was placed halfway down each arm. Once the rats were habituated to the maze, testing began. Trials began by placing a single rat in the center of the maze facing away from the experimenter. The trial ended when the rat had obtained all 4 pellets or 5min had elapsed, whichever occurred first. Rats were run until they achieved criterion performance for task acquisition. Criterion was attained when the rat collected 3 out of the 4 food pellets within their first 4 arm entries within a trial (while still completing the trial) with this level of performance being maintained for 5 consecutive criterion performance. The number of trials up to and including the last of these 5 criterion performance formed the "number of trials to criterion" measure. Experimental subjects were tested under blind conditions. The time of testing was consistent from day to day for each subject but testing of the various treatment groups was distributed randomly throughout the day.

Morris water maze Test

Water maze experiment was conducted during post-treatment. The custom-constructed water maze pool measured 100 cm in diameter by 50 cm in depth and was filled with water to a depth of 35 cm. A 10-cm-diameter white platform was located 1.5 cm below the surface of the water. Nontoxic white paint was added to the water to visually obscure the location of the platform. The pool had been divided into four quadrants of homogeneous size and the platform was located in the center of one of the quadrants, halfway between the center and the wall of the pool. All swim latencies were recorded with a manual stopwatch, a technique routinely employed by others (Hort *et al.*, 1999). The water maze task

consisted of 15 sessions conducted once daily over 15 successive days. Each session consisted of four trials separated by approximately 60 seconds. Rats were placed manually into the pool, facing the pool wall in the center of one of the quadrants that did not contain the platform. . For any given rat, the location of the platform remained fixed across all trials and all sessions. The latency to find the platform was recorded as the time from release into the pool until the rat had reached the platform. A maximum of 60 seconds was allowed for each trial. Rats not reaching the platform within 60 seconds were guided to the platform and a score of 60 seconds was recorded for each of these experimenter-terminated trials. The rat was allowed to remain on the platform for the duration of the inter-trial interval. A 60-seconds probe test to determine the time spent in the platform quadrant after removing the platform from pool was conducted on the 13^{th}, 14^{th} and 15^{th} day of the study (24 h after the last hidden platform session). Rats were released into the pool in the quadrant opposite to that previously associated with the escape platform. A manual time-sampling procedure (one measurement per second) was utilized to record the swimming bias of the rat in each of the four quadrants of the pool.

STATISTICS

Statistical evaluations were done by ANOVA using InStat (Ver.2.04a) computer programme. Linear regression Scatchard plots were made using SIGMA PLOT (Ver 2.03). Sigma Plot software (version 2.0, Jandel GmbH, Erkrath, Germany). Competitive binding data were analysed using non-linear regression curve-fitting procedure (GraphPad PRISM™, San Diego, USA). Empower software were used for HPLC analysis. Relative Quantification Software was used for analyzing Real-Time PCR results.

Results

BODY WEIGHT AND BLOOD GLUCOSE LEVEL

There was no significant change in the body weight and blood glucose levels in the control and experimental groups of neonatal rats (Table- 1).

SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN HEART OF CONTROL AND EXPERIMENTAL NEONATAL RATS

The SOD activity showed a significant increase in V_{max} ($p<0.001$) and a significant decrease in K_m ($p<0.01$) in adult rats compared to neonates, showing the poorly developed enzyme system in the neonates. In hypoxic group (Hx), a significant decrease in V_{max} ($p<0.01$) and a significant increase in K_m ($p<0.01$) was observed, showing decreased activity of SOD with less affinity under hypoxic condition. In glucose supplemented hypoxic group, (Hx + G), an increase in V_{max} ($p<0.001$) and a decrease in K_m ($p<0.001$) was observed compared to hypoxic group, showing the enhanced free radical scavenging capability due to glucose supplementation. In Hx + O group both V_{max} ($p<0.001$) and K_m ($p<0.05$) showed a significant increase compared to control neonate. In Hx +G + O group, V_{max} and K_m showed a reversal to near control value with significant increase in V_{max} ($p<0.001$) and a significant decrease in K_m ($p<0.001$) compared to hypoxic group was observed. In Hx + G + E + O group, V_{max} ($p<0.001$) showed a significant decrease to near hypoxic value with a decreased K_m ($p<0.001$) when compared to control neonate (Figure- 1, Table- 2).

SUPEROXIDE DISMUTASE ACTIVITY IN CEREBRAL CORTEX OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

In cerebral cortex, SOD activity increased ($p<0.001$) with an increased affinity ($p<0.01$) in adult rats compared to neonates. In hypoxic group (Hx), SOD

activity showed a significant decrease in V_{max} ($p<0.001$), whereas in oxygen supplemented hypoxic rats (Hx + O) showed significant increase in V_{max} ($p<0.001$) was observed compared to control. The SOD activity in glucose supplemented group (Hx + G) showed reversal to the near control level with a significant increase in V_{max} ($p<0.01$) compared to the hypoxic group. In Hx +G + O group V_{max} showed a significant increase ($p<0.001$) compared to hypoxic group, showing a reversal to near control level. Hx + G + E + O group showed a significant decrease in V_{max} ($p<0.001$) to near hypoxic groups (Figure- 2, Table- 3).

CATALASE (CAT) ACTIVITY IN THE HEART OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

In heart of adult Wistar rats, CAT showed a higher activity ($p<0.001$) with a higher affinity ($p<0.05$) compared to neonatal control rats. In hypoxic group (Hx), a significant decrease in V_{max} ($p<0.01$) with a decreased affinity ($p<0.05$) was observed, which accounted for the free radial injury during hypoxic shock. In glucose supplemented hypoxic group, (Hx + G), V_{max} showed a significant increase ($p<0.01$) compared to hypoxic group, showing a reversal of the activity to control. In Hx + O group both V_{max} ($p<0.01$) exhibited a significant decrease compared to control. In Hx +G + O group V_{max} and K_m showed a reversal to near control value with significant increase in V_{max} ($p<0.05$) compared to hypoxic group. In Hx + G + E + O group V_{max} ($p<0.001$) showed a significant decrease to near hypoxic rats (Figure- 3, Table- 4).

CATALASE ACTIVITY IN THE CEREBRAL CORTEX OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

In cerebral cortex the CAT activity showed a significant increase in V_{max} ($p<0.001$) and a significant decrease in K_m ($p<0.001$) in adult rats compared to neonates. In hypoxic group (Hx) and oxygen supplemented hypoxic group (Hx + O), CAT activity showed a significant decrease in V_{max} ($p<0.001$) compared to

control. The V_{max} of glucose supplemented Hx + G and Hx +G + O groups showed reversal to the near control level with a significant increase in V_{max} ($p<0.001$) compared to the hypoxic group, showing a reversal to near control level. Hx + G + E + O group showed a significant decrease in V_{max} ($p<0.001$) to near hypoxic groups (Figure- 4, Table- 5).

TRIIODOTHYRONINE (T3) CONTENT IN THE HEART OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

Hypoxic neonatal rats showed a significant ($p<0.001$) decrease in T3 content in the heart compared to the control. The T3 content in Hx + G and Hx + G + O showed a reversal to near control. In Hx+O, Hx + E and Hx + G + E + O no reversal in to control was observed in the T3 content (Figure- 5, Table- 6).

TRIIODOTHYRONINE (T3) CONCENTRATION IN THE SERUM OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

Hypoxic neonatal rats showed a significant ($p<0.001$) decrease in T3 content in serum compared to the control. The T3 content in Hx + G and Hx + G + O showed a reversal to near control. In Hx+O, Hx + E and Hx + G + E + O the altered T3 content showed no reversal (Figure- 6, Table- 7).

CIRCULATING INSULIN LEVEL IN THE PLASMA OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

Hypoxic neonatal rats showed a significant increase ($p<0.001$) in circulating insulin level compared to control. There was a significant increase ($p<0.001$) in circulating insulin level of Hx + G, Hx + G + O, Hx + E and Hx + G + E + O compared to hypoxic and control neonates (Figure- 7, Table- 8).

INSULIN RECEPTOR GENE EXPRESSION IN THE HEART OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

The gene expression of insulin receptor showed a significant up regulation ($p<0.01$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant up regulation of the gene expression to near control. In Hx + O, there was a significant down regulation whereas in epinephrine supplemented groups the expression showed a significant up regulation compared to control (Figure- 8, Table- 9).

TRIIODOTHYRONINE (T3) RECEPTOR FUNCTION IN THE HEART OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

[^{125}I] Triiodothyronine binding parameters in the heart of hypoxic neonatal rats showed a significant decrease in B_{max} ($p<0.001$), with a significant increase ($p<0.001$) in K_d compared to control. This reflected a decreased receptor number with lower affinity in the heart of hypoxic neonatal rats compared to control. Glucose supplementation to hypoxic neonates, Hx + G and Hx + G + O, reversed the altered receptor status to near control. In Hx + O, Hx + E and Hx + G + E + O there was a significant increase in B_{max} ($p<0.001$) compared to control with no reversal in B_{max} (Figure- 9, 10 & Table- 10, 11).

GABA CONTENT (µMOLES/G WET WT.) IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL GROUPS OF NEONATAL RATS.

GABA content in the cerebral cortex, cerebellum, brain stem and corpus striatum showed a significant decrease ($p<0.001$) in Hx, Hx+O, Hx+E and Hx+G+E+O compared to control. Glucose resuscitation, Hx+G and Hx+G+O significantly ($p<0.001$) reversed the GABA content to near control (Table- 12).

SEROTONIN AND ITS METABOLITES CONTENT (NANOMOLES/G WET WT.) IN THE CEREBELLUM OF CONTROL AND EXPERIMENTAL NEONATAL RATS

5-HT content in the cerebellum showed a significant increase ($p<0.001$) in Hx, Hx+O, Hx+E and Hx+G+E+O compared to control. Glucose resuscitation, Hx+G and Hx+G+O significantly ($p<0.001$) reversed the 5-HT contents to near control. 5-HIAA content significantly decreased ($p<0.01$) in Hx, Hx+O, Hx+E and Hx+G+E+O compared to control. The 5-HIAA content was reversed to near control in Hx+G and Hx+G+O (Table- 13).

SEROTONIN AND ITS METABOLITES CONTENT (NANOMOLES/G WET WT.) IN THE BRAIN STEM OF CONTROL AND EXPERIMENTAL NEONATAL RATS

5-HT content in the brain stem showed a significant increase ($p<0.001$) in Hx, Hx+O, Hx+E and Hx+G+E+O compared to control. Hx+G and Hx+G+O showed a significant ($p<0.001$) reversal of 5-HT contents to near control. 5-HIAA content significantly decreased ($p<0.01$) in Hx, Hx+O, Hx+E and Hx+G+E+O compared to control. The 5-HIAA content was reversed to near control in Hx+G and Hx+G+O (Table- 14).

NEUROTRANSMITTERS, HIF1A, SUPEROXIDE DISMUTASE, GLUTHATIONE PEROXIDASE, BAX, CREB, AND PHOSPHOLIPASE C EXPRESSION IN THE BRAIN REGIONS OF EXPERIMENTAL NEONATAL RATS

CEREBRAL CORTEX

TOTAL GABA RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the cerebral cortex of control and experimental neonatal rats

Scatchard analysis of [^{3}H] GABA against GABA in the cerebral cortex of hypoxic rats showed a significant decrease ($p<0.01$) in B_{max} with significantly increased ($p<0.001$) K_d compared to control. It shows the decreased GABA receptor number with less affinity in neonatal rats exposed to hypoxia. In Hx + G and Hx + G + O, the receptor number ($p<0.001$) and affinity ($p<0.001$, $p<0.01$ respectively) showed a significant reversal to near control. In Hx + O, the B_{max} was significantly decreased ($p<0.001$) compared to control with a significant increase in affinity ($p<0.001$). Epinephrine supplemented groups, Hx + E and Hx + G + E + O, did not show significant reversal in the receptor number whereas the affinity of the receptor was significantly increased ($p<0.001$) compared to control (Table- 15, 16 & Figure- 11, 12).

Displacement Analysis of GABA in the cerebral cortex

The competition curve for GABA against [^{3}H] GABA fitted for two-sited model in all the groups with Hill slope value away from Unity. The $Ki_{(H)}$ increased in hypoxic neonatal rats along with an increase in the log (EC_{50})-1 indicating a shift in high affinity towards low affinity. $Ki_{(L)}$ also showed an increase in hypoxic neonatal rats with an increase in log (EC_{50})-2 denoting a shift in the low affinity site towards much lower affinity. (Figure- 13, Table- 17).

$GABA_A$ RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the cerebral cortex of control and experimental neonatal rats.

Scatchard analysis of [^{3}H] bicuculline against bicuculline in the cerebral cortex of hypoxic rats showed a significant decrease ($p<0.001$) in B_{max} and a significant increase ($p<0.001$) in K_d compared to control. In glucose supplemented group, Hx + G the receptor number showed a significant reversal ($p<0.01$) to near control with significantly decreased ($p<0.001$) affinity. In Hx + G + O, both receptor number ($p<0.001$) and affinity ($p<0.001$) reversed to near control. In Hx + O, the B_{max} was significantly decreased ($p<0.001$) with significant increase ($p<0.001$) in K_d compared to control. In Hx + E and Hx + G + E + O, there was no significant reversal in the altered receptor number to control whereas in Hx + G + E + O the affinity showed a reversal to near control. (Figure- 14, 15 & Table- 18, 19).

$GABA_B$ RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the cerebral cortex of control and experimental neonatal rats.

In hypoxic group, $GABA_B$ receptors was significantly decreased ($p<0.001$) with a significant increase in K_d ($p<0.001$) compared to control. Hx + G, Hx + G + O and Hx + O showed a significant reversal of B_{max} ($p<0.001$, $p<0.001$, $p<0.05$ respectively) to near control. In epinephrine supplemented groups, Hx + E and Hx + G + E + O the receptor number and affinity were not significantly reversed to near control (Figure- 16, 17 & Table- 20, 21).

REAL-TIME PCR ANALYSIS OF $GABA_A$, $GABA_B$ RECEPTOR SUBTYPES AND GAD

Real Time PCR analysis of $GABA_A$ receptor subtypes mRNA.

The gene expression studies showed that $GABA_{A\alpha1}$, $GABA_{A\alpha5}$ and $GABA_{A\gamma3}$ receptor subunits mRNA were significantly down regulated ($p<0.001$) whereas $GABA_{A\delta}$ was significantly up regulated ($p<0.05$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal of the gene expression of $GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\delta}$ and $GABA_{A\gamma3}$ to near control ($p<0.001$). In Hx + O, $GABA_{A\alpha5}$ and $GABA_{A\gamma3}$ expression showed a significant reversal ($p<0.001$, $p<0.01$ respectively) to near control. Epinephrine supplemented groups, Hx + E and Hx + G + E + O showed no significant reversal in $GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\gamma3}$ and $GABA_{A\delta}$ receptor gene expression to near control. (Figure- 18 - 21 & Table- 22 - 25).

Real Time PCR analysis of $GABA_B$ receptor subtypes mRNA.

The gene expression of $GABA_B$ receptor showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal of the gene expression to near control ($p<0.001$). In Hx + O, Hx + E and Hx + G + E + O there was a significant down regulation in $GABA_B$ receptor gene expression ($p<0.05$, $p<0.001$, $p<0.001$ respectively) compared to control. Epinephrine supplemented groups did not show significant reversal in the altered $GABA_B$ receptor expression to near control (Figure- 22, Table- 26).

Real Time PCR analysis of GAD mRNA.

The expression of glutamate decarboxylase showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O, the GAD gene expression was reversed to near control ($p<0.001$). In Hx + O, Hx + E and Hx + G + E + O, the gene expression was significantly down

regulated ($p<0.01$, $p<0.01$, $p<0.001$ respectively) compared to control without a reversal of altered enzyme expression to near control. (Figure- 23, Table- 27).

CONFOCAL STUDIES OF $GABA_{A\alpha1}$ RECEPTOR ANTIBODY STAINING.

$GABA_{A\alpha1}$ receptor antibody staining in the cerebral cortex of control and experimental neonatal rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the cerebral cortex showed a significant decrease ($p<0.001$) in the mean pixel value in hypoxic neonatal rats compared to control. Glucose resuscitation, alone and along with oxygen to hypoxic neonatal rats significantly reversed ($p<0.001$) the $GABA_{A\alpha1}$ receptor expression in the cerebral cortex to near control. The mean pixel intensity of Hx + O, Hx + E and Hx + G + E + O was significantly decreased ($p<0.001$) compared to control, showing that the expression of $GABA_{A\alpha1}$ receptor was not reversed to control in these groups (Figure- 24 Table- 28,).

TOTAL 5-HT RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] 5-HT binding against 5-HT to total 5-HT receptor in the cerebral cortex of control and experimental neonatal rats

Scatchard analysis of [^{3}H] 5-HT against 5-HT in the cerebral cortex of hypoxic neonatal rats showed a significant increase ($p<0.01$) in B_{max} with significantly increased ($p<0.001$) K_d compared to control. In Hx + G and Hx + G + O, the receptor number ($p<0.001$) and affinity ($p<0.001$) showed a reversal to near control. In Hx + O, the B_{max} was significantly increased ($p<0.001$) compared to control with a reversal of affinity ($p<0.001$) to near control. Epinephrine supplemented groups, Hx + E and Hx + G + E + O, did not show significant reversal in the receptor number to near control whereas the affinity of the receptor was significantly increased ($p<0.01$). (Figure- 25, 26 & Table- 29, 30).

5-HT_{2A} RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the cerebral cortex of control and experimental neonatal rats

Scatchard analysis of [^{3}H] ketanserin against ketanserin in the cerebral cortex of hypoxic neonatal rats showed a significant increase ($p<0.01$) in B_{max} with significantly decreased ($p<0.001$) K_d compared to control. In Hx + G and Hx + G + O, B_{max} was significantly increased ($p<0.01$, $p<0.001$ respectively) compared to control. In Hx + O, the B_{max} was significantly increased ($p<0.001$) with a significant decrease ($p<0.001$) in K_d compared to control. In Hx + E and Hx + G + E + O, B_{max} did not show significant reversal to near control (Figure- 27, 28 & Table- 31, 32).

REAL-TIME PCR ANALYSIS OF 5-HT_{2A} RECEPTOR SUBTYPE AND 5-HTT.

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

The gene expression studies showed that 5-HT_{2A} receptor subunit mRNA was significantly up regulated ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the gene expression of 5-HT_{2A} to near control. In Hx + E and Hx + G + E + O there was a significant up regulation ($p<0.001$) in 5-HT_{2A} receptor gene expression compared to control, indicating that the altered expression was not reversed to near control. (Figure- 29, Table- 33).

Real Time PCR analysis of 5-HTT mRNA.

The gene expression studies showed that 5-HTT mRNA was significantly up regulated ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant down regulation ($p<0.01$, $p<0.001$ respectively) of the gene expression compared to control. In Hx + O, the gene expression of 5-HTT was up regulated ($p<0.001$) compared to control. In Hx + E

and Hx + G + E + O no significant reversal was observed in 5-HTT expression compared to control. (Figure- 30, Table- 34).

CONFOCAL STUDIES OF 5-HT_{2A} RECEPTOR AND 5-HTT ANTIBODY STAINING.

5-HT_{2A} receptor antibody staining in the cerebral cortex of control and experimental neonatal rats.

5-HT_{2A} receptor subunit antibody staining in the cerebral cortex showed a significant increase ($p<0.001$) in the mean pixel value in hypoxic neonatal rats compared to control. Glucose resuscitation, alone and along with oxygen to hypoxic neonatal rats significantly reversed ($p<0.001$) the 5-HT_{2A} receptor expression in the cerebral cortex to near control. The mean pixel intensity of Hx + O, Hx + E and Hx + G + E + O was significantly increased ($p<0.001$) compared to control indicating that these resuscitation did not reverse the altered 5-HT_{2A} receptor expression (Figure- 31, Table- 35).

5-HTT antibody staining in the cerebral cortex of control and experimental neonatal rats.

5-HTT antibody staining in the cerebral cortex showed a significant increase ($p<0.001$) in the mean pixel value in hypoxic neonatal rats compared to control. Glucose resuscitation, alone and along with oxygen to hypoxic neonatal rats significantly reversed ($p<0.001$) the 5-HTT expression in the cerebral cortex to near control. The mean pixel intensity of Hx + O, Hx + E and Hx + G + E + O was significantly increased ($p<0.001$) compared to control with out significant reversal in the transporter expression to control (Figure- 32, Table- 36).

TOTAL MUSCARINIC RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the cerebral cortex of control and experimental neonatal rats

Scatchard analysis of [^{3}H] QNB against atropinne in the cerebral cortex of hypoxic neonatal rats showed a significant decrease (p<0.001) in B_{max} compared to control. In Hx + G and Hx + G + O, the receptor number (p<0.001) and affinity (p<0.001) showed a reversal to near control. In Hx + O, the B_{max} was significantly decreased (p<0.001) compared to control. The altered receptor number and affinity were not reversed by epinephrine resuscitated groups, Hx + E and Hx + G + E + O (Figure- 33, 34 & Table- 37, 38).

REAL-TIME PCR ANALYSIS OF MUSCARINIC RECEPTOR SUBTYPES, ChAT, AChE, HIF, SOD, GPx, BAX, CREB AND PHOSPHOLIPASE C

Real Time PCR analysis of muscarinic M1 receptor mRNA.

The gene expression studies showed that muscarinic M1 receptor subunit mRNA was significantly down regulated (p<0.001) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal (p<0.001) of the gene expression compared to control. In Hx + O, the gene expression was down regulated (p<0.01) compared to control. In Hx + E and Hx + G + E + O there was no significant reversal in the gene expression to near control. (Figure- 35, Table- 39).

Real Time PCR analysis of muscarinic M2 receptor mRNA.

The gene expression studies showed that muscarinic M2 receptor subunit mRNA was significantly down regulated (p<0.001) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal (p<0.001) of the gene expression compared to control. In Hx + O, the gene expression was down regulated (p<0.01) compared to control. Epinephrine

resuscitated groups, Hx + E and Hx + G + E + O showed no significant reversal in the gene expression to near control. (Figure- 36, Table- 40).

Real Time PCR analysis of muscarinic M3 receptor mRNA.

The gene expression studies showed that muscarinic M3 receptor subunit mRNA was significantly down regulated ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal ($p<0.001$) of the gene expression compared to control. In Hx + O, the gene expression was down regulated ($p<0.01$) compared to control. In Hx + E and Hx + G + E + O no significant reversal was observed in the altered gene expression to control. (Figure- 37, Table- 41).

Real Time PCR analysis of choline acetyl transferase mRNA.

The gene expression of choline acetyl transferase showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression to near control ($p<0.001$, $p<0.001$, $p<0.01$ respectively). In epinephrine supplemented groups, Hx + E and Hx + G + E + O there was no significant reversal in choline acetyl transferase gene expression to control. (Figure- 38, Table- 42).

Real Time PCR analysis of acetyl choline esterase mRNA.

The gene expression of acetyl choline esterase showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression to near control ($p<0.001$, $p<0.001$, $p<0.01$ respectively). Acetyl choline esterase gene expression was not reversed significantly to control by epinephrine resuscitation, Hx + E and Hx + G + E + O. (Figure- 39, Table- 43).

Real Time PCR analysis of HIF1 mRNA.

The gene expression of transcription factor HIF1 showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression to near control ($p<0.001$). In Hx + E and Hx + G + E + O there was a significant down regulation in HIF1 gene expression ($p<0.001$) compared to control without a significant reversal in the altered HIF1 expression (Figure- 40, Table- 44).

Real Time PCR analysis of SOD mRNA.

The gene expression of antioxidant enzyme SOD showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal of the gene expression to near control ($p<0.001$). In Hx + O, there is a significant up regulation ($p<0.001$) in the gene expression of SOD. Resuscitation with epinephrine, Hx + E and Hx + G + E + O, did not significantly reverse the altered SOD gene expression to control. (Figure- 41, Table- 45).

Real Time PCR analysis of GPx mRNA.

The gene expression of antioxidant enzyme GPx showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal of the gene expression to near control ($p<0.001$). In Hx + O, there is a significant up regulation ($p<0.001$) in the gene expression of GPx. The altered GPx expression was not reversed significantly in Hx + E and Hx + G + E + O. (Figure- 42, Table- 46).

Real Time PCR analysis of Bax mRNA.

Pro-apoptotic gene Bax mRNA expression showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant down regulation ($p<0.001$) of the Bax expression

compared to control. In Hx + O, Hx + E and Hx + G + E + O there was a significant up regulation ($p<0.001$) in the gene expression compared to control indicating that the alterations due to hypoxic insult was not reversed to near control (Figure- 43, Table- 47).

Real Time PCR analysis of CREB mRNA.

Transcription factor CREB expression showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the CREB expression compared to near control. In epinephrine resuscitated groups, Hx + E and Hx + G + E + O there was no significant reversal in CREB mRNA expression to near control (Figure- 44, Table- 48).

Real Time PCR analysis of Phospholipase C mRNA.

The gene expression of phospholipase C showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the expression to near control. In Hx + E and Hx + G + E + O there was a significant up regulation ($p<0.001$) in the gene expression compared to control without a significant reversal in the expression to near control (Figure- 45, Table- 49).

CEREBELLUM

TOTAL GABA RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the cerebellum of control and experimental neonatal rats

Scatchard analysis of [^{3}H] GABA against GABA in the cerebellum of hypoxic rats showed a significant decrease ($p<0.01$) in B_{max} with significantly increased ($p<0.001$) K_d compared to control. In Hx + G and Hx + G + O, the receptor number ($p<0.001$) and affinity ($p<0.001$) showed a reversal to near

control. In Hx + O, the B_{max} was significantly decreased ($p<0.001$) with a significant increase in K_d ($p<0.001$) compared to control. Epinephrine supplemented groups, Hx + E and Hx + G + E + O, showed no significant reversal in the receptor number to near control whereas the affinity showed a slight reversal ($p<0.001$) to control (Figure- 46, 47 & Table- 50, 51).

Displacement Analysis of [^{3}H] GABA in the cerebellum

The competition curve for GABA against [^{3}H] GABA fitted for two-sited model in all the groups with Hill slope value away from Unity. The Ki(H) increased in hypoxic neonatal rats along with an increase in the log (EC_{50})-1 indicating a shift in high affinity towards low affinity. Ki(L) also showed an increase in hypoxic neonatal rats with an increase in log (EC_{50})-2 denoting a shift in the low affinity site towards much lower affinity (Figure- 48, Table- 52).

$GABA_A$ RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the cerebellum of control and experimental neonatal rats.

Scatchard analysis of [^{3}H] bicuculline against bicuculline in the cerebellum of hypoxic rats showed a significant decrease in B_{max} ($p<0.001$) and K_d ($p<0.001$) compared to control. In glucose supplemented group, Hx + G and Hx + G + O, there was a significant reversal in receptor number ($p<0.01$) to near control with significantly decreased ($p<0.001$) affinity. In Hx + O, the B_{max} was significantly decreased ($p<0.001$) with a reversal in K_d ($p<0.001$) compared to control. The receptor number was not reversed significantly in epinephrine resuscitated groups, Hx + E and Hx + G + E + O. (Figure- 49, 50 & Table- 53, 54).

$GABA_B$ RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the cerebellum of control and experimental neonatal rats.

In hypoxic group, $GABA_B$ receptors was significantly decreased ($p<0.001$) with a significant increase in affinity ($p<0.001$) compared to control. Hx + G and Hx + G + O showed a significant reversal of B_{max} ($p<0.001$) and K_d ($p<0.01$) to near control. In Hx + O, B_{max} ($p<0.001$) and K_d ($p<0.001$) was significantly decreased compared to control. In Hx + E and Hx + G + E + O the $GABA_B$ receptor number showed no significant reversal to near control. (Figure- 51, 52 & Table- 55, 56).

REAL-TIME PCR ANALYSIS OF $GABA_A$, $GABA_B$ RECEPTOR SUBTYPES AND GAD

Real Time PCR analysis of $GABA_A$ receptor subtypes mRNA.

The gene expression studies showed that $GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\gamma3}$ and $GABA_{A\delta}$ receptor subunits mRNA were significantly down regulated ($p<0.001$) in the hypoxic neonatal rats compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the gene expression of $GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\delta}$ and $GABA_{A\gamma3}$ to near control. Resuscitation with epinephrine, Hx + E and Hx + G + E + O, did not reverse the altered $GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\gamma3}$ and $GABA_{A\delta}$ receptor expression to near control. (Figure- 53 – 56 & Table- 57 - 60).

Real Time PCR analysis of $GABA_B$ receptor subtypes mRNA.

The gene expression of $GABA_B$ receptor showed a significant down regulation ($p<0.001$) in the hypoxic neonates compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression ($p<0.001$, $p<0.001$ and $p<0.05$ respectively) to near control. Epinephrine

supplementation, Hx + E and Hx + G + E + O, showed no significant reversal in $GABA_B$ receptor gene expression to near control. (Figure- 57, Table- 61).

Real Time PCR analysis of GAD mRNA.

The expression of GAD showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O, GAD expression was reversed to near control ($p<0.001$, $p<0.001$, $p<0.01$ respectively). In Hx + E and Hx + G + E + O, the gene expression was significantly down regulated ($p<0.01$, $p<0.001$ respectively) without a significant reversal in the expression to near control. (Figure- 58, Table- 62).

CONFOCAL STUDIES OF $GABA_{A\alpha1}$ RECEPTOR ANTIBODY STAINING.

$GABA_{A\alpha1}$ receptor antibody staining in the cerebellum of control and experimental neonatal rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the cerebellum showed a significant decrease ($p<0.001$) in the mean pixel value in hypoxic neonatal rats compared to control. Glucose resuscitation, alone and along with oxygen to hypoxic neonatal rats significantly reversed ($p<0.001$) the $GABA_{A\alpha1}$ receptor expression in the cerebellum to near control. The mean pixel intensity of Hx + O, Hx + E and Hx + G + E + O was significantly decreased ($p<0.001$) compared to control showing that $GABA_{A\alpha1}$ receptor expression was not reversed to control by resuscitation with 100% oxygen and epinephrine. (Figure- 59, Table- 63).

TOTAL 5-HT RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] 5-HT binding against 5-HT to total 5-HT receptor in the cerebellum of control and experimental neonatal rats

Scatchard analysis of [^{3}H] 5-HT against 5-HT in the cerebellum of hypoxic neonatal rats showed a significant increase ($p<0.01$) in B_{max} with a significant decrease ($p<0.001$) in K_d compared to control. In Hx + G and Hx + G +

O, the receptor number ($p<0.001$) and affinity ($p<0.001$) showed a reversal to near control. In Hx + O, the B_{max} and affinity was significantly increased ($p<0.001$) compared to control. Hx + E and Hx + G + E + O showed no significant reversal in the receptor number to near control whereas the affinity of the receptor was reversed ($p<0.001$) to near control (Figure- 60, 61 & Table- 64, 65).

5-HT_{2A} RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the cerebellum of control and experimental neonatal rats

Scatchard analysis of [^{3}H] ketanserin against ketanserin in the cerebellum of hypoxic neonatal rats showed a significant increase ($p<0.01$) in B_{max} with significantly increased ($p<0.001$) affinity compared to control. In Hx + G and Hx + G + O, B_{max} showed a reversal ($p<0.001$) to near control. In Hx + O, the B_{max} was significantly decreased ($p<0.001$) with a reversal ($p<0.001$) in K_d compared to control. In Hx + E and Hx + G + E + O no significant reversal in the receptor number was observed to near control (Figure- 62, 63 & Table- 66, 67).

REAL-TIME PCR ANALYSIS OF 5-HT_{2A} RECEPTOR SUBTYPE AND 5-HTT.

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

The gene expression studies showed that 5-HT_{2A} receptor subunit mRNA was significantly up regulated ($p<0.001$) in the hypoxic neonatal rats compared to control. In Hx + G and Hx + G + O there was a significant reversal ($p<0.001$) of 5-HT_{2A} receptor expression to near control. Hx + O showed a significant down regulation ($p<0.001$) in the receptor gene expression compared to control. In Hx + E and Hx + G + E + O there was a no significant reversal in 5-HT_{2A} receptor gene expression to near control. (Figure- 64, Table- 68).

Real Time PCR analysis of 5-HTT mRNA.

The gene expression studies showed that 5-HTT mRNA was significantly up regulated ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal ($p<0.001$) of the gene expression to near control. In Hx + O the gene expression of 5-HTT was significantly down regulated ($p<0.001$) compared to control. Resuscitation with epinephrine, Hx + E and Hx + G + E + O, showed no significant reversal in the altered 5-HTT gene expression to near control. (Figure- 65, Table- 69).

CONFOCAL STUDIES OF 5-HT_{2A} RECEPTOR AND 5-HTT ANTIBODY STAINING.

5-HT_{2A} receptor antibody staining in the cerebellum of control and experimental neonatal rats.

5-HT_{2A} receptor subunit antibody staining in the cerebellum showed a significant increase ($p<0.001$) in the mean pixel value in hypoxic neonatal rats compared to control. Hx + G and Hx + G + O showed a significant reversal ($p<0.001$) in 5-HT_{2A} receptor expression in the cerebellum to near control. The mean pixel intensity of Hx + O, Hx + E and Hx + G + E + O was significantly increased ($p<0.001$) compared to control indicating that 5-HT_{2A} receptor expression was not altered to near control (Figure- 66, Table-70).

5-HTT antibody staining in the cerebellum of control and experimental neonatal rats.

5-HTT antibody staining in the cerebellum showed a significant increase ($p<0.001$) in the mean pixel value in hypoxic neonatal rats compared to control. Glucose resuscitation, alone and along with oxygen to hypoxic neonatal rats significantly reversed ($p<0.001$) the 5-HTT expression in the cerebellum to near control. The mean pixel intensity of Hx + O, Hx + E and Hx + G + E + O showed no significant reversal to near control (Figure- 67, Table- 71).

TOTAL MUSCARINIC RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the cerebellum of control and experimental neonatal rats.

Scatchard analysis of [^{3}H] QNB against atropinne in the cerebellum of hypoxic neonatal rats showed a significant decrease (p<0.001) in B_{max} and K_d compared to control. In Hx + G and Hx + G + O, the receptor number (p<0.001) showed a reversal to near control. In Hx + O, the B_{max} and K_d was significantly decreased (p<0.001) compared to control. In epinephrine resuscitated groups, Hx + E and Hx + G + E + O, the receptor number and affinity did not reverse significantly to near control (Figure- 68, 69 & Table- 72, 73).

REAL-TIME PCR ANALYSIS OF MUSCARINIC RECEPTOR SUBTYPES, ChAT, AChE, HIF, SOD, GPx, BAX, CREB AND PHOSPHOLIPASE C

Real Time PCR analysis of muscarinic M1 receptor mRNA.

The gene expression studies showed that muscarinic M1 receptor subunit mRNA was significantly down regulated (p<0.001) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal (p<0.001) of the gene expression compared to control. The gene expression of muscarinic M1 receptor in Hx + O, Hx + E and Hx + G + E + O showed a significant down regulation (p<0.001) indicating that there is no significant reversal in the receptor subunit expression to near control. (Figure- 70, Table- 74).

Real Time PCR analysis of muscarinic M2 receptor mRNA.

The gene expression studies showed that muscarinic M2 receptor subunit mRNA was significantly down regulated (p<0.001) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal (p<0.001) of the gene expression compared to control. Epinephrine

supplemented groups, Hx + E and Hx + G + E + O, did not reverse the altered gene expression to near control. (Figure- 71, Table- 75).

Real Time PCR analysis of muscarinic M3 receptor mRNA.

The gene expression studies showed that muscarinic M3 receptor subunit mRNA was significantly down regulated ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$, $p<0.001$, $p<0.01$ respectively) of the gene expression compared to control. Gene expression of muscarinic M3 receptor subunit showed no significant reversal in Hx + E and Hx + G + E + O to near control. (Figure- 72, Table- 76).

Real Time PCR analysis of acetyl choline esterase mRNA.

The gene expression of acetyl choline esterase showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression to near control ($p<0.001$, $p<0.001$, $p<0.05$ respectively). Epinephrine resuscitation, Hx + E and Hx + G + E + O did not show a significant reversal in the up regulated acetyl choline esterase gene expression to control. (Figure- 73, Table- 77).

Real Time PCR analysis of choline acetyl transferase mRNA.

The gene expression of choline acetyl transferase showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression to near control ($p<0.001$, $p<0.001$, $p<0.05$ respectively). In Hx + E and Hx + G + E + O there was no significant reversal in the down regulated choline acetyl transferase gene expression to control. (Figure- 74, Table- 78).

Real Time PCR analysis of HIF1 mRNA.

The gene expression of transcription factor HIF1 showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression to near control ($p<0.001$) whereas in Hx + E and Hx + G + E + O there was no significant reversal in the up regulated HIF1 gene expression to near control. (Figure- 75, Table- 79).

Real Time PCR analysis of SOD mRNA.

The gene expression of antioxidant enzyme SOD showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal of the gene expression to near control ($p<0.001$). In Hx + O, there is a significant up regulation ($p<0.001$) in the gene expression of SOD compared to control. The down regulated SOD gene expression in hypoxic group showed no significant reversal in epinephrine resuscitated groups, Hx + E and Hx + G + E + O. (Figure- 76, Table- 80).

Real Time PCR analysis of GPx mRNA.

The gene expression of antioxidant enzyme GPx showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal ($p<0.001$) of the gene expression to near control. In Hx + O, there is a significant up regulation ($p<0.001$) in the gene expression of GPx. Epinephrine resuscitation, Hx + E and Hx + G + E + O, showed no significant reversal in the altered GPx expression to near control. (Figure- 77, Table- 81).

Real Time PCR analysis of Bax mRNA.

Bax mRNA expression showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a

significant reversal ($p<0.001$) of Bax expression to near control. In Hx + O, Hx + E and Hx + G + E + O the Bax expression was up regulated ($p<0.001$) without a significant reversal of gene expression to near control. (Figure- 78, Table- 82).

Real Time PCR analysis of CREB mRNA.

The gene expression of transcription factor CREB mRNA showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the CREB expression to near control. Epinephrine resuscitation, Hx + E and Hx + G + E + O, showed no significant reversal in the altered gene expression to control. (Figure- 79, Table- 83).

Real Time PCR analysis of Phospholipase C mRNA.

The gene expression of phospholipase C mRNA showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the expression to near control whereas in Hx + E and Hx + G + E + O there was no significant reversal of the altered expression to near control. (Figure- 80, Table- 84).

BRAIN STEM

TOTAL GABA RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the brain stem of control and experimental neonatal rats

Scatchard analysis of [^{3}H] GABA against GABA in the brain stem of hypoxic rats showed a significant decrease in B_{max} ($p<0.001$) and K_d ($p<0.001$) compared to control. In Hx + G and Hx + G + O, the receptor number ($p<0.001$) showed a reversal to near control. In Hx + O, the B_{max} was significantly decreased ($p<0.001$) with a reversal in K_d ($p<0.001$) to near control. The receptor number in

epinephrine supplemented groups, Hx + E and Hx + G + E + O, showed no significant reversal to near control (Figure- 81, 82 & Table- 85, 86).

Displacement Analysis of [^{3}H] GABA in the brain stem

The competition curve for GABA against [^{3}H] GABA fitted for two-sited model in all the groups with Hill slope value away from Unity. The $Ki_{(H)}$ increased in hypoxic neonatal rats along with an increase in the log (EC_{50})-1 indicating a shift in high affinity towards low affinity. $Ki_{(L)}$ also showed an increase in hypoxic neonatal rats with an increase in log (EC_{50})-2 denoting a shift in the low affinity site towards much lower affinity (Figure- 83, Table- 87).

$GABA_A$ RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the brain stem of control and experimental neonatal rats.

Scatchard analysis of [^{3}H] bicuculline against bicuculline in the brain stem of hypoxic rats showed a significant decrease in B_{max} (p<0.001) and K_d (p<0.001) compared to control. In glucose supplemented group, Hx + G and Hx + G + O, there was a significant reversal in receptor number (p<0.01) to near control with a significant decrease (p<0.001) in affinity. In Hx + O, the B_{max} was significantly decreased (p<0.001) with a reversal in K_d (p<0.001) to near control. In Hx + E the B_{max} increased significantly (p<0.001) with out a significant reversal in the receptor affinity to control whereas in Hx + G + E + O the K_d decreased significantly (p<0.001) without a significant reversal in the receptor number to near control. (Figure- 84, 85 & Table- 88, 89).

$GABA_B$ RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the brain stem of control and experimental neonatal rats.

In hypoxic group, $GABA_B$ receptors was significantly decreased ($p<0.001$) with a significant increase in K_d ($p<0.05$) compared to control. Hx + G and Hx + G + O showed a significant reversal of B_{max} ($p<0.01$, $p<0.001$ respectively) to near control. In Hx + O, B_{max} ($p<0.001$) and affinity ($p<0.001$) was significantly decreased compared to control. In epinephrine resuscitated groups, Hx + E and Hx + G + E + O, the receptor number and affinity of $GABA_B$ receptors showed no significant reversal to near control. (Figure- 86, 87 & Table- 90, 91).

REAL-TIME PCR ANALYSIS OF $GABA_A$, $GABA_B$ RECEPTOR SUBTYPES AND GAD

Real Time PCR analysis of $GABA_A$ receptor subtypes mRNA

The gene expression studies showed that $GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\gamma3}$ and $GABA_{A\delta}$ receptor subunits mRNA were significantly down regulated ($p<0.001$) in the hypoxic neonatal rats compared to control. In Hx + G and Hx + G + O there was a significant reversal ($p<0.001$) of the gene expression of $GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\delta}$ and $GABA_{A\gamma3}$ receptors to near control. In Hx + O, the gene expression of $GABA_{A\alpha1}$ receptor showed a significant reversal ($p<0.001$) to near control. In Hx + E the gene expression of $GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\gamma3}$ and $GABA_{A\delta}$ receptors showed up regulation ($p<0.001$) without a significant reversal to control. In Hx + G + E + O the gene expression of $GABA_{A\alpha1}$, $GABA_{A\alpha5}$ and $GABA_{A\delta}$ receptors showed significant down regulation ($p<0.001$) without a significant reversal in the expression to near control. (Figure- 88 – 91 & Table- 92 - 95).

Real Time PCR analysis of $GABA_B$ receptor subtypes mRNA.

The gene expression of $GABA_B$ receptor showed a significant down regulation ($p<0.001$) in the hypoxic neonates compared to control. In Hx + G, Hx + G + O there was a significant reversal of the gene expression ($p<0.001$) to near control. In Hx + O, Hx + E and Hx + G + E + O showed a down regulated $GABA_B$ receptor gene expression ($p<0.01$, $p<0.001$, $p<0.001$ respectively) with out a significant reversal to near control. (Figure- 92, Table- 96).

Real Time PCR analysis of GAD mRNA.

The expression of GAD showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O, GAD expression was reversed to near control ($p<0.001$) whereas in Hx + E and Hx + G + E + O, there was no significant reversal to near control. (Figure- 93, Table- 97).

CONFOCAL STUDIES OF $GABA_{A\alpha1}$ RECEPTOR ANTIBODY STAINING.

$GABA_{A\alpha1}$ receptor antibody staining in the brain stem of control and experimental neonatal rats

$GABA_{A\alpha1}$ receptor subunit antibody staining in the brain stem showed a significant decrease ($p<0.001$) in the mean pixel value in hypoxic neonatal rats compared to control. Glucose resuscitation, alone and along with oxygen to hypoxic neonatal rats significantly reversed ($p<0.001$) the $GABA_{A\alpha1}$ receptor expression in the cerebellum to near control. The mean pixel intensity of Hx + O, Hx + E and Hx + G + E + O showed significant decrease ($p<0.001$) without a significant reversal of reversal of $GABA_{A\alpha1}$ receptor expression to control. (Figure- 94, Table- 98).

TOTAL 5-HT RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] 5-HT binding against 5-HT to total 5-HT receptor in the brain stem of control and experimental neonatal rats

Scatchard analysis of [^{3}H] 5-HT against 5-HT in the brain stem of hypoxic neonatal rats showed a significant increase ($p<0.01$) in B_{max} with a significant decrease ($p<0.001$) in K_d compared to control. In Hx + G and Hx + G + O, the receptor number ($p<0.001$) and affinity ($p<0.001$) showed a reversal to near control. In Hx + O, the B_{max} and affinity was significantly increased ($p<0.001$) compared to control. Epinephrine resuscitated groups, Hx + E and Hx + G + E + O showed a significant increase in affinity ($p<0.001$) without a reversal of receptor number to near control (Figure- 95, 96 & Table- 99, 100).

5-HT$_{2A}$ RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT$_{2A}$ receptor in the brain stem of control and experimental neonatal rats

Scatchard analysis of [^{3}H] ketanserin against ketanserin in the brain stem of hypoxic neonatal rats showed a significant increase ($p<0.01$) in B_{max} with significantly increased ($p<0.001$) K_d compared to control. In Hx + G and Hx + G + O, B_{max} showed a reversal ($p<0.001$) to near control. In Hx + O, the B_{max} was significantly decreased ($p<0.001$) with a reversal ($p<0.001$) in K_d compared to control. In Hx + E and Hx + G + E + O, there was no significant reversal in B_{max} and K_d to near control (Figure- 97, 98 & Table- 101, 102).

REAL-TIME PCR ANALYSIS OF 5-HT$_{2A}$ RECEPTOR SUBTYPE AND 5-HTT.

Real Time PCR analysis of 5-HT$_{2A}$ receptor subtypes mRNA.

The gene expression studies showed that 5-HT$_{2A}$ receptor subunit mRNA was significantly up regulated ($p<0.001$) in the hypoxic neonatal rats compared to control. In Hx + G and Hx + G + O there was a significant reversal ($p<0.001$) of

5-HT_{2A} receptor expression to near control. Hx + O showed a significant down regulation ($p<0.05$) in the receptor gene expression compared to control. In Hx + E and Hx + G + E + O there was no significant reversal in the up regulated 5-HT_{2A} receptor gene expression to near control. (Figure- 99, Table- 103).

Real Time PCR analysis of 5-HTT mRNA.

The gene expression of 5-HTT mRNA showed that there was a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the gene expression to near control. Epinephrine supplementation, Hx + E and Hx + G + E + O, showed no significant reversal in the up regulated 5-HTT gene expression to near control. (Figure- 100, Table- 104).

CONFOCAL STUDIES OF 5-HT_{2A} RECEPTOR AND 5-HTT ANTIBODY STAINING.

5-HT_{2A} receptor antibody staining in the brain stem of control and experimental neonatal rats.

5-HT_{2A} receptor subunit antibody staining in the brain stem showed a significant increase ($p<0.001$) in the mean pixel value in hypoxic neonatal rats compared to control. Hx + G and Hx + G + O showed a significant reversal ($p<0.001$) the 5-HT_{2A} receptor expression in the brain stem to near control. The mean pixel intensity of Hx + O, Hx + E and Hx + G + E + O showed a significant increase ($p<0.001$) without a significant reversal in 5-HT_{2A} receptor subunit expression to near control. (Figure- 101, Table- 105).

5-HTT antibody staining in the brain stem of control and experimental neonatal rats.

5-HTT antibody staining in the brain stem showed a significant increase ($p<0.001$) in the mean pixel value in hypoxic neonatal rats compared to control.

Glucose resuscitation, alone and along with oxygen to hypoxic neonatal rats significantly reversed ($p<0.001$) the 5-HTT expression in the brain stem to near control. The mean pixel intensity of Hx + O, Hx + E and Hx + G + E + O was significantly increased ($p<0.001$) compared to control indicating that there was no reversal in 5-HTT expression to near control. (Figure- 102, Table -106).

TOTAL MUSCARINIC RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the brain stem of control and experimental neonatal rats

Scatchard analysis of [^{3}H] QNB against atropinne in the brain stem of hypoxic neonatal rats showed a significant decrease ($p<0.001$) in B_{max} and K_d compared to control. In Hx + G, B_{max} showed a significant reversal ($p<0.01$) to near control with a significantly decreased Kd ($p<0.001$) compared to control whereas Hx + G + O showed a significant reversal in the receptor number and affinity ($p<0.001$) to near control. In Hx + O, the B_{max} and K_d was significantly decreased ($p<0.001$) compared to control. In Hx + E and Hx + G + E + O, the receptor number showed no significant reversal to near control (Figure- 103, 104 & Table- 107, 108).

REAL-TIME PCR ANALYSIS OF MUSCARINIC RECEPTOR SUBTYPES, ChAT, AChE, HIF, SOD, GPx, BAX, CREB AND PHOSPHOLIPASE C

Real Time PCR analysis of muscarinic M1 receptor mRNA.

The gene expression studies showed that muscarinic M1 receptor subunit mRNA was significantly down regulated ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the gene expression compared to control whereas in Hx + E and Hx + G + E + O no significant reversal was observed compared to control. (Figure- 105, Table- 109).

Real Time PCR analysis of muscarinic M2 receptor mRNA.

The gene expression studies showed that muscarinic M2 receptor subunit mRNA was significantly down regulated ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$, $p<0.001$, $p<0.01$ respectively) of the gene expression compared to control. In epinephrine resuscitated groups, Hx + E and Hx + G + E + O, there was no significant reversal in the gene expression to near control. (Figure- 106, Table- 110).

Real Time PCR analysis of muscarinic M3 receptor mRNA.

The gene expression studies showed that muscarinic M3 receptor subunit mRNA was significantly down regulated ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$, $p<0.001$, $p<0.01$ respectively) of the gene expression compared to control. Epinephrine resuscitation, Hx + E and Hx + G + E + O, did not significantly reverse the altered muscarinic M3 receptor gene expression to near control. (Figure- 107, Table- 111).

Real Time PCR analysis of acetyl choline esterase mRNA.

The gene expression of acetyl choline esterase showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression to near control ($p<0.001$, $p<0.001$, $p<0.01$ respectively) whereas in Hx + E and Hx + G + E + O there was no significant reversal in the up regulated acetyl choline esterase gene expression to near control. (Figure- 108, Table- 112).

Real Time PCR analysis of choline acetyl transferase mRNA.

The gene expression of choline acetyl transferase showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression to near control ($p<0.001$, $p<0.001$, $p<0.01$ respectively). In Hx + E and Hx + G + E + O no significant reversal in choline acetyl transferase gene expression was observed to near control. (Figure- 109, Table- 113).

Real Time PCR analysis of HIF1 mRNA.

The gene expression of transcription factor HIF1 showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression to near control ($p<0.001$). Resuscitation with epinephrine, Hx + E and Hx + G + E + O, did not significantly reverse the HIF1 gene expression to control. (Figure- 110, Table- 114).

Real Time PCR analysis of SOD mRNA.

The gene expression of antioxidant enzyme SOD showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal of the gene expression to near control ($p<0.001$). In Hx + O, there is a significant up regulation ($p<0.001$) in the gene expression of SOD compared to control. In Hx + E and Hx + G + E + O no significant reversal in the altered SOD gene expression was observed to control. (Figure 111, Table. 115).

Real Time PCR analysis of GPx mRNA.

The gene expression of antioxidant enzyme GPx showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the gene

expression to near control whereas Hx + E and Hx + G + E + O showed no significant reversal in GPx expression to control. (Figure- 112, Table- 116).

Real Time PCR analysis of Bax mRNA.

Bax mRNA expression showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal ($p<0.001$) of Bax expression to near control. In Hx + O, Hx + E and Hx + G + E + O a significant up regulation ($p<0.001$) in the Bax expression was observed without a significant reversal to near control. (Figure- 113, Table- 117).

Real Time PCR analysis of CREB mRNA.

The gene expression of transcription factor CREB mRNA showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the CREB expression to near control. In epinephrine supplemented groups, Hx + E and Hx + G + E + O, there was no significant reversal in the gene expression to near control. (Figure- 114, Table- 118).

Real Time PCR analysis of Phospholipase C mRNA.

The gene expression of phospholipase C mRNA showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the expression to near control. In Hx + E and Hx + G + E + O no significant reversal in phospholipase C gene expression was observed to near control. (Figure- 115, Table- 119).

CORPUS STRIATUM

TOTAL GABA RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the corpus striatum of control and experimental neonatal rats

Scatchard analysis of [^{3}H] GABA against GABA in the corpus striatum of hypoxic rats showed a significant decrease in B_{max} (p<0.001) and K_d (p<0.001) compared to control. In Hx + G and Hx + G + O, the receptor number and affinity (p<0.001) showed a reversal to near control. In Hx + O, the B_{max} and K_d was significantly decreased (p<0.001) compared to control. Epinephrine supplemented groups, Hx + E and Hx + G + E + O, showed no significant reversal in B_{max} to near control (Figure- 116, 117 & Table- 120- 121).

Displacement Analysis of [^{3}H] GABA in the corpus striatum

The competition curve for GABA against [^{3}H] GABA fitted for two-sited model in all the groups with Hill slope value away from Unity. The $Ki_{(H)}$ increased in hypoxic neonatal rats along with an increase in the log (EC_{50})-1 indicating a shift in high affinity towards low affinity. $Ki_{(L)}$ also showed an increase in hypoxic neonatal rats with an increase in log (EC_{50})-2 denoting a shift in the low affinity site towards much lower affinity (Figure- 118, Table- 122).

$GABA_A$ RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the corpus striatum of control and experimental neonatal rats.

Scatchard analysis of [^{3}H] bicuculline against bicuculline in the corpus striatum of hypoxic rats showed a significant decrease in B_{max} (p<0.001) and K_d (p<0.001) compared to control. In glucose supplemented group, Hx + G and Hx + G + O, there was a significant reversal in receptor number (p<0.001) and affinity

($p<0.001$) to near control. In Hx + O, the B_{max} and K_d was significantly decreased ($p<0.001$) compared to near control. No significant reversal of the receptor number to near control was observed in epinephrine supplemented groups, Hx + E and Hx + G + E + O (Figure- 119, 120 & Table- 123 - 124).

$GABA_B$ RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the corpus striatum of control and experimental neonatal rats.

In hypoxic group, $GABA_B$ receptors was significantly decreased ($p<0.001$) with a significant decrease in K_d ($p<0.001$) compared to control. Hx + G and Hx + G + O showed a significant reversal of B_{max} ($p<0.001$) to near control. In Hx + O, Hx + E and Hx + G + E + O, K_d was significantly decreased ($p<0.001$) compared to control without a significant reversal of B_{max} to near control. (Figure- 121,122 & Table- 125, 126).

REAL-TIME PCR ANALYSIS OF $GABA_A$, $GABA_B$ RECEPTOR SUBTYPES AND GAD

Real Time PCR analysis of $GABA_A$ receptor subtypes mRNA.

The gene expression studies showed that $GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\gamma3}$ and $GABA_{A\delta}$ receptor subunits mRNA were significantly down regulated ($p<0.001$) in the hypoxic neonatal rats compared to control. In Hx + G and Hx + G + O there was a significant reversal ($p<0.001$) of the gene expression of $GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\delta}$ and $GABA_{A\gamma3}$ receptors to near control. In Hx + O, the gene expression of $GABA_{A\gamma3}$ and $GABA_{A\delta}$ receptor subunits showed a significant reversal ($p<0.01$, $p<0.001$ respectively) to near control. In Hx + E and Hx + G + E + O the gene expression of $GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\gamma3}$ and $GABA_{A\delta}$ receptors showed no significant reversal to near control. (Figure- 123 - 126 & Table- 127 - 130).

Real Time PCR analysis of $GABA_B$ receptor subtypes mRNA.

The gene expression of $GABA_B$ receptor showed a significant down regulation ($p<0.001$) in the hypoxic neonates compared to control. In Hx + G, Hx + G + O there was a significant reversal of the gene expression ($p<0.001$) to near control. In Hx + O, Hx + E and Hx + G + E + O no significant reversal was observed in the down regulated $GABA_B$ receptor gene expression to near control. (Figure- 127, Table- 131).

Real Time PCR analysis of GAD mRNA.

The expression of GAD mRNA showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O, GAD expression was reversed ($p<0.001$) to near control. In Hx + O, Hx + E and Hx + G + E + O, the GAD gene expression showed no significant reversal to near control. (Figure- 128, Table- 132).

TOTAL 5-HT RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] 5-HT binding against 5-HT to total 5-HT receptor in the corpus striatum of control and experimental neonatal rats

Scatchard analysis of [^{3}H] 5-HT against 5-HT in the corpus striatum of hypoxic neonatal rats showed a significant increase in B_{max} ($p<0.01$) with a significant decrease in K_d ($p<0.001$) compared to control. In Hx + G and Hx + G + O, the receptor number ($p<0.001$) showed a reversal to near control. In Hx + O, the B_{max} and affinity was significantly increased ($p<0.05$, $p<0.001$ respectively) compared to control. Hx + E and Hx + G + E + O showed a significant increase in the affinity ($p<0.001$) without any significant reversal in receptor number to near control (Figure- 129, 130 & Table- 133, 134).

5-HT_{2A} RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the corpus striatum of control and experimental neonatal rats

Scatchard analysis of [^{3}H] ketanserin against ketanserin in the corpus striatum of hypoxic neonatal rats showed a significant increase in B_{max} and K_d ($p<0.001$) compared to control. In Hx + G and Hx + G + O, B_{max} showed a reversal ($p<0.001$) to near control. In Hx + O, the B_{max} and affinity was significantly decreased ($p<0.001$) compared to control. In Hx + E and Hx + G + E + O, no significant reversal in the receptor number and affinity was observed (Figure- 131, 132 & Table- 135, 136).

REAL-TIME PCR ANALYSIS OF 5-HT_{2A} RECEPTOR SUBTYPE AND 5-HTT.

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

The gene expression studies showed that 5-HT_{2A} receptor subunit mRNA was significantly up regulated ($p<0.001$) in the hypoxic neonatal rats compared to control. In Hx + G and Hx + G + O there was a significant reversal ($p<0.001$) of 5-HT_{2A} receptor expression to near control. Hx + O showed a significant down regulation ($p<0.05$) in the receptor gene expression compared to control. In Hx + E and Hx + G + E + O there was no significant reversal in 5-HT_{2A} receptor gene expression to near control. (Figure- 133, Table- 137).

Real Time PCR analysis of 5-HTT mRNA.

The gene expression of 5-HTT mRNA showed that there was a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the gene expression to near control whereas Hx + E and Hx + G + E + O showed no significant reversal in 5-HTT gene expression to near control. (Figure- 134, Table- 138).

TOTAL MUSCARINIC RECEPTOR ANALYSIS:

Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the corpus striatum of control and experimental neonatal rats

Scatchard analysis of [^{3}H] QNB against atropinne in the corpus striatum of hypoxic neonatal rats showed a significant decrease (p<0.001) in B_{max} and K_d compared to control. In Hx + G and Hx + G + O, B_{max} showed a significant reversal (p<0.01) to near control with a significantly decreased K_d (p<0.001) compared to control. In Hx + O, the B_{max} and K_d was significantly decreased (p<0.001) compared to control. In Hx + E and Hx + G + E + O, the K_d was significantly decreased (p<0.001) compared to control without significant reversal in receptor number to near control (Figure- 135,136 & Table- 139, 140).

REAL-TIME PCR ANALYSIS OF MUSCARINIC RECEPTOR SUBTYPES, ChAT, AChE, HIF, SOD, GPx, BAX, CREB AND PHOSPHOLIPASE C

Real Time PCR analysis of muscarinic M1 receptor mRNA.

The gene expression studies showed that muscarinic M1 receptor subunit mRNA was significantly down regulated (p<0.001) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal (p<0.001, p<0.001, p<0.01 respectively) of the gene expression to near control. In Hx + E and Hx + G + E + O there was no significant reversal in the altered gene expression to near control. (Figure- 137, Table- 141).

Real Time PCR analysis of muscarinic M2 receptor mRNA.

The gene expression studies showed that muscarinic M2 receptor subunit mRNA was significantly down regulated (p<0.001) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal (p<0.001, p<0.001, p<0.01 respectively) of the gene expression to near

control whereas in Hx + E and Hx + G + E + O there was no significant reversal in the gene expression to near control. (Figure- 138, Table- 142).

Real Time PCR analysis of muscarinic M3 receptor mRNA.

The gene expression studies showed that muscarinic M3 receptor subunit mRNA was significantly down regulated ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$, $p<0.001$, $p<0.01$ respectively) of the gene expression to near control. Epinephrine resuscitation, Hx + E and Hx + G + E + O, showed no significant reversal in the altered gene expression to near control. (Figure- 139, Table- 143).

Real Time PCR analysis of acetyl choline esterase mRNA.

The gene expression of acetyl choline esterase showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression to near control ($p<0.001$, $p<0.001$, $p<0.01$ respectively). Acetyl choline esterase gene expression showed no significant reversal in epinephrine supplemented groups, Hx + E and Hx + G + E + O. (Figure- 140, Table- 144).

Real Time PCR analysis of choline acetyl transferase mRNA.

The gene expression of choline acetyl transferase showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression to near control ($p<0.001$, $p<0.001$, $p<0.01$ respectively). Resuscitation with epinephrine, Hx + E and Hx + G + E + O showed no significant reversal in choline acetyl transferase gene expression to near control. (Figure- 141, Table- 145).

Real Time PCR analysis of HIF1 mRNA.

The gene expression of transcription factor HIF1 showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal of the gene expression to near control ($p<0.001$). In Hx + E and Hx + G + E + O no significant reversal was observed in the up regulated HIF1 gene expression to near control. (Figure- 142, Table- 146).

Real Time PCR analysis of SOD mRNA.

The gene expression of antioxidant enzyme SOD showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal of the gene expression to near control ($p<0.001$). In Hx + O, there is a significant up regulation ($p<0.001$) in the gene expression of SOD compared to control. Epinephrine supplementation, Hx + E and Hx + G + E + O, no significant reversal in SOD gene expression was observed to near control. (Figure- 143, Table- 147).

Real Time PCR analysis of GPx mRNA.

The gene expression of antioxidant enzyme GPx showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G and Hx + G + O there was a significant reversal ($p<0.001$) of the gene expression to near control. In Hx + O, there is a significant up regulation ($p<0.001$) in the gene expression of GPx compared to control. In Hx + E and Hx + G + E + O there was no significant reversal in GPx expression ($p<0.001$) compared to control. (Figure- 144, Table- 148).

Real Time PCR analysis of Bax mRNA.

Bax mRNA expression showed a significant up regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + G + E +

O there was a significant reversal ($p<0.001$) of Bax expression to near control. In Hx + O and Hx + E there was a significant up regulation ($p<0.001$) in the gene expression compared to control. (Figure- 145, Table- 149).

Real Time PCR analysis of CREB mRNA.

The gene expression of transcription factor CREB mRNA showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the CREB expression to near control. Epinephrine resuscitated groups, Hx + E and Hx + G + E + O, showed no significant reversal in the gene expression to near control. (Figure- 146, Table- 150).

Real Time PCR analysis of Phospholipase C mRNA.

The gene expression of phospholipase C mRNA showed a significant down regulation ($p<0.001$) in the hypoxic group compared to control. In Hx + G, Hx + G + O and Hx + O there was a significant reversal ($p<0.001$) of the expression to near control. Phospholipase C gene expression showed no significant reversal in Hx + E and Hx + G + E + O to near control. (Figure- 147, Table- 151).

SECOND MESSENGER cGMP, cAMP and IP3 CONTENT IN THE CORPUS STRIATUM

cGMP content in the corpus striatum of experimental groups of neonatal rats

The cGMP content in the corpus striatum increased significantly ($p<0.001$) in Hx, Hx+O, Hx + E and Hx+G+E+O compared to control. Glucose treatment to hypoxic rats – Hx+G ($p<0.001$), Hx+G+O ($p<0.001$) significantly reversed the cGMP content to near control (Figure- 148, Table- 152).

cAMP content in the corpus striatum of experimental groups of neonatal rats

The cAMP content in the corpus striatum increased significantly ($p<0.001$) in Hx, Hx+O, Hx + E and Hx+G+E+O compared to control. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed ($p<0.001$) the cAMP levels to near control (Figure- 149, Table- 153).

IP3 content in the cerebral cortex of experimental groups of neonatal rats

The IP3 content in the corpus striatum increased significantly ($p<0.001$) in Hx, Hx+O, Hx + E and Hx+G+E+O compared to control. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed ($p<0.001$) the IP3 levels to near control (Figure- 150, Table- 154).

BEHAVIOURAL STUDIES

Body weight of experimental animals used for behavioural studies

Experimental rats on postnatal day 30 were used for behavioural study. Body weight of experimental animals used for behavioural studies showed a significant decrease in Hx ($p<0.01$), Hx + O ($p<0.01$), Hx + E ($p<0.001$) and Hx+G+E+O ($p<0.001$) when compared to control. There was no significant change in the body weight of Hx+G, Hx+O and Hx+G+O groups compared to control (Table- 155).

Y MAZE TEST

Behavioural response of one month old control and experimental rats on Y-maze.

The percentage of visits to the novel arm was found to be significantly decreased ($p<0.001$) in hypoxic rats compared to control. Glucose supplemented groups, Hx + G and Hx + G + O showed a significant increase ($p<0.01$) in the percentage of visits to the novel arm. In Hx + O, Hx + E and Hx + G + E + O, the

percentage of visits to novel arm was significantly decreased ($p<0.01$, $p<0.001$, $p<0.01$ respectively) compared to control. (Figure- 151, Table- 156).

The percentage duration of arm visits was found to be significantly decreased ($p<0.001$) in hypoxic rats compared to control. Glucose supplemented groups, Hx + G and Hx + G + O showed a significant increase ($p<0.01$, $p<0.001$ respectively) in percentage duration of arm visits. In Hx + O, Hx + E and Hx + G + E + O, the percentage duration of arm visits was significantly decreased ($p<0.001$) compared to control. (Figure- 152, Table- 157).

RADIAL ARM MAZE TEST

Number of trials to criteria was found to be significantly increased ($p<0.001$) in hypoxic rats compared to control. Glucose supplemented groups, Hx + G and Hx + G + O showed a significant decrease ($p<0.01$) in the number of trials to criteria compared to hypoxic group. In Hx + O, Hx + E and Hx + G + E + O, the number of trials to criteria was significantly increased ($p<0.01$, $p<0.001$, $p<0.01$ respectively) compared to control. (Figure- 153, Table- 158).

The mean reference memory error in all the groups of rats decreased over trial from first to the fourth trial. A significant increase in mean reference memory error over trial was observed in Hx, Hx + O, Hx + E and Hx + G + E + O ($p<0.01$) compared to control. Hx + G and Hx + G + O showed a significant reversal ($p<0.01$) in the mean reference memory error to near control. (Figure- 154, Table- 159).

The mean working memory error in all the groups of rats decreased over trial from first to the fourth trial. A significant increase in mean working memory error over trial was observed in Hx, Hx + O, Hx + E and Hx + G + E + O ($p<0.01$) compared to control. Hx + G and Hx + G + O showed a significant reversal ($p<0.01$) in the mean working memory error to near control. (Figure- 155, Table- 160).

MORRIS WATER MAZE TEST

The escape latency in all the groups of rats decreased over trial from first to the fourth day. A significant increase in escape latency over trial was observed in Hx, Hx + O, Hx + E and Hx + G + E + O ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$ respectively) compared to control. Hx + G and Hx + G + O showed a significant reversal ($p<0.001$) in the escape latency to near control. (Figure- 156, Table- 161).

A significant decrease in the time spent in platform quadrant was observed in Hx, Hx + O, Hx + E and Hx + G + E + O ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$ respectively) compared to control. Hx + G and Hx + G + O showed a significant reversal ($p<0.001$) in the time spent in platform quadrant to near control. (Figure 157, Table 162).

Discussion

Respiration proves to be a highly integrated process that involves a complex network of interplay between the brain, brain stem, spinal cord, cranial and spinal nerves, diaphragm, intercostal muscles, laryngeal and pharyngeal structures, lungs, and the vasculature. It also involves diverse sets of neurotransmitters, neuromodulators, receptors, second messengers and transcription factors. Hypoxic injury induces early changes in cerebral energy that later lead to the presence and extension of brain damage and subsequently to severe neurodevelopmental impairments. Kranjc *et al* (1994) reported that maximal neurotransmitter changes occurred 15 minutes after the hypoxic insult. Vulnerability of the neonatal rat brain to hypoxia/ischemia appears to peak at the end of the first postnatal week and then progressively diminish (Ikonomidou *et al.,* 1989). Hyperoxia triggers diffused apoptosis in the immature rodent brain peaking at 3-7 postnatal days, a particularly vulnerable period corresponding to the brain growth spurt of rodents (Sola *et al.*, 2007). Hypoxia induces catecholamine secretion by 2- 6 days after birth and initiates the chemoreceptor response as well as nerve activity to hypoxia (Donnelly & Doyle, 1994).

The end of second postnatal week is a highly plastic narrow window of respiratory development. This time window is regarded as the 'critical period' previously described as a period 'devoted to structural and/or functional shaping of the neural system subserving respiratory control' (Carroll, 2003). This window also marks other bodily changes, such as the opening of eyelids, the opening of the external auditory canal, the onset of non-REM sleep, the onset of the power-law distribution of wake bout distribution, the thickening of fur, a switch from polyneuronal to mononeuronal innervation of muscle fibres, the pruning of synapses onto Purkinje cells of the cerebellum and a change from crawling to walking (Jouvet-Mounier *et al.,* 1970; Brown *et al.,* 1976; Crepel *et al.,* 1976;

Hoath, 1986; Petrosini *et al.,* 1990; Blumberg *et al.,* 2005). Other neurochemical and hormonal changes also contribute to dynamic homeostatic regulation at this time. If such a critical period exists in humans (weeks or months in humans instead of days in rats) and if respiratory insults are introduced at this time to a vulnerable infant, especially during sleep when the respiratory control system is further suppressed (Olson & Simon, 1996; Moss, 2002), it is possible that catastrophic events, such as Sudden Infant Death Syndrome, may result. The response to acute hypoxia is indeed biphasic, especially from P3 onward, with ventilation in the first 30 s to 1min being higher than the rest of the 5-min period. Significantly, this biphasic response undergoes developmental changes during the first 3 postnatal weeks. From P0 to P11, the ratio of ˙minute ventilation (VE) in hypoxia to that in normoxia throughout the 5-min period is >1, indicating that the system is able to respond adequately to hypoxia. However, between P12 and P16, much of the late phase of HVR is <1, suggesting that the depressive effect of hypoxia is much more prominent at this time, despite the fact that ventilation during normoxia is quite high (Liu *et al*., 2006). This reduction is due mainly to decreased frequency response, but on P13, when HVR is at its lowest, the decrease is caused by a distinct suppression of both frequency and tidal volume (VT). P13 is the only time during development when the *V*T (hypoxia): *VT* (normoxia) ratio falls below 1. The marked reduction in HVR at the end of the second postnatal week is consistent with a predominance of inhibitory neurotransmitter expression, a switch in $GABA_A$ receptor subunits from neonatal to an adult form, as well as heightened expression of GluR2 receptors occurring at the same time in multiple brain stem respiratory nuclei (Wong-Riley & Liu, 2005), all of which point to a stronger inhibitory suppression that dampen the hypoxic ventilatory response. Hence, in our experiments the neonatal rats were subjected to single episode of hypoxia for 30 minutes and sacrificed on day 13 after birth.

Prolonged hypoxemia during infancy is frequently the result of a congenital cyanotic heart defect or lung disease resulting from pre-maturity. A

constant supply of oxygen is indispensable for cardiac viability and function. However, the role of oxygen and oxygen-associated processes in the heart is complex, and they can be either beneficial or contribute to cardiac dysfunction and death. As oxygen is a major determinant of cardiac gene expression and a critical participant in the formation of ROS and numerous other cellular processes, consideration of its role in the heart is essential in understanding the pathogenesis of cardiac dysfunction (Giordano, 2005). As myocardial O_2 levels decrease, either during isolated hypoxia or ischemia-associated hypoxia, gene expression patterns in the heart are significantly altered (Huang *et al*., 2004). Prior studies have revealed that respiratory as well as cardiovascular function and control at maturity were abnormal after hypoxemia experienced in early life (Sorensen & Severinghaus., 1968; Okubo & Mortola., 1990., Rohlicek *et al*., 2002). Considering the impact of hypoxia on cardiac tissue, we evaluated the free radical scavenging system, insulin and T3 receptor expression in the heart of experimental groups of rats. The dysfunction of antioxidant and hormonal systems in the heart under neonatal hypoxia further points to the need for devising an immediate and proper resuscitation method to overcome the stress.

GABA, the principal inhibitory neurotransmitter in the CNS and peripheral nervous system (PNS), maintains the inhibitory tones that counter balances neuronal excitation. Glutamate decarboxylase enzyme (GAD) is the rate limiting enzyme of GABA synthesis and it is used as a marker for GABAergic activity. Glucoregulatory control is mediated by the interplay between inhibitory GABAergic neurotransmission and excitatory glutamatergic neurotransmission within the CNS. Serotonin or 5-Hydroxytryptamine (5-HT) is a monoamine neurotransmitter, which act through serotonin receptors. It influences various biological and neurological processes such as aggression, anxiety, appetite, cognition, learning, memory, mood, nausea, sleep and thermoregulation. 5-HT transporter (5-HTT) is an integral membrane protein that transports serotonin from synaptic spaces into presynaptic neurons. This transport of serotonin by the 5-HTT

terminates the action of serotonin and recycles it in a sodium-dependent manner. Acetylcholine, a major neurotransmitter in the autonomic nervous system plays an integral role in normal muscle functions, motor activity, attention, fear, anxiety and learning through interactions with muscarinic and nicotinic receptors. Acetylcholine level in synapse is regulated by choline acetyltransferase, the acetylcholine synthesizing enzyme and acetylcholine esterase which degrade the acetylcholine. Hypoxia damages the GABAergic, serotonergic and cholinergic functions. Hypoxic insult during neonatal period is associated with an apoptotic cell death in the CNS resulting in behavioural changes in the later stages of life.

Several neurotransmitters and neuromodulators, such as GABA, 5-HT, adenosine and platelet-derived growth factor – β play important roles in hypoxic ventilatory decline (Kazemi & Hoop., 1991; Elnazir *et al*., 1996). Endogenous GABA acting on $GABA_A$ or $GABA_B$ receptors modulates ventilation during room air breathing as well that the ventilatory response to acute and sustained hypoxia (Zhang *et al*., 2002). Serotonin is one of neurotransmitters participating in the development of hypoxia-induced pulmonary hypertension. Pulmonary vasoactive responses to hypoxia are intensified by 5-HT (Eddahibi, 1997). Muscarinic receptors are also present on rhythm-generating neurons in the brain stem. The muscarinic receptor stimulation by acetylcholine and $5HT_{2A}$ leads to activation of phospholipase C (PLC), which in turn hydrolyses phosphatidylinositol 4, 5-bisphosphate (PIP2) to produce Inositol triphosphate (IP3) and diacylglycerol (DAG).

The signaling from the neurotransmitters is carried to the cell nucleus by second messengers like cAMP, cGMP and IP3. Their expression and changes play a major role in the signaling cascade. Different transcription factors are modulated during hypoxia in order to overcome the stressful condition. The most important among them is hypoxia inducible factor 1 (HIF 1) and cAMP responsive element binding protein (CREB). HIF-1α plays an essential role in cellular oxygen homeostasis by regulating the expression of genes involved in glycolysis,

erythropoiesis and angiogenesis. HIF-1α is also a key component of the cellular response to hypoxia and ischemia under pathophysiological conditions. CREB plays an important role in a variety of cellular processes, including proliferation, differentiation and adaptive responses. Increased CREB phosphorylation during post-ischemic recovery is associated with neuronal survival.

Free radical toxicity mediated neurodegeneration and apoptotic cell death are two important factors which contributes to brain damage in hypoxic condition. So an effective resuscitation method should help in enhancing the free radical scavenging capability and reducing the expression of pro-apoptotic genes. The free radical scavenging system consists of enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These enzymes were reported to show decreased activity under hypoxic condition. An effective resuscitation programme to encounter neonatal hypoxia should ensure minimum brain damage and free radical formation with a balanced expression of various neurotransmitters and its signaling pathways, so that the harmony of the body functions is not disturbed.

BODY WEIGHT AND BLOOD GLUCOSE LEVEL

There was no significant change in body weight of hypoxic rats when compared to control on postnatal day 13. Behavioural study was conducted on postnatal day 30. The 1 month old hypoxic rats showed a significant decrease in the body weight compared to control. When animals are acutely subjected to hypoxia, food intake declines and hence results in decreased body weight. Golan *et al.*, (2004) reported that hypoxic episode in early life stage caused impairment in the morphogenic parameters and motor strength in the newborns during the first month of age.

Hypoxic insult to four day old neonate did not cause any significant change in the blood glucose compared to control. Supplementation of glucose, oxygen and epinephrine does not cause significant change in blood glucose level

after one week. Maintaining blood glucose level continues to be important throughout the pregnancy, but it is particularly important during early developmental stage, when an embryo's organs are forming. Oxygen is needed by cells to break down glucose and produce energy. The oxygen requirement is more during embryonic development for all the tissues. Insufficient oxygen supply cause developmental abnormalities and birth defects. Thus hypoxia has a potential to cause damage to cells (Rulin *et al*., 2005). Epinephrine stimulated mechanical performance and heart rate of hypoxic hearts, but decreased myocardial glycogen and ATP. Though glucose utilization remained unchanged, the release of lactate increased from hypoxic hearts treated with epinephrine. However, epinephrine failed to stimulate myocardial lipolysis in hypoxic hearts. These metabolic changes due to epinephrine would lead to accelerated depletion of energetic reserves in hypoxic heart and its earlier deterioration.

FREE RADICAL SCAVENGING IN HYPOXIA.

Oxygen or glucose deprivation alters electrical transmission in the brain and generates free radicals, which mediate neuronal death (Pedersen et al., 1998). Free radical production has been proposed to be involved in the pathogenesis of the ischemia-reperfusion neuronal damage (Globus *et al*., 1995; Ozben, 1998). Damage to lipids, proteins and nucleic acids has been observed concomitantly with their production, ultimately resulting in cell function impairment and death (Halliwell & Gutteridge, 1990). Reactive oxygen is a signalling molecule and its levels in tissue determine the aging process and lifespan (Sasaki *et al*., 2010). Increased ventilatory drive following chronic intermittent hypoxia represents a form a neural plasticity which is a ROS dependent phenomenon (Edge *et al*., 2010)

Free radical scavenging enzymes like SOD, CAT and GPx play important role in protection against oxygen toxicity in mammalian systems (Liu *et al*., 1977). Studies have demonstrated that free radicals are formed under hypoxic

conditions in newborn piglet brain (Torres *et al*., 2004). Hypoxia induces superoxide accumulation in pulmonary artery myocytes through inhibition of mitochondrial SOD activity, promoting peroxynitrite-induced generation of 8-isoprostane (Gong *et al*., 2010). In the cerebral cortex of rats subjected to 24 hours of reperfusion following 2 h of cerebral ischemia, the SOD activity was decreased (Tsinghua, 2002). The rat endothelial cells produce SOD inhibittable ROS which are augmented by hypoxia/reoxygenation (Strasser *et al*., 1997). The present study showed that both SOD and CAT activities are significantly less in hypoxic neonatal rats compared to control in the heart and cerebral cortex with a down regulated gene expression of SOD and GPx mRNA, indicating that limitation in oxygen supply is associated with reduction in antioxidant enzymes like SOD, CAT and GPx.

Hyperoxia with 100% oxygen after hypoxic-ischemia cause more damage in the cerebral cortex than room air in newborn rats (Shimabuku, 2005). 100% oxygen generates abnormally high levels of ROS which cause dysfunction of defensive antioxidant system of cells by altering enzyme activity (Bandypadhyay *et al*., 1999) and act as a factor for neurodegeneration (Matharan *et al*., 2004). Hypoxemic piglets resuscitated with 100% oxygen also showed increased cerebral injury, cortical damage and early neurological disorders (Shimabuku *et al*., 2000). In the present study, the increased SOD and CAT activities with decreased affinity observed in hypoxic rats resuscitated with oxygen indicates the decreased function of free radical scavenging enzymes, which add to more damage due to the free radical formation. The gene expression of SOD and GPx showed an up regulation in the brain regions as a homeostatic response to counter act the excess oxygen free radicals formed. This highlights the role of free radicals in causing damage to brain and heart in neonatal hypoxic rats on 100% oxygen resuscitation.

The present study showed that glucose supplementation to hypoxic and hypoxic treated with oxygen has an efficient free radical scavenging capability compared to all other experimental groups. Hypoxic neonates treated with glucose

have shown a higher activity of SOD and CAT, showing an increased antioxidant capability in presence of glucose. The combination of glucose, epinephrine and oxygen as resuscitation in hypoxic condition has shown a decreased SOD and CAT activity, indicating that free radical toxicity is high in heart and cerebral cortex, due to the administration of epinephrine. Reduction in blood glucose levels and substantially increased cerebral glucose utilization was observed as a result of hypoxic stress in experimental rats (Hattori & Wasterlain, 2004; Vannucci & Hagberg, 2004). Unlike the adult, where glucose supplementation prior to or during hypoxic-ischemia accentuates tissue injury, glucose treatment of perinatal animals subjected to a similar insult substantially reduces the extent of tissue injury (Vannucci & Hagberg, 2004). Hypoxia induced expressional and functional changes in NMDAR1 receptors of neuronal cells in neonatal rats are corrected by supplementation of glucose alone or glucose, followed by oxygen during the resuscitation to prevent the glutamate related neuronal damage (Paulose *et al.*, 2007). Post hypoxic glucose supplement also reduces an elevated brain lactate level which is responsible for cerebral infarction occurring during the hypoxia (Hattori & Wasterlain, 2004). The present study reported the protective role of glucose supplementation by ameliorating the free radical mediated toxicity in cerebral cortex and heart.

INSULIN LEVEL AND INSULIN RECEPTOR

Hypoxic stress is well known to decrease appetite and weight gain in growing rats and to induce weight loss in humans at high altitude (Tschop & Morrison, 2001; Raff, 2003). It also increases the expression of a variety of genes with products that act in synergy to facilitate the supply of metabolic energy (Yasumasu *et al.*, 2002). Insulin levels are expected to drop with weight loss; however, in the hypoxic group despite weight loss, insulin levels were higher than in the control group. This implies that hypoxia has a direct effect on insulin independent of weight changes. Hypoxia was previously shown to increase insulin

messenger RNA (Tillmar & Welsh, 2002). Baum (1969), Baum and Porte (1976) were the only group to report the inhibition of insulin release during hypoxia when studying hypoxic puppies. Their results are in contradiction with our study and with most of previously published results (Bitar *et al*., 1994; Raff *et al*., 2001; Tillmar & Welsh, 2002; Meissner *et al*., 2003). Insulin inhibits hypoxia-induced cleavage of apoptotic factor caspase-3, mediated by p38 mitogen-activated protein kinase (MAPK). Insulin-induced MKP-1 expression was mediated by extracellular signal-regulated protein kinases (ERK) 1/2, c-Jun NH2-terminal kinases (JNK), MAPK and phosphatidylinositol 3-kinase (PI3K)/Akt pathways. Concomitant activation of Akt, ERK 1/2 and JNK was required for insulin to exert its protective effect against the hypoxia-induced cleavage of caspase-3 (Morisco *et al*., 2007). The increased serum insulin level with up regulated insulin receptors in heart observed in the present study is suggested to be an adaptive modification in the body to overcome energy depletion and to prevent apoptotic cell death.

Both hypoxia and insulin induce common target genes, including vascular endothelial growth factors and several glycolytic enzymes. However, these two signals eventually trigger quite different metabolic pathways. Hypoxia induces glycolysis, resulting in anaerobic ATP production, while insulin increases glycolysis for energy storage. Energy stores are maintained during hypoxic pulmonary vasoconstriction and that this is dependent on glucose availability and up regulation of glycolysis (Leach *et al*., 2002). Severe hypoxia results in ATP depletion to cells and hence to provide immediate energy glucose is used in the resuscitation practice. Insulin enables the body cells to take glucose from the bloodstream. Circulating insulin increased in response to increased glucose content, which is observed in our glucose supplemented groups. The increased insulin helps in metabolising glucose to derive energy to encounter hypoxic stress. To prevent the on set of cardiac ischemia due to hypoxic stress, heart underwent adaptive changes like increased expression of insulin receptors. Qin *et al*., (2010) postulated that in mice intermittent hypoxia reduces body weight and serum

glucose by increasing erythropoietin synthesis which secondarily increases leptin and insulin production in liver.

In oxygen supplemented group (Hx + O), the circulating insulin was significantly less with a significant down regulation of insulin receptor gene compared to control. It has been reported that during hypoxia, oxygen supplementation results in the free radical formation and glutamate mediated excitotoxicity (Finla *et al*., 2008). Reactive oxygen species and reactive nitrogen species act as negative regulators of insulin signalling, rendering them putative mediators in the development of insulin resistance, a common endocrine abnormality that accompanies many diseases (Bashan *et al*., 2009). In epinephrine supplemented groups (Hx + E, Hx + G + E + O), a very high circulating insulin was observed which contribute to insulin resistance. The insulin receptor expression was significantly up regulated in these groups. Insulin resistance in cells reduces glucose uptake thereby increasing the stress during hypoxia. Epinephrine is known to inhibit insulin secretion (Deibert & DeFronzo, 1980) but will also cause hyperglycemia, which can secondarily increase insulin secretion (Eigler *et al*., 1979). An increase in plasma epinephrine concentration causes insulin resistance (Porte, 1967; Saccà *et al*., 1979).

TRIIODOTHYRONINE AND ITS RECEPTOR

Thyroid hormones are the most significant factors in regulating energy transformations. Thyroid hormones are involved in setting the basal metabolic rate in many target tissues, such as liver, heart, kidney and brain. Corresponding changes in respiratory activity occur in mitochondria isolated from tissues of hypo- and hyperthyroid animals.

The evaluation of T3 content in serum and heart revealed a significant decrease in hypoxic neonatal rats. This may be an adaptive response of the body to encounter the hypoxic insult. Due to decreased T3 level there will be a reduction in carbohydrate, fat and protein metabolism, so that energy can be utilised for

compensating the ATP depletion during hypoxia. Sawhney and Malhotra (1990) reported a decline in thyroid hormone levels and their production in rabbits under hypoxic stress. Simonides *et al.,* (2008) also reported a mechanism of metabolic regulation during hypoxic-ischemic injury in which HIF-1 reduces local thyroid hormone signalling through induction of type 3 deiodinase. The deficiency of the thyroid hormones results in decreased metabolism and lowers the basal metabolic rate. Thyroid deficiency severely impaired the oxidative energy metabolism in rat brain mitochondria which was accompanied by decrease in cytochromes aa3 and cc1 contents and delayed pattern of mitochondrial protein turnover (Rajwade *et al*., 1975; Katyare *et al*., 1977). Hypoxia-induced signalling appears to drive type 3 deiodinase expression in the hypertrophic cardiomyocyte. Many cardiac genes are transcriptionally regulated by T3 and impairment of T3 signaling will not only reduce energy turnover, but also lead to changes in gene expression that contribute to contractile dysfunction in pathologic remodelling (Pol *et al*., 2010).

In glucose supplemented groups, Hx + G and Hx + G + O, an increased T3 content was observed compared to hypoxic group. T3 increases metabolism including carbohydrate utilisation and hence it increases glucose uptake. Thyroid hormone induces an increase in cardiac contractility and frequency, resulting in a greater cardiac output (Polikar *et al*., 1993; Toft & Boon; 2000) and a proportional change in energy turnover (Clausen *et al*., 1991). Kvetny *et al* (1990) showed that glucose feeding increases serum T3 levels, decreases nuclear T3 binding and enhances thyroid hormone stimulated oxygen consumption and glucose uptake. Since glucose administration is reported to have a role in enhancing T3 stimulated oxygen consumption glucose supplementation during hypoxic stress will help to increase the ventilatory response. The supplementation of oxygen alone showed a decreased T3 content in heart and serum. The decreased T3 is an adaptation of the body to increase the oxygen availability of blood. Resuscitation of hypoxic neonates with 100% oxygen was reported to cause free radical mediated injury in heart and brain. T3 induces a state of oxidative stress mainly by stimulating

mitochondrial ROS generation and not by decreasing mitochondrial antioxidant systems. The increase in O^{2-} production observed by T3 is due to the interaction of the hormone with the mitochondrial membrane lipid bilayer leading to alterations in membrane structure and increased electron leakage at the respiratory chain (Castilho *et al*., 1998). The activation of mitochondrial respiration by 100% oxygen resuscitation to hypoxic rats releases ROS. Since T3 causes ROS production, its level is decreased as an adaptive response to diminish free radical toxicity. Epinephrine administration stimulates lipolysis, which causes energy utilisation by cells. In order to reduce energy need T3 level was decreased in epinephrine supplemented groups as a homeostatic response.

Thyroid hormone dependent stimulation of target genes requires the interaction of thyroid hormone receptors with specific nucleotide sequences called thyroid-hormone-responsive elements. Lowering of available oxygen (hypoxia) caused a decrease in binding capacity of T3 in the cerebral cortex measured *in vitro* (Thrall, 1983). T3 receptor can recognize specific DNA sequences and suggest that it can act directly as a positive transcriptional regulatory factor (Koenig *et al*., 1987). In the present study, T3 receptor showed a significant decrease under hypoxic condition. Glucose supplementation showed a reversal of the receptor status to near control. The receptor binding data is in accordance to the T3 level observed in heart.

CENTRAL GABAERGIC RECEPTOR ALTERATIONS.

The ventilatory response to hypoxia is influenced by the balance between inhibitory (GABA, glycine, and taurine) and excitatory (glutamate and aspartate) amino acid neurotransmitters. During and after hypoxia, extracellular levels of amino acid neurotransmitters increase in many brain regions (Hagberg *et al*., 1987), causing an imbalance of excitatory and inhibitory neurotransmission leading to excitotoxicity (Yue *et al*., 1997). $GABA_A$ receptor subunit composition determines the affinity for and efficacy of GABA with binding sites at the $GABA_A$

receptor (Hevers & Luddens 1998; Whiting *et al* 1999; Belelli *et al*., 2002; Jurd *et al* 2003). $GABA_A$ receptor subunits exhibit distinct anatomical specificity in the neural regions (Laurie *et al* 1992a; Wisden *et al* 1992; Pirker *et al* 2000), the types of neurons (Gao & Fritschy 1994; Fritschy & Mohler 1995; Schwarzer *et al* 2001), and the specific parts of neurons in which they are expressed (Nusser *et al* 1996; Fritschy *et al* 1998; Brunig *et al* 2002). Subunit expression exhibits remarkable plasticity across development (Laurie *et al* 1992b), in response to changes in physiological states (Fenelon & Herbison, 1996), and in response to environmental challenges (Devaud *et al* 1995; Cullinan & Wolfe 2000). In particular, $GABA_{A\alpha}$ subunits differentially contribute to various physiologic/psychological/behavioral functions mediated by $GABA_A$ receptors (Rudolph *et al*., 1999; Low *et al*., 2000; McKernan *et a l.,* 2000; Tauber *et al*., 2003) and to the cortical plasticity which in turn mediates environmental influence on neuronal function (Fagiolini *et al*., 2004).

GABA and glutamate are the two important neurotransmitters involved in hypoxic ventilatory response. GABA in the nucleus tractus solitarii has a pivotal role in the hypoxic ventilatory decline (HVD) and this mechanism is not activated without chemoreceptor stimulation (Tabata *et al.*, 2001). Anoxia-tolerant vertebrates decrease their metabolic rate by 70% or more during anoxia, with an increase in concentration of GABA (Nilsson & Lutz, 2004). Hypoxia has been a selective pressure in conserving GABA and glutamate as major inhibitory and excitatory neurotransmitters in vertebrates as well as invertebrates (Nilsson & Lutz., 1993).

GABA CONTENT IN BRAIN REGIONS

GABA is one of the most abundant neurotransmitters in the vertebrate central nervous system and is involved in neuroendocrine processes such as development, reproduction, feeding and stress (Martyniuk *et al*., 2005). A decrease in GABA content was observed during neonatal hypoxic insult in

cerebral cortex, brain stem, cerebellum and corpus striatum. The decreased content in the brain regions were reversed to basal level in hypoxic neonates resuscitated with glucose. A reduction of GABAergic input onto caudal hypothalamic neurons in spontaneously hypertensive rats was reported earlier (Shonis *et al*., 1993). Diminished GABAergic inhibition in this region would ultimately translate into an elevated arterial pressure since activation of neurons in the caudal hypothalamus increases sympathetic nerve discharge to several vascular beds and to the heart (Waldrop & Bauer, 1989). Reductions in GABA concentrations and glutamic acid decarboxylase gene expression are consistent with a reduced GABA receptor function in different brain regions. Reduction in GABAergic inhibition in brain regions contribute to stimulation of heart functions and sympathetic innervations to overcome hypoxia. Hypoxia stimulates caudal hypothalamic neurons with a cardiovascular or sympathetic related discharge in both intact and peripherally chemodenervated cats (Dillon & Waldrop, 1993). Blockade of excitatory input onto the caudal hypothalamus was reported to attenuate the respiratory response to hypoxia in the anesthetized rat (Horn & Waldrop, 1994). It is known that hypoxia rapidly suppresses excitatory transmission between glutamatergic terminals and the inhibitory interneurons, leading to the subsequent failure of inhibitory GABAergic transmission to pyramidal cells (Congar *et al*., 1995).

Cerebral cortex

GABA increases total cerebral blood flow, acting on specific receptor sites in the cerebral blood vessels (Alborch *et al*., 1984). Long-term hypoxia produces a significant but reversible reduction on GABA binding to $GABA_A$ receptor sites in cerebral cortex, which reflect an adaptive response to this sustained pathophysiological state (Viapiano *et al.*, 2001). Hypothermic newborn piglets have a depressed ventilatory response to hypoxia due to an increase in central nervous system GABA levels (Qiming xiao *et al*., 2000). Infusion of the

GABA antagonist bicuculline caused augmentation of the hyperventilatory response to acute hypoxia (Homayoun kazem, 2006). $GABA_A$ receptors mediate the majority of fast inhibitory synaptic interactions in the mammalian brain. In the adult brain, networks of neurons containing $GABA_A$ergic receptors have been implicated in the maintenance of rhythmic activities of neuronal circuits (Whittington *et al*., 1995; Wang & Buzsaki, 1996) and the precise control of the timing of excitability in individual neurons (Tsubokawa & Ross, 1996). GABA neurotransmission serve both excitatory and inhibitory roles during early development (Chen *et al*., 1996). Subunit diversity appears to underlie distinctive roles for $GABA_A$ receptors in the developing nervous system (Laurie *et al*., 1992; Poulter *et al*., 1992; Ma *et al.*, 1993).

The binding studies of total GABA and its subtypes – $GABA_A$ and $GABA_B$ in the cerebral cortex revealed a significant decrease in the receptor number with a decreased affinity in hypoxic neonates. The expression of GAD was significantly down regulated in hypoxic neonatal rats. The data presented here suggests that decreased levels of key proteins in the GABA pathway in the cerebral cortex lead to high susceptibility to seizures and epilepsy in newborns after prenatal hypoxia (Louzoun-Kaplan *et al*., 2008). The observed decrease in GAD expression in hypoxic neonates significantly decrease GABA level, which is observed by displacement analysis for GABA quantification. Altered cortical GABA neurotransmission contribute to disturbances in diverse functions through affecting the generation of cortical oscillations in conditions like schizophrenia. These oscillatory activities have been proposed to play critical roles in regulating the efficiency of information transfer between neurons and neuronal networks in the cortex (Hashimoto *et al*., 2010).

Encountering the hypoxic insult by supplementation of glucose alone and glucose along with 100% oxygen showed a reversal in the receptor number and GAD expression to near control in the cerebral cortex. The combination of glucose and oxygen was found to be the most effective resuscitation method. Glucose is

supplemented during hypoxia to provide an immediate resuscitation to the stress condition by acting as an instant source of energy to the brain. Hattori and Wasterlain (2004) observed a reduction in the blood glucose levels and substantially increased cerebral glucose utilization (Vannucci & Hagberg., 2004) as a result of hypoxic stress in experimental rats. We observed that supplementation of glucose is effective in increasing the GABA, $GABA_A$ and $GABA_B$ receptor status in the cortex. Sine glucose provides an immediate and instant energy to tissues it helps in encountering the ATP depletion induced inhibition of respiration. Ito *et al* (1994) observed a dose-dependent reduction in the cerebral glucose utilization after intravenous administration of various doses of muscimol, an agonist of $GABA_A$. A linear relationship was observed between the $GABA_A$ receptor occupancy of muscimol and the decrease in the cerebral glucose utilization (Ito *et al*., 1994). Bailey *et al* (2007) reported that glucose dose-dependently increased the expression of $GABA_A$ receptor subunits in pancreatic α-cells.

One of the routine methods of resuscitation for severe hypoxia is the immediate administration of oxygen. We observed that 100% oxygen supplementation for neonatal hypoxia is not as effective as the combination of glucose and oxygen or administration of glucose alone. In the cerebral cortex of oxygen supplemented group, total GABA and $GABA_B$ receptors showed a significant decrease compared to control whereas $GABA_A$ receptors showed a decrease below the hypoxic level. Thus, the binding analysis of total GABA, $GABA_A$ and $GABA_B$ showed that administration of 100% oxygen to hypoxic neonates did not bring balance the GABA level to encounter HVD. Hundred percentage of oxygen generated abnormally high levels of reactive oxygen species (ROS) which causes dysfunction of defensive antioxidant system of cells by altering enzyme activity (Bandyopadhyay *et al*., 1999) and act as a factor for neurodegeneration (Matharan *et al*, 2004). Hypoxemic piglets resuscitated with 100% O_2 also showed increased cerebral injury, cortical damage and early

neurologic disorders (Temesvari *et al*., 2001; Munkeby *et al*., 2004; Shimabuku *et al*., 2005). Based on behavioural studies and the studies on acetylcholinesterase, Finla *et al.,* (2008) reported the efficiency of glucose and combination of glucose and oxygen resuscitation methods and the damaging effects of oxygen supplementation alone. The reduction in GABA and its subtypes receptor function in the cortex during oxygen supplementation is suggested to be due to tissue damage caused by the formation of free radicals or reactive oxygen species. Oxidative stress potentiate presynaptic GABA release through the mechanism of cAMP-dependent protein kinase A (PKA)-dependent pathways, which result in the inhibition of the cerebral cortex neuronal activity (Hahm *et al*., 2010).

Epinephrine and GABA acts antagonistically in a biological system. The over stimulation of brain caused by epinephrine innervation is antagonized by the activity of GABA. Resuscitation of hypoxic neonatal rats with epinephrine triggers its receptors and pathways. The stimulatory action of epinephrine causes a feedback regulation of GABAergic pathways thereby decreasing the GABA receptor function in epinephrine resuscitated groups.

Cerebellum

Cerebellum significantly differs with respect to ischemia and hypoxia, this response being directly related to the duration and intensity of the injury. Cerebellar hypoxia is responsible for important aspects of cognitive deterioration and motor disturbances in neurological disorders, such as stroke, vascular dementia and neurodegeneration. The cerebellum plays important role by boosting the nitric oxide synthase activity to cover the eventual need for nitric oxide during hypoxia (Rodrigo *et al*., 2004). Hypoxia retards the development of neuronal processes, resulting in a smaller cerebellum (Yu & Wan-Hua Yu, 1980).

In the present study, a decreased total GABA, $GABA_A$ and $GABA_B$ receptor number with a down regulated $GABA_{A\alpha1,}$ $GABA_{A\alpha5,}$ $GABA_{A\gamma3}$, $GABA_{A\delta}$ and $GABA_B$ receptor subunits gene expression was observed in the cerebellum of

neonatal rats exposed to hypoxic stress. During hypoxic insult, body tries to encounter the stress by increasing its metabolic rate, thereby resulting in ATP depletion. The high metabolic cost for various cellular adaptations to hypoxia results in a decrease in neuronal activity and synaptic transmission. GABA receptors were decreased as an adaptive response of the body to prevent energy depletion. Hypoxia activates c-Jun NH (2)-terminal kinase (JNK) signaling pathway. Stimulation of GABA receptors attenuate this apoptotic pathway (Han *et al*., 2008). Since the receptors were decreased during hypoxic stress, the apoptotic pathway will be triggered resulting in brain cell death. An effective resuscitation method should inhibit JNK apoptotic pathway to prevent brain injury.

Hypoxia induced expressional and functional changes in GABA receptors in the cerebellum of neonatal rats are corrected by supplementation of glucose alone or glucose, followed by oxygen during the resuscitation, which prevented the GABA induced ventilatory decline. A significant reversal in receptor number to near control was observed in glucose supplemented groups. The gene expression of GABA subunits also showed a reversal to near control in a differential manner. The reversal of $GABA_A$ receptors to near control will augment Akt activation and thereby inhibit the JNK cascade. Differential expression of $GABA_A$ receptor subunits represents a major aspect of homeostatic synaptic plasticity and contributes to the excitation/ inhibition balance under physiological conditions and upon pathological challenges.

Hypoxia is a general factor affecting glucose metabolism. Gilleben and Kammermeier (1999) reported that moderate glucose elevation exerted beneficial effects on hypoxic hearts: the depressed contraction was improved, the action potential shortening partly reversed and the percentage of irreversibly damaged myocytes diminished. Reduction in blood glucose levels and substantially increased cerebral glucose utilization was observed as a result of hypoxic stress in experimental rats (Hattori & Wasterlain, 2004). The neuroprotective effect of

glucose supplementation to hypoxic neonates on dopamine receptor and glutamate receptors was already reported (Joseph *et al*., 2010).

Resuscitation of hypoxic neonates with 100% oxygen decreased the GABA receptor number and down regulated the gene expression of $GABA_A$ and $GABA_B$ subunits. Hundred percentage of oxygen generated abnormally high levels of reactive oxygen species (ROS) which causes dysfunction of antioxidant system of cells by altering enzyme activity. GABA neurotransmission is sensitive to reactive oxygen species. ROS contributes to $GABA_A$-mediated neuronal inhibition *via* interaction with pre and postsynaptic sites. A reduction in $GABA_A$-gated Cl^- channel function during periods of oxidative stress contribute to the development of neuronal damage (Sah *et al*., 2000). This resuscitation method was already reported to cause glutamate and dopamine mediated excitotoxicity. Our study points out the adverse effects of epinephrine supplementation, alone and even in combination with glucose and oxygen. The resuscitation attempt using epinephrine cause a hyperactivity of excitatory stimulus, which might have affected the various neurotransmitter levels thereby declining body's adaptations to overcome hypoxia.

Brain stem

Respiratory control centers in the brain stem are located in three main areas: pons, dorsal medulla and ventrolateral medulla (Bianchi *et al*., 1995; Feldman *et al*., 2003). GABA and glycine are the two major inhibitory neurotransmitters in the network of respiratory neurons in the brain stem and spinal cord (Haji *et al*., 2000). GABA, acting mainly on $GABA_A$ receptors and glycine mediate fast synaptic inhibition *via* chloride channels and help shape the discharge patterns of all respiratory neurons (Bianchi *et al*., 1995). $GABA_B$ receptors, on the other hand, are slower-acting metabotropic receptors coupled to Ca^{2+} and K^+ channels *via* G proteins and second messengers (Kerr & Ong, 1995;

Takahashi *et al*., 1998). They reportedly modulate respiratory rhythm in adult mammals (Gray *et al*., 1999).

Binding studies using radio labeled ligands showed a significant decrease in total GABA, $GABA_A$ and $GABA_B$ receptor number with a down regulated expression of receptor subtypes and GAD in hypoxic neonatal rats. The increased glutamate and 5-HT activation for transmission of inspiratory drive in the respiratory centers of brain stem negatively regulated the GABA receptors. The resuscitation with glucose alone and with oxygen effectively reversed the changed parameters to near control. Resuscitation with epinephrine and oxygen alone did not show any significant reversal of GABAergic function to control. The alterations in GABA receptors under hypoxic stress are due to negative regulation by other neurotransmitters and also a response of ATP depletion. Since glucose supplementation along with oxygenation provides an immediate energy source the imbalance in GABAergic function can be reversed by timely supplementation of glucose.

Corpus striatum

The striatum is involved in mediating habit learning (Berke & Hyman, 2000) and optimizing control of motor behavior and cognitive function (Graybiel, 2005) and is susceptible to oxygen deprivation. Approximately 90% of striatal neurons are medium spiny neurons (Calabresi *et al*., 2003; Baquet *et al*., 2004), which are selectively targeted during glucose (Calabresi *et al*., 1995[a]) and oxygen deprivation (Calabresi *et al*., 1995[b], 2000). GABA is considered as the main transmitter released from the axons of striatal neurons projecting to the output structures of the basal ganglia. It also plays a central role in the processing of information in the striatum (Groves, 1983). The striatum also receives GABAergic afferents from globus pallidus and substantia nigra (pars reticulata) (Kita, 1993). Another important intrastriatal source of GABA is represented by an extensive network of axon collaterals originating from spiny neurones (Preston *et al*., 1979;

Park *et al*., 1980; Wilson & Groves, 1980; Kita, 1993). Striatal neurons are known to have both $GABA_A$ and $GABA_B$ receptors (Ng & Yung, 2001), activation of which decrease potentiation evoked-glutamate and dopamine release in many brain regions including hippocampus, cortex and spinal cord (Tanaka *et al*., 2002; Matsumoto *et al*., 2003; Tanaka *et al*., 2003). GABA protect neurons not only by directly hyperpolarizing neurons but also by exerting an inhibitory influence on glutamate-mediated neuronal activity (Costa *et al*., 2004).

Neonatal hypoxic stress was observed to decrease the GABA receptor number – both $GABA_A$ and $GABA_B$ with a down regulated gene expression of its subunits. Under hypoxic condition the striatal neurons innervate the glutamergic system to facilitate a respiratory trigger to other regions of brain. This accounts for the decline in GABA receptors in striatum. This in turn leads to hypoxic ventilatory decline and glutamate mediated excitotoxicity in hypoxic neonatal rats. Since glucose resuscitated groups reversed the altered GABA receptor function to near control it was suggested to be an effective method to prevent ventilatory decline and neurotoxicity mediated through glutamate excitation. Immediate resuscitation with 100% oxygen and administration of epinephrine resulted in a sudden change of the cellular environment from anaerobic to aerobic. This resulted in the formation of more reactive oxygen species and glutamergic innervation, which in turn leads to decreased GABA receptors and ventilatory roll off. This points to the fact that 100% oxygen or epinephrine resuscitation to hypoxic neonatal rats was not able to reverse the disturbances and alterations in the molecular level due to hypoxic stress.

DIFFERENTIAL EXPRESSION OF $GABA_A$ RECEPTORS IN BRAIN REGIONS

$GABA_A$ receptors are composed of five protein subunits that belong to different subunit classes. There are 19 genes for $GABA_A$-R subunits (Simon *et al*., 2004). These include 16 subunits (α1–6, β1– 3, γ1–3, δ, ε, θ, π) combined as

$GABA_A$, and 3 rho (ρ) subunits which contribute to what have sometimes been called $GABA_C$ receptors. The assembly of $GABA_A$-R as heteropentamers produces complex heterogeneity in their structure, which is the major determinant of their pharmacological profile (Barnard *et al*., 1998; Olsen & Sieghart, 2008). The various receptor subtypes differ in abundance in cells throughout the nervous system and thus in functions related to the circuits involved. An important criterion for association of subunit isoforms into oligomeric native receptors is co-localization of the subunits. Immunocytochemical studies investigating the co-localization of subunits in $GABA_A$ receptor clusters on neuronal membranes (Fritschy *et al*., 1992; Bohlhalter *et al*., 1996), as well as electron microscopic studies (Nusser *et al*., 1995; Somogyi *et al*., 1996), indicate that the majority of $GABA_A$ receptors present in the brain are composed of α, β, and γ subunits.

We observed a differential regulation of $GABA_A$ receptor subunits in different brain regions under neonatal hypoxic insult and its resuscitation conditions. In cerebellum, brain stem and corpus striatum $GABA_{A\alpha1,}$ $GABA_{A\alpha5,}$ $GABA_{A\gamma3}$ and $GABA_{A\delta}$ receptor expression was down regulated under hypoxic stress. In cerebral cortex $GABA_{A\alpha1,}$ $GABA_{A\alpha5}$ and $GABA_{A\gamma3}$ receptor expression was down regulated with an up regulated $GABA_{A\delta}$ expression. The up regulation of $GABA_{A\delta}$ receptor subunit in cortex is an adaptive modification to increase conductance and tonic inhibition. Receptors containing δ subunits exhibit a smaller single channel conductance and a much longer open time and do not desensitize on the prolonged presence of GABA (Saxena & Macdonald, 1994). Together with the exclusive extrasynaptic localization of these receptors, these properties indicate that tonic inhibition observed in these cells is mediated mainly by the persistent activation of α6βδ receptors by GABA that is present in the extracellular space of glomeruli (Nusser *et al*., 1998; Brickley *et al*., 1999).

In glucose resuscitated groups, the $GABA_{A\alpha1,}$ $GABA_{A\alpha5,}$ $GABA_{A\gamma3}$ and $GABA_{A\delta}$ receptor subunits expression was reversed to near control in all the brain regions. When hypoxic neonatal rats were resuscitated with 100% oxygen alone, a

reversal in subunit receptor expression was evident only in the cerebellum, a region which is highly sensitive to oxygen availability. In cortex, $GABA_{A\alpha5}$ and $GABA_{A\gamma3}$ subunits expression was reversed. Synaptic alpha5-GABA(A)Rs play a role in the phasic GABAergic inhibition of pyramidal neurons in hippocampus and cerebral cortex (Serwanski *et al*., 2006). $GABA_{A\alpha1}$ and $GABA_{A\delta}$ receptor subunits expression was decreased in the cortical region which may contribute to inhibition of cortical plasticity and associated learning impairments. An increased expression of $GABA_{A\alpha1}$ mRNA was reported at the cortical site where the plastic changes were found which contributes to learning associated activation of the cerebral cortex (Lech *et al*., 2001). In brain stem, the seat of respiratory network, $GABA_{A\alpha1}$ and $GABA_{A\gamma3}$ receptor subunits expression was reversed to near control whereas $GABA_{A\alpha5}$ and $GABA_{A\delta}$ receptor subunits expression were decreased. Wei *et al.,* (2003) reported a perisynaptic localization of δ subunit-containing $GABA_A$ receptors in the brain stem and that these receptors are activated by GABA overspill in the molecular layer. α5 subunit-containing $GABA_A$ receptors mediate tonic inhibition and are important regulators of the expression of locomotor exploration (Hauser *et al*., 2005).

In epinephrine resuscitated groups $GABA_{A\alpha1,}$ $GABA_{A\alpha5,}$ $GABA_{A\gamma3}$ and $GABA_{A\delta}$ receptor subunits expression was reduced in cortex, cerebellum and striatum whereas in brain stem a differential subunit expression was observed. In the brain stem of hypoxic neonatal rats resuscitated with epinephrine alone, the expression of $GABA_{A\alpha5,}$ $GABA_{A\gamma3}$ and $GABA_{A\delta}$ receptor subunits was increased. A reversal in $GABA_{A\alpha1}$ receptor subunit expression on epinephrine administration indicates the attempt of the CNS respiratory circuit to adapt to the diminished oxygen availability by enhancing cardiac pumping and other peripheral changes mediated by epinephrine. The expression of $GABA_{A\alpha1}$ subunits in brain stem supports a role for GABA in the brainstem circuit controlling esophageal peristalsis (Broussard *et al*., 1996). In hypoxic neonates resuscitated with epinephrine along with glucose and oxygen, $GABA_{A\gamma3}$ receptor subunit showed

increased expression which promotes tonic inhibition. Tonic inhibition was also produced by gamma subunit-containing $GABA_A$ receptors (Semyanov *et al*., 2004). Thus the difference in regional and subunit expression of $GABA_A$ receptors observed in the present study points to the effectiveness of glucose resuscitation to neonatal hypoxic rats in modulating the various respiratory responses in a molecular level.

CENTRAL SEROTONERGIC RECEPTOR ALTERATIONS

Serotonin plays a pivotal role in the control of breathing. Brain and spinal cord regions involved in respiratory control receive 5HT input primarily from the medullary raphe nuclei including nucleus raphe magnus, pallidus and obscurus (Holtman, 1988; Li *et al*., 1993; Manaker & Tischler, 1993). Caudal raphe neurons are activated during hypoxia, and presumably release 5HT in the vicinity of respiratory premotor and motoneurons (Erickson & Millhorn, 1994; Teppema *et al*., 1997; Kinkead *et al*., 2001). Serotonin has an excitatory effect on upper airway and phrenic motoneurons (Berger *et al*., 1992; Lindsay & Feldman, 1993; Di Pasquale *et al*., 1997). Serotonergic facilitation of excitatory drive in respiratory motoneurons is largely mediated by $5HT_2$ receptors (Kubin *et al*., 1992; Lindsay & Feldman, 1993). One model of 5HT dependent plasticity in respiratory motor control is long term facilitation (LTF). LTF is a long lasting increase in respiratory motor output following either electrical stimulation of chemoafferent neurons (Fregosi & Mitchell, 1994) or intermittent hypoxia (Mitchell *et al*., 2001). The specific 5HT receptor involved appears to be of the $5HT_2$ family mainly $5HT_{2A}$ receptors (Kinkead & Mitchell, 1999; Fuller *et al*., 2001[a]; Mitchell *et al*., 2001). The serotonin transporter (5-HTT) plays a key role in central serotoninergic neurotransmission by controlling its intensity and duration through the reuptake of 5-HT that has been released from serotoninergic terminals, somas and/or dendrites.

5-HT CONTENT IN THE BRAIN REGIONS.

Brain serotonin content increases in brain stem and cerebellum during neonatal hypoxia. This increase is due to an increase in the rate of 5-HT synthesis (Crandall *et al.,* 1981). Changes in serotonin neurotransmission have demonstrated to alter 5-HT and 5-HIAA concentrations (Kwok & Juorio, 1987; Sandirini *et al.,* 1997). In cerebellum and brain stem, conversion of tryptophan by pyridoxal phosphate increased the 5-HT content. Serotonin is metabolised to 5- hydyoxy indole acetic acid (5-HIAA) by the mitochondrial enzyme monoamine oxidase (MAO, primarily MAO-A). The increase in 5-HT content in cerebellum and brain stem is brought about by significant increase in 5-HT synthesis and decrease in its breakdown to 5-HIAA. Caudal raphe neurons are activated during hypoxia, and presumably release 5HT in the vicinity of respiratory premotor and motoneurons (Kinkead et al., 2001). Serotonin has an excitatory effect on upper airway and phrenic motoneurons (Di Pasquale *et al*., 1997). Thus the elevated levels of 5-HT observed in the respiratory centers of brain is a response of the body to stimulate respiratory output from the lungs.

Cerebral cortex

In cerebral cortical development, early manipulations of the serotonergic innervation lead to altered development and plasticity in sensory areas in a variety of species (Gu & Singer, 1995; Osterheld-Haas & Hornung, 1996; Janusonis *et al*., 2004). Several studies have revealed the presence of 5-HT_{2A} receptors in cortical pyramidal neurons (Willins *et al*., 1997; Amargos-Bosch *et al*., 2004; Santana *et al*., 2004). 5-HT influence the descending excitatory input into limbic and motor structures, where the prefrontal cortex projects through the activation of pyramidal 5-HT_{2A} receptors.

The present study on binding parameters of total 5-HT and 5-HT_{2A} receptors in the cerebral cortex showed that the receptor number was significantly increased in hypoxic neonatal rats. Even though the receptor affinity of 5-HT was

decreased, serotonin subtype 5-HT_{2A} showed a higher affinity in hypoxic group. This suggests that the increase in 5-HT receptors may be directed towards 5-HT_{2A} receptors, which protect the respiratory network against hypoxic impairment of brain function. Earlier studies reported changes in synthesis and metabolism of 5-HT during short-term (i.e. 30 min to 2 h) hypoxia (Davis & Carlsson 1973; Olson *et al*., 1983). A number of studies have demonstrated that serotoninergic system participates in the compensatory responses to hypoxia, such as hypothermia and hyperventilation (Poncet *et al*., 1997; Richter *et al*., 1999; Steiner & Branco 2002; McGuire *et al*., 2004; Gargaglioni *et al*., 2005). Richter *et al*., (1999) reported an elevation of extracellular 5-HT levels in the ventral respiratory group in response to hypoxic challenge, suggesting that 5-HT is involved in the elaboration of the HVR. The gene expression of 5-HT receptor and 5-HTT also showed a significant up regulation in hypoxic group. The increased 5-HT receptors with decreased affinity observed under hypoxic condition results in pulmonary vasoconstriction and hypertension as an adaptive response to the stress. The ability of 5-HT to prolong survival in anoxia revealed a 5-HT-activated metabolic pathway that liberates an alternative energy source (Shartau *et al*., 2010).

The alterations in 5-HT and 5-HT_{2A} receptor status and 5-HTT was brought back to near control by the supplementation of glucose alone and glucose with oxygen to the sufferers of hypoxic insult during early neonatal period. The availability of glucose as a universal substrate for aerobic and anaerobic metabolism is of critical importance in cellular homeostasis. The enhancement of glucose transport has been deemed to be essential for the adaptive response to hypoxia. Hyperglycemic effects of 5-HT are closely related to the release of adrenaline from the adrenal gland, mediated by 5-HT_{2A} receptors (Yamada *et al*., 1995). But 5-HT_{2A} was also reported to decrease brain glucose utilization (Hommer *et al*., 1997). So during glucose resuscitation, 5-HT and 5-HT_{2A} receptor function decreased and brought back to near control to facilitate maximum brain glucose utilization. Hypoxia induced ATP depletion causes a reduction in blood

glucose levels which is encountered by glucose administration and immediate oxygenation helps in overcoming the anaerobic condition. Supplementation of 100% oxygen alone to hypoxic rats did not show any reversal in the receptor status and its gene expression to control. The supplementation of 100% oxygen generates reactive oxygen species (ROS) which have been well documented as causative mediators of excitotoxicity (Choi., 1988; Coyle & Puttfarcken 1993; Li *et al*., 1998). Enzymatic oxidation of 5-HT at physiological pH forms tryptamine-4,5-dione (Wrona & Dryhurst, 1991). Tryptamine-4,5-dione is also formed *in vivo*, possibly by an oxidative enzyme or potent oxidizing agent, such as oxygen free radicals. If tryptamine-4,5-dione is formed *via* a free-radical reaction in the central nervous system, it participate in the pathological process of neuronal damage induced by ischemia or traumatic brain injury (Ikeda *et al*., 1989; Sakamoto *et al*., 1991). Supplementation of 100% oxygen cause oxidation of 5-HT, which along with free radicals can contribute to neuronal toxicity in this group.

It is a usual practice of clinicians to resuscitate neonatal hypoxia with epinephrine. The adverse effects of epinephrine supplementation, alone or even in combination with glucose and oxygen, was observed by studying the changes in 5-HT receptor, expression of 5-HT receptor and its transporter. The 5-HT and 5-HT_{2A} receptor was significantly increased compared to control in epinephrine treated groups. The gene expression of 5-HT_{2A} receptor and 5-HTT also showed a significant up regulation.

Epinephrine induces a hypoxia-neovascularization cascade and plays a primary role in vascular proliferation within soft tissues (Karacaoglu *et al*., 2007). It is reported that repetitive hypoxic stress induced by labour is a powerful stimulus for catecholamine release in fetus and is accompanied by typical alterations of fetal heart rate (Jensen *et al*., 2009). Epinephrine mediated platelet aggression is potentiated by 5-HT (Shah *et al*., 1999). Activation of serotonergic system on epinephrine supplementation has a synergistic effect on platelet aggregation, which negatively affects oxygen transport. Hence the increased 5-HT

function on epinephrine supplemention to hypoxic neonates facilitates platelet aggression, which in turn reduces the cellular oxygen transport to overcome hypoxia.

Cerebellum

The cerebellar neurotransmitters significantly differ with respect to ischemia and hypoxia, this response being directly related to the duration and intensity of the injury. Lee *et al*., (2001) reported that hypoxic insult resulted in considerable neurocytological deficits of the Purkinje cells and altered glial fibrillary acid protein immunoreactivity in the fetal cerebellum. Ischemic hypoxia was reported to provoke alterations in the production system of nitric oxide in the cerebellum (Xu *et al.*, 1995). Cerebellum facilitate the respiratory response to hypoxia and that the fastigial nucleus is an important region in the modulation of the hypoxic respiratory responses, presumably *via* its effects on inputs from peripheral chemoreceptors (Carmeliet *et al.,* 1998). Maeshima *et al.,* (1998) reported a dense concentration of 5-HT_{2A} receptors in rat cerebellum. Eddahibi *et al.,* (2000) showed that hypoxia in rats up regulates 5-HT transporter mRNA and it is required directly in the development of hypoxic pulmonary condition. Enhanced long term facilitation after chronic intermittent hypoxia involves an up regulation of a non-5-HT_2 serotonin receptor subtype or subtypes (Ling *et al.*, 2001).

A significant increase in total 5-HT and 5-HT_{2A} receptor number and gene expression in the cerebellar region was observed in the present study under hypoxic stress. The confocal imaging of 5-HT_{2A} receptor immnuohistochemical analysis in cerebellum also showed increased pixel intensity under hypoxic condition. Hypoxia evidently mobilizes 5-HT and stimulates its biosynthesis. The increased 5HT helps in facilitating pulmonary vasoconstriction thereby equipping the body to encounter the stress. This will result in pulmonary hypertension in hypoxic neonatal rats. Serotonin is also reported to be important for effective

stimulation of cAMP levels and initiation of plasticity mediated by adenylyl cyclase (Lin *et al*., 2010).

The up regulated gene expression of $5HT_{2A}$ and the increased receptor status was brought back to near control by the supplementation of glucose alone and glucose with oxygen to the sufferers of hypoxic insult during early neonatal period. Unlike the adult, where glucose supplementation prior to or during hypoxia-ischemia accentuates tissue injury, glucose treatment of perinatal animals subjected to a similar insult substantially reduces the extent of tissue injury (Baron *et al*, 1987). On supplementation of 100% oxygen alone to hypoxic rats, there was an increase in the receptor status and its gene expression. During 100% oxygenation 5-HT function increases to facilitate more blood flow and oxygenation to the cells. But the supplementation of 100% oxygen generates reactive oxygen species (ROS) which have been well documented as causative mediators of excitotoxicity (Choi *et al.*, 1988; Coyle & Puttfarcken, 1993; Li *et al.*, 1998). Both free radicals and glutamate has been suggested to be involved in tandem in the neurotoxicity induced by hypoxia, whereas glutamate alone is involved in ischemic neurotoxicity (Omata *et al.*, 2000). NMDA neurotoxicity and oxidative stress have also been well documented as mechanisms underlying hypoxic–ischemic brain injuries. The adverse effects of epinephrine supplementation, alone and even in combination with glucose and oxygen was reported by studying the changes in 5HT receptor, expression of 5HT receptor and transporter.

Brain stem

Serotonin containing cell bodies are localized in mesencephalic and rhombencephalic raphe nuclei in the brain stem. It has been proposed that 5-HT could be involved in neuronal development and plasticity. Serotonergic fibres have a pervasive innervation of hypoxic-ischemic affected areas in the neonatal brain and serotonin is pivotal in numerous neurobehaviours that match many

hypoxic-ischemia induced deficits. Neonatal hypoxic-ischemia injury caused significant disruption of the brainstem serotonergic system that can persist for up to six weeks after the insult. The different vulnerabilities of serotonergic populations in specific raphe nuclei suggest that certain raphe nuclei underpin neurological deficits in HI-affected neonates through to adulthood (Reinebrant *et al*., 2010). The brain stem serotoninergic system is important for early brain growth and development, including the development of the central respiratory rhythm (Richter *et al*., 2003). 5-HT depletion is concomitant with changes in nitric oxide synthase activity without affecting nitric oxide synthase expression in the dorsal raphe nucleus (Tagliaferro *et al*., 2001). Hypoxia decreased serotonin turnover (5-HIAA/5-HT) in the brain stem (Henley *et al*., 1992). In the central respiratory network, 5-HT_{1A}R efficiently reduces the excitability of respiratory neurons as indicated by the suppression of hypoxic activation (Richter *et al*., 1999) and decreased ventilatory responses to hypercapnia (Taylor *et al*., 2005); thus, 5-HT_{1A}R has inhibitory actions. 5-HT_{2A}R on the other hand, has an excitatory role and animal studies have shown that 5-HT_{2A}R receptor stimulation excites the respiratory motor system at the level of the pre- Botzinger complex resulting in increased gasping (Tryba *et al*., 2006) and increased frequency of two types of respiratory bursts representative of fictive eupneic activity and fictive sigh activity (Pena & Ramirez, 2002).

The binding studies of total 5-HT and 5-HT_{2A} receptors showed a significantly increase in receptor number with an up regulation of its gene expression under hypoxic stress in the brain stem. The altered parameters observed under the stress was effectively reversed back to near control by resuscitating with glucose alone and in combination with oxygen. No significant reversal was found in hypoxic rats resuscitated with oxygen alone and epinephrine. The increased expression of 5-HT_{2A} receptor excites the respiratory centers of the brain stem resulting in increased gasping and increased respiratory bursts. Since continuous innervation of 5-HT causes pulmonary vasoconstriction

and reduction of GABA receptors an effective resuscitation should bring back the elevated 5-HT receptor function to near control.

Corpus striatum

Broderick and Gibson (1989) reported that *in vivo* hypoxia increases rat striatal extracellular dopamine and to a lesser extent, extracellular serotonin. Furthermore, after repeated, mild hypoxic episodes or moderate hypoxia, the increases in rat striatal extracellular dopamine and serotonin continue even during normoxia. These studies support a role for dopamine and serotonin in hypoxic-induced changes in brain function. The hypoxic-induced elevation of these two neurotransmitters during normoxia are important in the production of hypoxic/ischemic-induced cell damage. Saligaut *et al*., (1986) reported an inhibition of 5- HIAA formation and a complex interaction between synthesis, release and uptake of 5-HT in the hypoxic striatum and hypothalamus.

The present study reported an elevated 5-HT and 5-HT_{2A} receptor number under hypoxic stress in the striatum. The gene expression of 5-HT_{2A} and 5-HTT also showed a significant up regulation in hypoxic neonatal rats. Glucose supplementation reversed the alterations in striatal serotonergic receptors to near control. The immediate energy source from glucose helps in reducing the free radical toxicity thereby preventing the neurotransmitter alterations. Resuscitation with oxygen alone and epinephrine showed no reversal in the altered parameters indicating that 100% oxygenation and administration of epinephrine have worsened the neurotransmitter imbalance caused by neonatal hypoxic stress. 5-HT_{2A} receptor-mediated signaling events are strengthened within the striatum under conditions of dopamine depletion to provide a more potent regulation of motor activity (Bishop *et al*., 2004).

CENTRAL MUSCARINIC RECEPTOR ALTERATIONS

Acetylcholine (ACh) is involved in the central control of respiration (Haji *et al*., 2000). Cholinergic systems originating in the pons (Dutschmann & Herbert, 1999) also control respiration in the sleep–wake states by affecting respiratory neurons and motoneurons (Bellingham & Berger, 1996; Bellingham & Ireland, 2002). Cholinergic control also occur through direct action on rhythm-generating neurons in the pre-Bötzinger complex, because muscarinic or nicotinic agonists affect these neurons and increase respiratory frequency when applied in the medullary slice *in vitro* (Shao & Feldman, 2000, 2001). Pharmacological evidence suggests that the muscarinic M1 and M3 receptor subtypes play a predominant role in respiratory control, primarily based on studies in the neonatal rodent brain stem preparation *in vitro* (Burton *et al*., 1994) and in the adult cat (Nattie & Li, 1990). The majority of respiratory neurons are cholinoceptive and are either excited or depressed by ACh or ACh agonists applied by iontophoresis *in vivo*, the proportion of depressed neurons being higher in anesthetized animals than in decerebrated preparations (Morin-Surun *et al*., 1984). It was shown that ACh increase respiratory frequency through muscarinic M3 receptor activation of rhythm-generating neurons in the pre-Bötzinger complex in the medullary slice *in vitro* (Shao & Feldman, 2000).

Cerebral cortex

In the central nervous system (CNS), the basal forebrain cholinergic system has been shown to be important in learning and memory. Cholinergic stimulation in regions of the brain such as cortex and hippocampus appear to have a modulatory role in facilitating responsiveness of cortical neurons to other inputs (Bartus *et al*., 1982). The RT-PCR and HPLC studies revealed that the muscarinic M1 receptor was present in a relatively high density in the cerebral cortex (Jian *et al*., 1994; Oki *et al*., 2005). It is hypothesized that the cerebral cortex participates in the memory, attention, perceptual awareness, thought, language and

consciousness which are necessary for the normal life style. The muscarinic M1, M3 and M5 receptors are located predominantly on postsynaptic nerve terminals and are thought to be responsible for the role of the muscarinic cholinergic system in cognition and long term potentiation in the hippocampus and cortex (Bartus, 2000). Immunoprecipitation and immunofluorescence studies indicate that muscarinic M1 and M3 receptors are expressed in cortex (Levey, 1993).

Binding studies using [^{3}H] QNB and muscarinic general antagonist, atropine revealed a significant decrease in total muscarinic receptor number in hypoxic neonatal rats. The decreased muscarinic receptors in the cortical region can attribute to the behavioural deficits observed in the later stages. Acetylcholine receptors are targeted to compartments rich in mitochondria, particularly postsynaptic domains and presynaptic terminals, exposing these receptors to reactive oxygen species. During neonatal hypoxic shock these receptors become exposed to ROS which resulted in a decrease in its number and affinity. Muscarinic AChRs are predominant cholinergic receptors in the central nervous system, where they play a major role in learning and memory (Gibbs, 1999), ingestion behavior (Rowland *et al*., 2003), and other central functions. Muscarinic M1/M3 receptors are involved in the control of tidal volume whereas M2/M4 receptors are involved in the control of breathing frequency and sensitivity to stress (Boudinot *et al.,* 2008*)*. The gene expression studies of muscarinic M1, M2 and M3 was down regulated in hypoxic neonatal rats indicating the reduction in tidal volume and breathing frequency under hypoxic stress.

Central cholinergic neurons participate in the complex neural events responsible for the hyperglycemic response to neurocytoglucopenia and to stressful situations. Atropine injected into the third cerebral ventricle suppressed epinephrine secretion and dose-dependently inhibited hepatic venous hyperglycemia induced by neostigmine in intact rats (Iguchi *et al*., 1990). The resuscitation with glucose alone and with oxygen effectively reversed the binding parameters and gene expression patterns to near control. Resuscitation with

oxygen alone and epinephrine did not show reversal of altered muscarinic function. Since glucose supplementation along with oxygenation provided an immediate energy source the imbalance in muscarinic function can be reversed by timely supplementation of glucose. Stabilising the muscarinic receptor function by glucose resuscitation improved the ventilatory response of hypoxic neonatal rats and reduced the behavioural deficits in the later stages of life.

Cerebellum

Cerebellum is a region of the brain that plays an important role in the integration of sensory perception, memory consolidation, coordination and motor control. In order to coordinate motor control, there are many neural pathways linking the cerebellum with the cerebral motor cortex and the spinocerebellar tract (Roberta & Peter, 2003). There is currently enough anatomical, physiological and theoretical evidence to support the hypothesis that cerebellum is the region of the brain for learning, basal ganglia for reinforcement learning and cerebral cortex for unsupervised learning (Doya, 1999). The cellular basis of motor learning has been mostly attributed to long term depression (LTD) at excitatory parallel fiber - purkinje cell synapses. LTD is induced when parallel fibers are activated in conjunction with a climbing fiber, the other excitatory input to Purkinje cells. Recently, by using whole-cell patch-clamp recording from Purkinje cells in cerebellar slices, a new form of synaptic plasticity was discovered.

Total muscarinic receptor number showed a significant decrease with a down regulated expression of muscarinic M1, M2 and M3 receptor subtypes in hypoxic neonatal rats. Cerebellum participates in the learning and coordination of anticipatory operations which are necessary for the effective and timely directing of cognitive and non-cognitive resources (Allen *et al*., 1997). The cholinergic dysfunction, impaired glucose transport and oxidative stress contributes to learning and memory deficits in diabetes (Peeyush *et al*., 2010). The dimished acetylcholine muscarinic receptor function under hypoxic stress in neonates

contribute to memory and learning deficits. The current study revealed the modulatory function of glucose alone and along with oxygen on total muscarinic receptors by normalising the altered receptor gene expression and binding parameters to near control. The cerebellum has generally been suggested to be involved in the control and integration of motor processes, as well as cognitive functions. In the current study, we observed the neuroprotective effect of glucose on muscarinic receptors and muscarinic M1, M2 and M3 receptor subtypes in cerebellum, which is responsible for the coordination of voluntary motor movement, balance and equilibrium and declarative memory. Epinephrine administration and oxygenation alone was observed to worsen the situation by further decreasing the receptor function, which lead to behavioural deficits.

Brain stem

The neurotransmitter acetylcholine, acting through muscarinic receptors is involved in many aspects of respiratory neuromodulation (Haji *et al*., 2000), notably central chemosensitivity in brain stem structures (Ballantyne &Scheid, 2000; Burton & Kazemi, 2000). Pharmacological evidence, based primarily on studies conducted *in vitro* in the neonatal rodent whole brain stem (Burton *et al*., 1994) or brain stem slice (Shao & Feldman, 2000) and in the adult cat (Nattie & Li, 1990), suggests that the most important role in respiratory control is played by muscarinic M1 and M3 receptor subtypes.

The total muscarinic receptors of the brainstem were found to be decreased with a down regulated expression of muscarinic M1, M2 and M3 receptor subtypes in hypoxic neonatal rats. Muscarinic alterations in brainstem during neonatal hypoxia result in memory problems, difficulty in concentrating and difficulty in staying focused. Our results showed that glucose resuscitation restored the altered muscarinic functions associated with brainstem whereas resuscitation with epinephrine and oxygenation alone worsened the receptor functions.

Corpus striatum

Cholinergic terminals within the striatum contain presynaptic muscarinic receptors that inhibit neurotransmitter release (Chesselet, 1984). Various anatomical, electrophysiological and pathological observations provide evidence that ACh plays a major role in the control of striatal function and in the regulation of motor control (Jabbari *et al*., 1989). Striatal ACh is released from a population of large cholinergic interneurons that establish complex synaptic contacts with dopamine terminals, originating from the substantia nigra and with several striatal neuronal populations (Lehmann & Langer, 1982, 1983; Wainer *et al*., 1984; Phelps *et al*., 1985; Izzo & Bolam, 1988; Vuillet *et al*., 1992). Corpus striatum regulates endocrine functions indirectly through the secretion of other hormones like thyroxine.

Binding studies of total muscarinic receptors showed that the receptor number was significantly decreased with a down regulated expression of muscarinic M1, M2 and M3 receptor subtypes in hypoxic neonatal rats. The resuscitation with glucose alone and with oxygen effectively reversed the changed parameters to near control. Resuscitation with oxygen alone and epinephrine did not show reversal of altered muscarinic function. Since striatal acetyl choline receptor function is regulated through nigrostriatal pathway the changes in dopaminergic, serotonergic and GABAergic receptors influence the muscarinic function. The disturbances in the pathway resulted in an altered muscarinic status which contributes to the impairment in memory and cognition. The neuroprotective role of glucose resuscitation was also observed.

CHOLINERGIC ENZYME ALTERATIONS IN BRAIN OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

Choline acetyltransferase (ChAT) is the rate-limiting enzyme of generating acetylcholine (ACh), which is synthesized in cholinergic neuronal cell

bodies and is often used in the studies of tissue localization and functional activity. The reduction of ChAT is correlated with the severity of dementia and pathologic changes (Rodrigo *et al*., 2004). Acetylcholine is the primary neurotransmitter of the cholinergic system and its activity is regulated by acetylcholine esterase (AChE). The termination of nerve impulse transmission is accomplished through the degradation of acetylcholine into choline and acetyl CoA by AChE (Weihua *et al*., 2000).

Central cholinergic activity was studied in the brain regions of experimental neonatal rats using ChAT and AChE as marker. The gene expression of ChAT in cerebral cortex, cerebellum, brain stem and corpus striatum was significantly down regulated under neonatal hypoxic stress. This indicates the drastic reduction in the anabolic pathway of ACh under hypoxic stress. Chang *et al* (2004) observed a down-regulation of ChAT immunoexpression in hypoxic rats which indicates the poor neurotransmission within the injured neurons. The pronounced reduction of ChAT in hypoxic rats result from a drastic shift of intracellular metabolic pathways, which in turn could lead to more metabolic loading to the severely damaged neurons. Severe hypoxic challenges are associated with decreased forebrain and brainstem ChAT immunohistochemistry and supression of hippocampal ChAT activity (Flavin *et al*., 1993; Tanaka *et al*., 1995; Nyakas *et al*., 1996). Acetylcholine and its receptors – both muscarinic and nicotinic are down regulated due to reduced synthesis of the neurotransmitter which lead to brain dysfunction in neonatal hypoxia. ACh prevented the hypoxia-induced apoptosis of mouse ES cells by inhibiting the ROS-mediated p38 MAPK and JNK activation as well as the regulation of Bcl-2, c-IAPs, and caspase-3 (Kim *et al*., 2008). Gibson and Duffy (2006) showed that even mild hypoxic hypoxia impairs ACh synthesis, which in turn account for the early symptoms of brain dysfunction associated with hypoxia.

The resuscitation with glucose and glucose along with oxygen to neonatal rats exposed to hypoxia effectively reversed the down regulated ChAT expression

to near control. Glucose forms the primary precursor for ACh synthesis. Supplementation of glucose provides the substrate for acetyl choline synthesis and also provides an immediate energy source to encounter ATP depletion. Resuscitating hypoxic neonatal rats with 100% oxygen showed a slight reversal in the expression of ChAT to near control. Epinephrine supplementation to hypoxic neonatal rats was not effective in restoring the altered ChAT expression. Epinephrine was reported to counteract completely the effect of acetylcholine in limited concentrations on contractile strength while only partially or weakly counteracting its effect on the action potential (Robert *et al*., 1960).

Acetylcholine esterase activity has been used as a marker for cholinergic activity (Ellman *et al*., 1961). AChE plays a very important role in the ACh-cycle, including the release of ACh (Kouniniotou- Krontiri & Tsakiris, 1989). The duration of action of ACh at the synaptic clefts is critically dependent on AChE activity (Cooper *et al*., 2003). In the present study, AChE expression in the brain regions was found to be up regulated in hypoxic neonatal rats. Extracellular and tissue cholinergic activity have been previously reported to be depressed during hypoxic insult (Freeman *et al*., 1987; Beley *et al*., 1991). Furthermore, hypoxia is reported to decrease the ACh content in the brain as a result of release of choline into extracellular space *via* activation of NMDA receptors (Zapata *et al*., 1998; Paulose *et al*., 2007) due to which the AChE expression was up regulated in neonatal hypoxic rats.

Glucose supplementation alone and along with oxygenation to hypoxic neonatal rats reversed the expression of AChE to near control. Decrease in the blood glucose level during hypoxia would lead to decrease in the brain ACh synthesis. Glucose, one of the main sources of acetyl CoA, is utilized by the cholinergic neurons to synthesize ACh (Willoughby *et al*., 1986) which reasonably explain the reversal of up regulated AChE expression in glucose supplemented hypoxic rats in our study.

Epinephrine supplementation and 100% oxygenation to hypoxic neonatal rats did not show reversal in AChE expression. This up regulation in the AChE expression in the brain regions of oxygen supplemented group is either due to the ROS induced tissue damage hindering the enzyme activity or due to decreased ACh content as a result of ROS. Epinephrine decreases the uptake of glutamate in the brain causing persistent activation of glutamate receptors (Paulose *et al*., 2007) which is capable of causing cholinergic dysfunction (Alkondon & Albuquerque, 2006) leading to a change in ACh content and AChE activity. Thus resuscitation with epinephrine is not effective in reversing the alterations in AChE expression in hypoxic neonatal rats.

SECOND MESSENGER ALTERATIONS IN CORPUS STRIATUM OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

Cyclic AMP is generated through the action of adenylyl cyclases, which is stimulated through appropriate G- protein coupled receptors (GPCRs) able to couple to the stimulatory guanine nucleotide regulatory protein, Gs (Wong & Scott, 2004). cGMP synthesis is catalyzed by guanylate cyclase (GC), which converts GTP to cGMP. Membrane-bound GC is activated by peptide hormones such as the atrial natriuretic factor, while soluble GC is typically activated by nitric oxide to stimulate cGMP synthesis. cGMP is a common regulator of ion channel conductance, glycogenolysis, and cellular apoptosis. In our studies we observed an elevated cAMP and cGMP level in the corpus striatum of hypoxic neonatal rats. The imbalance in the redox system of oxidative phospholyration results in ROS production under hypoxic stress, which in turn activates the second messenger pathways as an adaptive modification. Millena *et al* (2006) reported that the increased cAMP levels in hypoxia are due to the ERK-mediated autocrine generation of prostaglandin E2. Consistent with such a role for ERK, MEK inhibitors was found to normalize cAMP levels in hypoxic hPASM cells presumably by curtailing this autocrine response (Millena *et al*., 2006).

Resuscitation with glucose brought back the altered cAMP and cGMP level to near control due to the reduced ROS production. Resuscitation with 100% oxygen forms ROS which triggers cAMP and cGMP production. In epinephrine resuscitated groups the cAMP and cGMP levels are high as epinephrine triggers cAMP formation. Epinephrine acts as a α_2- and β-adrenoceptor agonist and α_2-adrenoceptors interact with β -adrenoceptors and vasopressin receptors for cAMP accumulation (Yasuda *et al*., 1997). It was concluded that cAMP and cGMP play an important role in neonatal hypoxia, participate in the cellular signal transducation and promote the homeostatic response of the body to the stress.

IP3 functions by binding to the membrane-associated IP3 receptors (IP3R) (Berridge *et al*., 2003). Binding of IP3 to the receptor increases its sensitivity to Ca^{2+}, and only after Ca^{2+} is bound can trafficking of the Ca^{2+} into the cytosol take place. Notably, Ca^{2+} has a biphasic action on the IP3R with a stimulatory effect at low Ca2+ concentrations and an inhibitory effect at higher Ca^{2+} concentrations (Nadif Kasri *et al*., 2002; Taylor & Laude, 2002). Acting as a signal transducer between two ubiquitous second messengers IP3 and Ca^{2+}, IP3R has been implicated in a variety of cellular and physiological processes as diverse as cell division, cell proliferation, apoptosis, fertilization, development, behaviour, memory and learning. In mammals, there are three distinct types of IP3R with splice variants observed among the types (Furuichi *et al*., 1994; Patel *et al*., 1999). IP3-receptor is dominantly expressed in neuronal cells throughout the central nervous system (Nakanishi *et al*., 1991; Furuichi *et al*., 1993). Throughout the brain, the IP3R1 is the predominantly expressed member of the family and its mRNA is widely distributed (Ross *et al*., 1992).

Our studies observed an elevated IP3 content in the striatum of hypoxic neonatal rats. The elevated IP3 level causes extra cellular release of Ca^{2+}, which in turn results in the activation of apoptotic pathways. Transfer of Ca^{2+} between intracellular stores and mitochondria provides physiological control of respiration. But this Ca^{2+} cycle also lead to cell death. If the matrix Ca^{2+} level rises too high,

then deleterious changes in mitochondrial structure occur. In particular, mitochondria swell and rupture or undergo permeability transition, thereby releasing several pro-apoptotic factors into the cytoplasm, such as cytochrome C, second mitochondrial activator of caspases (SMAC/Diablo) or apoptosis-inducing factor (AIF) (Orrenius *et al*., 2003). This leads to the generation of the 'apoptosome' and activation of caspases from inactive zymogens. It is well established that Ca^{2+} released through IP3 receptors is sequestered by mitochondria (Rizzuto *et al*., 2004) Furthermore, it has been demonstrated that the flow of Ca^{2+} specifically from IP3 receptors can cause mitochondrial permeability transition and activate the apoptotic cascade (Szalai *et al*., 1999). Alterations in phosphoinositide-mediated signal transduction lead to the loss of mAChR sensitivity, which is also observed in the present study. Glucose resuscitation, alone and along with oxygen effectively brought back the elevated IP3 level to near control. The intracellular glucose level acts as a regulator of IP3 formation and signaling.

EXPRESSION OF HYPOXIA INDUCIBLE FACTOR IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

HIF-1α plays an essential role in cellular oxygen homeostasis by regulating the expression of genes involved in glycolysis, erythropoiesis and angiogenesis (Semenza, 2000[a, b]). HIF-1 is a transcription factor specifically activated by hypoxia (Chavez & LaManna, 2002). The accumulation of HIF-1α in ischemic or hypoxic tissues promote adaptive mechanisms for cell survival (Bergeron *et al*., 1999) and was found to be an important mediator of hypoxia-induced tolerance to ischemia (Bergeron *et al*., 2000; Jones & Bergeron, 2001; Bernaudin *et al*., 2002). Although HIF-1α is essential for adaptation to low oxygen levels, it has also been shown *in vitro* to mediate hypoxia-induced growth arrest and apoptosis (Goda *et al*., 2003). HIF-1α is also a key component of the cellular response to hypoxia and ischemia under pathophysiological conditions. HIF-1 is

reported to involve in the induction of cardioprotective molecules, such as inducible nitric oxide synthase (iNOS), hemeoxygenase 1 (HO-1), and erythropoietin (EPO), which in turn alleviate myocardial damages caused by harmful events such as ischemia-reperfusion injury (Tekin *et al*., 2010).

Our studies showed an up regulated HIF 1 expression in the cerebral cortex, cerebellum, brain stem and corpus striatum of hypoxic neonatal rats. The increased Hif 1 mRNA expression under hypoxia is suggested to be an adaptive response of the body to encounter the stress by facilitating angiogenesis, vasodialation and erythropoiesis. But in severe hypoxic cases, HIF-1α is accumulated and leads to cell death by activating different target genes (Semenza *et al*., 2000). It bind to pro apoptotic members of the Bcl-2 family such as BNIP3 (Bruick, 2000), Nix (Sowter *et al*., 2001) and p53 aswell as caspases (Li *et al*., 2005), which contribute to cell death or apoptosis. The role of HIF-1α in mediating pro death and pro survival responses, is dependent on the duration (Halterman & Federoff, 1999) and types of pathological stimuli (Aminova *et al*., 2005) as well as the cell type that it induces (Vangeison *et al*., 2008).

Suppression of HIF-1α is important for exploring HIF-1-dependent processes and for interfering with hypoxia-induced pathophysiological events. Hence the effectiveness of a resuscitation method for severe hypoxia depends on its capability to reverse the up regulated HIF expression. The present study reported a reversal in the elevated HIF gene expression in the brain regions by resuscitating the hypoxic neonates with glucose and oxygen. This points the significance of immediate resuscitation in encountering hypoxic stress without leading to cell death.

The traditional practice of epinephrine supplementation during prolonged hypoxia was found to up regulate the HIF expression to stimulate body functions that facilitate more oxygen transport to cells. Since HIF acts as a pro death response under severe hypoxia, epinephrine resuscitation can worsen the situation by activating cell death promoting pathways.

EXPRESSION OF BAX IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

The survival and effective functioning of the brain and brain cells are highly dependent on the balance between the brain's ability to utilize oxygen and oxygen's relative availability in the vascular system in the immediate surroundings. The human brain consumes some 20% of the body's oxygen intake, but constitutes just 2% of total body weight (Erecinska & Silver, 2001). An extreme reduction of brain oxygen supply is known to lead to neuronal death (Hopkins & Haaland, 2004; Xu & LaManna, 2006), yet many situations of reduced oxygen do not lead to death of the organism, or even overt damage. Bax is a pro-apoptotic protein allowing apoptosis to occur through the intrinsic, damage-induced pathway and amplifying that one occurring *via* the extrinsic, receptor mediated pathway. Bax is present in viable cells and activated by pro-apoptotic stimuli. Bax has multiple functions: it releases different mitochondrial factors such as cytochrome c, SMAC/diablo; it regulates mitochondrial fission, the mitochondrial permeability transition pore; it promotes Ca^{2+} leakage through ER membrane (Ghibelli & Diederich, 2010). The expression of proapototic protein BAX can be taken as an index of cell death.

The present study observed a significant up regulation of Bax expression in the cerebral cortex, cerebellum, brain stem and corpus striatum of hypoxic neonatal rats. Bax is one of the key proteins that turn on the apoptotic cascade. The Bcl-2 family of proteins is an important determinant of apoptotic cell death. It consists of pro-apoptotic (Bax, Bcl-Xs, Bak and Bad) and anti-apoptotic (Bcl-2, Bcl-XL and Bcl-w) proteins (Adams & Cory, 1998). Bcl-2 family members determine cell death and survival by controlling mitochondrial membrane ion permeability, cytochrome *c* release and the subsequent activation of caspase (caspase 3, caspase 9) executor functions (Allen *et al*, 1998; Banasiak *et al*, 2000; Glasgow & Perez-Polo, 2000). Bax homodimers facilitate mitochondrial release

of cytochrome *c via* a process requiring Bax translocation to mitochondria (Crompton, 2000). The up regulated Bax expression in the present study indicates the high cell death in the cortical, cerebellar, straital and brain stem regions of neonatal rats exposed to hypoxic insult. The cell death in the brain regions in the early stage of development drastically affect the memory and cognition in the later stages of life. The standard approach to resuscitate neonatal hypoxia is to use 100% O_2. Further, resuscitation with 100% is recommended as a beneficial short-term therapy that is generally thought to be non-toxic (Martin *et al*, 2005; Kuisma *et al*, 2006). Although the use of 100% O_2 appears intuitive to maximize the gradient required to drive O_2 into hypoxic cells (Corff & McCann, 2005), a building body of evidence derived from animal models, has demonstrated that although resuscitation with 100% O_2 improves restoration of cerebral and cortical perfusion, occur at the price of greater biochemical oxidative stress (Martin *et al*, 2005). Further, results from investigations by Munkeby *et al.,* (2004) suggest that resuscitation of asphyxiated piglets with 100% O_2 is detrimental to the brain. Our studies showed that the cell death in the brain regions of 100% O_2 resuscitated hypoxic rats is significantly high compared to control. This indicates that hyperoxygenation can trigger the apoptotic pathways resulting in cellular loss.

Immediate resuscitation with glucose, alone and along with oxygen, on the event of neonatal hypoxic stress was found to be effective in minimizing the cell death by down regulating the Bax mRNA expression. In the central nervous system, metabolic stress such as oxygen–glucose deprivation and hypoxic hypoglycemia *in vitro* deplete intracellular ATP stores and lead to the development of irreversible neuronal tissue damage (Lipton & Whittingham, 1979; Yoneda & Okada, 1989). Cell death caused by O_2 lack begins when anaerobic ATP production fails to meet the energetic maintenance demands of ionic and osmotic equilibrium. One of the earliest signs of neuronal responsiveness during a hypoxia-induced fall in ATP levels, coupled with extracellular accumulation of adenosine, a depression of synaptic transmission,

mainly due to presynaptic inhibition of L-glutamate release resulting from suppression of voltage-gated calcium currents (Krnjevic, 1999; Coelho *et al*, 2000). Hypoxia induced ATP depletion causes a reduction in blood glucose levels which can be encountered by glucose administration and immediate oxygenation helps in overcoming the anaerobic condition. Since glucose acts as an immediate energy source it can counteract the deleterious effects of ATP depletion induced cell death and other metabolic changes. In epinephrine supplemented groups the Bax expression was found to be up regulated in neonatal rats which points the adverse effects of epinephrine supplementation to hypoxic rats.

EXPRESSION OF CREB IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

Cyclic AMP response element binding (CREB) protein, a transcription factor, mediates responses to a number of physiological and pathological signals such as neurotransmitters, synaptic activity, depolarization, mitogens, hypoxia and other stress factors (Sheng *et al*., 1991; Ghosh & Greenberg, 1995; Ginty, 1997; Vo & Goodman, 2001). CREB drives cell survival signalling (Walton *et al*., 1999; Walton & Dragunow, 2000; Ciani *et al*., 2002), and activation of muscarinic acetylcholine receptors promote cell survival. Indeed, many previous in vitro studies have shown that muscarinic agonists (primarily muscarinic M1 andM3, but also muscarinic M4) promote cell survival (Lindenboim *et al*., 1995; Yan *et al*., 1995; Itano *et al*., 1996; Budd *et al*., 2003; De Sarno *et al*., 2003; Budd *et al*., 2004; Wu & Wong, 2006). Interestingly, both the muscarinic receptor-mediated activation of CREB and cell survival appear independent of MAP kinase signalling (Leloup *et al*., 2000; Greenwood & Dragunow, 2002; Budd *et al*., 2003) suggesting that the CREB signaling pathway is involved in this cytoprotective effect. Previous studies of oligodendrocyte progenitors showed that stimulation of mAChR (Ragheb *et al*., 2001) phosphorylates CREB in a calcium-dependent manner (Pende *et al*., 1997; Sato-Bigbee *et al*., 1999). This transcription factor

promotes cell survival (Bonni *et al*., 1999; Watt *et al*., 2004) through the PI3K/Akt pathway to up regulate the expression of the anti-apoptotic factor Bcl-2 (Du &Montminy, 1998; Pugazhenthi *et al*., 2000).

In the present study the gene expression of CREB was down regulated in cerebral cortex, cerebellum, brain stem and corpus striatum of hypoxic neonatal rats compared to control. Eventhough cAMP level was increased in hypoxic neonatal rats, the CREB expression declined. Adaptive response of the body to hypoxia activates the second messengers to encounter the stress. But acute and prolonged hypoxia triggers the cell death pathways by activating pro apoptotic genes like bax, bad and destabilizing jun- fos complex. The activation of apoptotic pathways down regulates the CREB expression thereby blocking the cAMP signaling cascade in hypoxic neonatal rats. Down regulation of CREB is a consequence of apoptotic pathway activation and down regulation of muscarinic receptor function. These findings suggest that decreased CREB expression is the result of cell loss. The fact that CREB expression is known to be regulated in a number of systems (Brecht *et al*., 1994; Widnell *et al*., 1994; Walker *et al*., 1995) suggests that post-translational modification is not the only mechanism involved in the control of its trans-activation potential. Ca^{2+} increase induced by hyperosmotic stress promotes cell survival by recruiting CREB-mediated signaling. The fate of cardiomyocytes under hyperosmotic stress will depend on the balance between Ca^{2+} induced survival and death pathways by regulating CREB (Bordukalo-Niksic *et al*., 2010)

Resuscitation with glucose alone and along with oxygen reversed the down regulated CREB expression to near control. Hypoxic neonatal rats resuscitated with epinephrine or 100% oxygen did not show any reversal. Since glucose acts as immediate source of ATP, the hypoxic stress related ROS production and apoptosis is limited by administrating glucose to hypoxic neonatal rats.

EXPRESSION OF PHOSPHOLIPASE C IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

. Phospholipase C (PLC) mediates transduction of neurotransmitter signals across membranes *via* hydrolysis of phosphatidylinositol-4,5-bisphosphate, leading to generation of second messengers inositol- 1,4,5-trisphosphate and diacylglycerol. In the CNS, neurotransmitter receptor coupling to phospholipase C has been extensively documented in [^{3}H] inositol-labeled tissue slices and synaptosomes obtained from animal brains (Fisher & Agranoff, 1987; Stephens & Logan, 1989; Chandler & Crews, 1990).

In the present study, we observed hypoxia-mediated alterations in phospholipase C expression in the brain regions- cerebral cortex, cerebellum, brain stem and corpus striatum. Further we extended the studies to phospholipase C regulation with glucose, oxygen and epinephrine resuscitation for potential therapeutics which modulate signal transduction pathway for preventing CNS dysfunction in neonatal hypoxia. Our results showed an increased expression of phospholipase C in the cerebral cortex, cerebellum, brain stem and corpus striatum of hypoxic neonatal rats when compared to control. Muscarinic receptors M1, M3, M5 typically couple *via* α subunits of the Gq/11 family to activate phospholipase C (PLC), stimulating phosphoinositide (PI) hydrolysis (Caulfield & Birdsall, 1998). In particular, reconstitution experiments with purified muscarinic M1 receptors, G protein subunits and PLC suggested that the β1 subtype of PLC serves as the primary effector for the muscarinic M1 receptor (Felder, 1995). We considered that the up regulation of the Phospholipase C in the brain regions of hypoxic neonatal rats contribute to the increased IP3 levels in hypoxic rats. Phospolipase C performs a catalytic mechanism, generating inositol triphosphate (IP3) and diacylglycerol (DAG). Altered phospholipase C expression fails to modulate the activity of down stream proteins important for cellular signaling. Defective expression of phospholipase C causes the impaired release of Ca^{2+} and brings down the level of intracellular calcium and thus failed to execute the

normal neuronal function in the brain regions. During hypoxia fructose-1,6-bisphosphate initiates a series of neuroprotective signals which include PLC activation, small increases in [Ca^{2+}] and increased activity of the MEK/ERK signaling pathway (Fahlmana *et al*., 2002). Previous studies reports that phospholipase C-mediated signaling initiated by growth factor receptor types, are involved in long-term memory formation, a process that requires gene expression (Paul *et al*., 1999). These evidences led us to propose that the enhancement of hypoxia-mediated phospholipase C gene expression could impart damage to the central cognitive functions, which has been effectively protected by glucose resuscitation.

BEHVIOURAL DEFICITS IN HYPOXIC NEONATAL RATS

The occurrence of hypoxic brain injury during fetal or neonatal development leads to damaged immature neurons and result in behavioural and/or cognitive dysfunction, including motor or learning disabilities, cerebral palsy, epilepsy or even death (Saikumar *et al.*, 1998; Delivoria-Papadopoulos & Mishra, 2000; Levison *et al*., 2001). Mild hypoxic–ischemia induces significant cerebral injury in neonates and is frequently accompanied by motor and cognitive impairments throughout life (Lindstrom *et al.*, 2006; van Handel *et al.*, 2007). There has been much interest in the acute neurological changes associated with neonatal hypoxia, along with the mechanisms of subsequent central nervous system dysfunction in the adult (Lindahl *et al.,* 1988; Berg, 1988; Soulier *et al.*, 1997; Peterson, 2003). Hypoxia during the first week of life induce neuronal death in vulnerable brain regions usually associated with an impairment of cognitive function that can be detected later in life (Casolini *et al.*, 2005).

We evaluated the spatial recognition and exploratory behaviour by Y-maze, spatial memory and learning by radial arm maze, memory and cognition by Morris water maze experiment in one month old rats exposed to neonatal hypoxia.

The reflex action and motor co-ordination was also evaluated by righting reflex test and wire maneuver test.

Y-maze is a simple recognition test for measuring spatial recognition memory. It is based on the innate tendency of rodents to explore novel environments (Dellu *et al*., 2000). In Y maze performance, the number of novel arm entries and time spent was significantly lower in hypoxic rats, showing the aversion for exploring new environments and defective spatial recognition. There are reports on the involvement of the cholinergic system abnormality in the impaired acquisition and/or retention of passive avoidance learning. In this respect, the observed behavioral abnormalities can be suggested to be a consequent of cholinergic dysfunction in hypoxic rats. However, in neonatal hypoxic rats resuscitated with glucose, alone and along with oxygen, the time spent and number of novel arm entries in the Y-maze was increased significantly. Y maze studies in epinephrine and 100% oxygen resuscitated groups showed decreased exploratory behaviour showing spatial recognition deficit. These findings indicate that glucose supplementation for neonatal hypoxia is an effective resuscitation programme in normalizing the cholinergic receptor dysfunction and thus improving the cognitive functions.

The radial arm maze is used primarily to measure spatial learning and memory. In radial arm maze, memory errors like working memory error and reference memory error were scored along with the number of trials needed to attain the criterion. Working memory is a transient form of memory that maintains task relevant information during conditions of competing demands (Baddeley, 1986). Working memory is operationalized as task accuracy in situations where information (or the appropriate response based on that information) changes frequently (i.e., from trial to trial), whereas reference memory reflects task accuracy when information (or the appropriate response based on that information) remains constant indefinitely (i.e. across trials and/or sessions) (Honig, 1978; Olton *et al*., 1979). The number of trials to attain five consecutive

criterion performances increased significantly in the hypoxic rats. Increased numbers of trials to criterion performance indicates the learning and memory deficit in hypoxic rats. A significant increase in memory errors was also scored in hypoxic rats indicating the impairment in coordination of tasks. High reference memory error in hypoxic rat points to the impairment with respect to procedural or reference memory i.e., what to do with the information they have. Glucose resuscitated rats showed a considerable reduction in the number of trials to attain the criterion performance and memory errors. The impairment in spatial learning, memory and task management was reversed by glucose supplementation to hypoxic neonatal rats, both alone and in combination with oxygen. This points the importance of a proper resuscitation programme to overcome neonatal hypoxia for a better intellect in the later stages of life.

The Morris water maze has been used extensively to measure cognitive deficits in brain-damaged rats (Brandeis *et al*., 1989). Performance in the Morris water maze depends on several elements, ranging from attention, learning and memory, to vision and motor coordination. Place navigation in the Morris water maze consists of two distinct components: declarative place representations as well as procedural learning (Morris *et al.,* 1999). The procedural aspects include learning to inhibit inborn nonadaptive behaviour, such as swimming along the wall (Whishaw & Mittleman 1986; Paylor & Rudy 1990), while selecting appropriate behavioural strategies, such as swimming across the pool or uniformly searching its surface. Other procedural components involved skills such as improved distance and angle judgment that are a necessary prerequisite for the cognitive demands of the task. The hippocampal formation is critical for computing place representations but is believed to be dispensable for procedural memories (O´Keefe & Nadel, 1978). Morris water maze performance in one month old rats exposed to neonatal hypoxia showed the deficits in spatial memory and cognition in these rats. Hypoxic rats scored high escape latency with less time spent in the platform quadrant. A proper resuscitation to prevent neonatal death

and adverse long-term neurodevelopment sequelae associated with neonatal hypoxia has to be established for better neonatal care. Radial arm maze, Y maze and Morris water maze tests showed that immediate resuscitation of neonatal hypoxia with glucose, alone and along with oxygen, showed an improved spatial recognition and memory in later stage of life.

The cognitive and memory deficits observed in one month old rats exposed to neonatal hypoxia is a consequence of a series of alterations in the brain regions at the molecular level. The decrease in GABA and muscarinic receptor subtypes function along with the activation of apoptotic pathways under hypoxic stress leads to functional disturbances in neonatal brain, which manifest in a later stage of life as behavioural deficits. $GABA_A$ receptors are involved in anxiety and convulsion (Matsumoto, 1989). where as $GABA_B$ receptors are related to depression and analgesia (Lloyd *et al*., 1985; Sawynok, 1990). $GABA_A$ as well as $GABA_B$ receptors play an important role in learning and memory (Castellano & McGaugh, 1991). GABA receptor systems modulate learning and memory through influences on cholinergic systems (Yutaka Nakagawa *et al*., 1995). Cholinergic system plays an important role during the early stages of memory formation (Naor & Dudai, 1996; Orsetti *et al*., 1996; Miranda & Berm_udez-Rattoni, 1999). Long-term potentiation (LTP) has been proposed as a model of induction of the synaptic plasticity underlying memory formation (Bliss & Collingridge, 1993; Escobar & Berm_udez-Rattoni, 2000). The neuronal plasticity needed for *in vivo* long-term potentiation is modulated by muscarinic acetylcholine receptors. Cholinergic system facilitate cortical plasticity by signaling stimulus relevance during memory formation. Improving the central cholinergic system and regulating the hippocampal monoamine neurotransmitters was reported to improve the learning and memory dysfunction (Wei *et al*., 2010). Since both GABAergic and cholinergic stimulation is important in learning, memory processing and cognition, along with the neuronal death the reduction in

GABAergic and muscarinic functions also contribute to the behavioural deficits observed in one month old rats exposed to neonatal hypoxia.

Evaluation of motor function by wire maneuver test showed that there is no significant difference in the grasping capability of hypoxic neonates with control and also within different groups. Righting reflex analysis showed that all groups of rats showed normal reflex action. In the present study, even though motor deficit was not observed due to hypoxic insult defective spatial memory and learning was observed by radial arm maze in 1- month old rats.

Hypoxic injury in term neonates, whether resulting from birth asphyxia, cardiac arrest or respiratory failure, is known to produce reduced brain growth and associated cognitive, motor and behavioural deficits later in life (Mercuri *et al*., 2000; Shalak & Perlman, 2004). There has been much interest in the acute neurological changes associated with neonatal hypoxia, along with the mechanisms of subsequent central nervous system dysfunction in the adult. Hypoxia during the first week of life induce neuronal death in vulnerable brain regions usually associated with an impairment of cognitive function that is detected later in life. Postnatal hypoxia resulting from lung immaturity and respiratory disturbances in infants is an important pathophysiological mechanism underlying the devastating neurological complications. This points the importance of a proper resuscitation programme to overcome neonatal hypoxia for a better intellect in the later stages of life.

Thus our results showed that neonatal hypoxia contributes to CNS disturbances mediated through the functional regulation of GABAergic, serotonergic and muscarinic receptors. Also, gene expression of transcription factors- HIF1 and CREB, pro- apoptotic protein BAX, second messenger enzyme phospholipase C, anti oxidant enzymes- superoxide dismutase and gluththione peroxidase and cholinergic enzymes were found to be altered in the CNS of hypoxic neonatal rats. Free radical scavenging capability, circulating insulin and triiodothyronine levels and second messengers- cAMP, cGMP and IP3 levels were

also functionally altered by neonatal hypoxic insult. Resuscitation with glucose, alone and along with oxygen exhibited a potential effect in improving the ventilatory response and reversing the altered functional regulation of receptors, transcription factors, second messengers and enzymes of hypoxic neonatal rats to near control. The deleterious effect of oxygen alone and epinephrine resuscitation in neuronal response through alterations in neurotransmitters receptors functional regulation was also observed. Thus it is suggested that glucose administration immediately after hypoxia with oxygenated air as a resuscitation programme will be of tremendous advantage especially in neonatal care. Deeper understanding of the mechanisms through which neonatal hypoxia regulates the neurotransmitters, second messengers and transcription factors could point towards the development of new therapeutic approaches to reduce or suppress the pathological effects of hypoxia.

Summary

1. Hypoxic neonatal rats were used as model to study the alterations in GABAergic, serotonergic, muscarinic receptors, second messengers cAMP, cGMP, IP3, second messenger enzyme phospholipase C, transcription factors HIF, CREB, anti oxidant enzymes superoxide dismutase, catalase, gluthathione peroxidase and proapoptotic gene Bax and their regulation by glucose, oxygen and epinephrine resuscitation.

2. Free radical scavenging capability of hypoxic neonatal rats and resuscitated groups were evaluated by SOD and CAT enzyme activity and SOD and GPx gene expression. Hypoxic neonatal rats showed decreased free radical scavenging capability whereas glucose resuscitated group reversed the condition to near control.

3. Serum T3 concentration was decreased and circulating insulin level was increased in hypoxic neonatal rats as an adaptation to overcome hypoxia mediated ATP depletion to the cells.

4. Total GABA receptor was analyzed in the brain regions of control and experimental neonatal rats. Total GABA receptor binding was decreased in cerebral cortex, cerebellum, brain stem and corpus striatum in hypoxic neonatal rats. The Scatchard analysis of $GABA_A$ and $GABA_B$ receptors revealed a decreased receptors in cerebral cortex, cerebellum, brainstem and corpus striatum of hypoxic neonatal rats. The gene expression of $GABA_B$ receptors was down regulated in hypoxic condition compared to control. $GABA_A$ receptor subunits- $GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\gamma3}$ and $GABA_{A\delta}$ showed differential expression pattern with regional variation in different brain regions. In cerebellum, brain stem and corpus striatum

$GABA_{A\alpha1}$, $GABA_{A\alpha5}$, $GABA_{A\gamma3}$ and $GABA_{A\delta}$ were down regulated whereas in cerebral cortex $GABA_{A\alpha1}$, $GABA_{A\alpha5}$ and $GABA_{A\gamma3}$ were down regulated and $GABA_{A\delta}$ was up regulated. Glucose resuscitation, alone and along with oxygen restored the binding and expression of total GABA, $GABA_A$ and $GABA_B$ receptors in brain regions to near control. Immunohistochemistry studies using specific antibodies confirmed the Scatchard analysis and Real Time PCR analysis of $GABA_{A\alpha}$ receptor expression at protein level in control and experimental rats.

5. Total 5-HT and 5-HT_{2A} receptors were analysed in the brain regions of control and experimental neonatal rats. Total 5-HT and 5-HT_{2A} receptor binding was increased in cerebral cortex, cerebellum, brain stem and corpus striatum in hypoxic neonatal rats. The gene expression studies of 5-HT_{2A} receptors and 5-HT transporter (5-HTT) revealed an up regulation in cerebral cortex, cerebellum, brainstem and corpus striatum in hypoxic neonatal rats. Glucose resuscitation, alone and along with oxygen reversed the altered binding parameters of 5-HT and 5-HT_{2A} receptor and expression of 5-HT_{2A} receptors and 5-HTT in brain regions to near control. Immunohistochemistry studies using specific antibodies confirmed the Scatchard analysis and Real Time PCR analysis of 5-HT_{2A} receptor and 5-HTT expression at protein level in control and experimental rats.

6. Total muscarinic receptors were analysed in the brain regions of control and experimental rats. Total muscarinic receptors binding was decreased in cerebral cortex, cerebellum, brain stem and corpus striatum in hypoxic neonatal rats. The gene expression studies of muscarinic M1, muscarinic M2 and muscarinic M3 receptors revealed a down regulation in cerebral cortex, cerebellum, brainstem and corpus striatum of neonatal rats

exposed to hypoxia. Glucose resuscitation restored the binding of total muscarinic receptors and expression of muscarinic M1, muscarinic M2 and muscarinic M3 receptors in brain regions to near control.

7. Acetylcholine esterase expression level has been used as a marker for cholinergic activity. Acetylcholine esterase expression was analysed in the brain regions of experimental neonatal rats. Hypoxic stress to neonatal rats triggered an up regulation of acetylcholine esterase expression in the cerebral cortex, cerebellum, brainstem and corpus striatum. Immediate resuscitation with glucose alone and along with oxygen to hypoxic neonatal rats reversed the altered expression to near control. 100% oxygen resuscitated group showed a reversal in the altered enzyme expression whereas epinephrine supplemented groups did not show any reversal in the altered expression.

8. Choline acetyltransferase expression level has been used as a marker for acetylcholine synthesis. The expression of choline acetyltransferase was decreased in the cerebral cortex, cerebellum, brain stem and corpus striatum of hypoxic neonatal rats. Resuscitation with glucose alone and along with oxygen to hypoxic neonatal rats restored the altered expression of acetylcholine synthesis enzyme to near control. 100% oxygen resuscitated group showed a reversal whereas epinephrine supplemented groups did not show any reversal in the altered expression.

9. Second messengers – cAMP, cGMP and IP3 levels in the corpus striatum was evaluated to track the signaling cascade of neurotrasmitters. Hypoxic insult to neonatal rats increased the striatal cAMP, cGMP and IP3 levels as an adaptive response. Resuscitation with glucose alone and along with oxygen revered the second messenger levels to near control. 100% oxygen

and epinephrine resuscitated groups showed no reversal in the altered level.

10. Hypoxia inducible factor expression was studied in the brain regions of experimental neonatal rats. The gene expression of this transcription factor was up regulated under hypoxic insult to neonatal rats. Resuscitation of hypoxic neonates with glucose and 100% oxygen alone and in combination reversed the altered expression to near control where as epinephrine resuscitation did not show any reversal.

11. Pro- apoptotic gene Bax expression in the cerebral cortex, cerebellum, brain stem and corpus striatum showed increased expression in hypoxic neonatal rats. Glucose resuscitation alone and along with oxygen reversed the altered expression. 100% oxygen and epinephrine resuscitation did not show any reversal in the Bax gene expression.

12. Transcription factor, CREB expression in the brain regions - cerebral cortex, cerebellum, brain stem and corpus striatum showed decreased expression in hypoxic neonatal rats to control. Hypoxia induced altered CREB expression in brain regions was reversed with glucose alone and in combination with oxygen and 100% oxygen resuscitation to hypoxic neonatal rats. Epinephrine resuscitation to hypoxic neonates did not show reversal in the altered CREB expression.

13. Second messenger enzyme - phospholipase C showed a increased expression in hypoxic neonatal brain regions - cerebral cortex, cerebellum, brain stem and corpus striatum. Resuscitation with glucose and 100% oxygen alone and in combination restored the altered enzyme expression

to near control. In epinephrine resuscitated groups, phospholipase C expression did not show any reversal to near control.

14. Behavioural studies were conducted in one month old rats exposed to neonatal hypoxia. Righting reflex and wire maneuver test was conducted to assess reflex action and motor coordination of experimental neonatal rats. Y maze, radial arm maze and water maze were conducted to assess the exploratory behaviour, spatial learning, memory and cognition in control and experimental neonatal rats. Hypoxic stress to neonatal rats caused impairment in exploratory behaviour, spatial learning, memory and cognition in a later stage of life whereas the reflex and motor coordination was not affected. Immediate glucose resuscitation to neonatal rats exposed to hypoxia was found to be effective in preventing the future deficits in spatial learning, memory and cognition due to neonatal hypoxic insult. 100% oxygen and epinephrine resuscitation for neonatal hypoxia was not effective in preventing the future deficits in spatial learning, memory and cognition.

In summary, we conclude that brain GABAergic, serotonergic, muscarinic receptors, enzymes of ACh metabolism- AChE and ChAT, second messengers- cAMP, cGMP and IP3, enzyme in second messenger pathway phospholipase C, transcription factors- HIF and CREB, free radical scavenging enzymes- superoxide dismutase, catalase and gluthathione peroxidase, pro-apoptotic gene Bax functional balance has a major role in regulating the ventilatory response to neonatal hypoxia and modulating behavioural and cognitive process. The present study demonstrates the role of glucose resuscitation, alone and in combination with oxygen, in ameliorating CNS dysfunctions and behavioural deficits in the later stages of life due to neonatal exposure to hypoxia. The present

study also demonstrates the adverse effects of 100% oxygen and epinephrine resuscitation to neonatal hypoxia. Thus our results confirmed the role of glucose in enhancing the ventilatory response without functional imbalance in CNS for a proper management of neonatal hypoxia. The study points to the importance of a proper resuscitation program to overcome neonatal hypoxia for a better intellect in the later stages of life.

Conclusion

Neonatal hypoxia a major cause of permanent neurological disabilities sustained during childhood has devastating consequences like cognitive dysfunctions, neurophysiological and structural changes in the CNS. Hypoxia during the first week of life can induce neuronal death in vulnerable brain regions usually associated with an impairment of cognitive function that can be detected later in life. Even though the body weight or blood glucose level was not altered due to neonatal hypoxic insult, it caused alterations in the brain GABA and serotonin contents. Brain requires continuous supply of oxygen for energy utilization and efficient functioning. Hypoxia leads to disruption of this energy utilization, resulting in neuronal functional failure. Structural and functional integrity of brain depends on regular glucose and oxygen supply. Our results showed that GABAergic, serotonergic and muscarinic functional regulations were impaired in neonatal hypoxia contributing to neurological dysfunction which is suggested to cause behavioural deficits in the later stage of life. Expression and activity of anti oxidant enzymes, superoxide dismutase, gluthathione peroxidase and catalase under hypoxic stress indicates the increased oxidative stress and decreased free radical scavenging capability. HIF mRNA expression was increased as a homeostatic response of the body to encounter hypoxia. Enhanced Bax expression along with decreased CREB mRNA expression induced by neonatal hypoxia triggers the apoptotic signaling cascade in the CNS. The second messenger study revealed the adaptive response of signal transduction pathway under hypoxia through alterations in cAMP, cGMP and IP3 levels. Up regulation of phospholipase C, a second messenger enzyme in the brain regions of neonatal hypoxic rats agrees with the altered second messenger level. Resuscitation of neonatal hypoxic rats with glucose alone and along with oxygen reversed the altered GABAergic, serotonergic, muscarinic receptors, transcription factors HIF

and CREB, pro-apoptotic gene Bax, anti oxidant enzymes SOD, GPx, CAT expression, second messengers cAMP, cGMP and IP3 levels in brain regions to near control. The adverse effects of 100% oxygen and epinephrine resuscitation were also evident from the present study. Thus our results showed that glucose administration immediately after neonatal hypoxia with oxygenation as a resuscitation method will be of tremendous advantage in neonatal care. This point to the importance of a proper resuscitation programme to overcome neonatal hypoxia for a better intellect in the later stages of life.

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2. **Anju T R**, Peeysh K T, Paulose C S. (2010). Decreased GABAA receptors functional regulation in the cerebral cortex and brainstem of hypoxic neonatal rats: effect of glucose and oxygen supplementation. Cell Mol Neurobiol. 30(4):599-606.
3. **Anju T R**, Jobin Mathew, Jayanarayanan S, Paulose C S. (2010). Cerebellar 5-HT_{2A} receptor function under hypoxia in neonatal rats: Role of glucose, oxygen, and epinephrine resuscitation. Respir Physiol Neurobiol. 172 (3):147-153.
4. **Anju T R**, Pretty Mary Abraham, Sherin Antony, Paulose CS. (2010). Alterations in cortical $GABA_B$ receptors in neonatal rats exposed to hypoxic stress: Role of glucose, oxygen and epinephrine resuscitation. Molecular and Cellular Biochemistry. doi: 10.1007/s11010-010-0491-9
5. **Anju T R**, Nandhu M S, Jes P, Paulose C S. (2010) Insulin and triiodothyronine regulation of neonatal hypoxia: Role of glucose, oxygen and epinephrine supplementation. Fetal and Pediatric Pathology (Accepted)
6. **Anju T R**, Smijin S, Korah P K, Paulose C S. Cortical $5HT_{2A}$ receptor function under hypoxia in neonatal rats: Role of glucose, oxygen and epinephrine resuscitation. Journal of Molecular Neuroscience. (Accepted)
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12. Peeyush Kumar T, Savitha Balakrishnan, Sherin Antony, **Anju T R**, and Jes Paul. (2010). Cholinergic, Dopaminergic and Insulin Receptors Gene Expression in the Cerebellum of Streptozotocin Induced Diabetic Rats: Functional Regulation with Vitamin D3 Supplementation. Pharmacology Biochemistry and behavior. 95: 216 – 222.

13. Pretty Mary Abraham, **Anju T R**, Jayanarayanan S, C. S. Paulose. (2010). Serotonergic receptor functional up regulation in cerebral cortex and down regulation in brain stem of Streptozotocin induced Diabetic Rats: Antagonism by pyridoxine and insulin. Neuroscience letters. doi:10.1016/j.neulet.2010.07.042.

14. Anitha M, Nandhu M S, **Anju T R**, Jes Paul and C S Paulose. Targeting Glutamate mediated excitotoxicity in Huntington's disease: Neural progenitors and partial Glutamate antagonist - Memantine. Medical Hypotheses (Accepted)

SEMINAR/CONFERENCE PRESENTATION

1. **Anju T R**, Athira Babu and C. S Paulose. "Superoxide dismutase functional regulation in neonatal hypoxia: Effect of glucose, oxygen and epinephrine." Society for Free Radical Research, India- Satellite Meeting, All India Institute of Medical Sciences (AIIMS), New Delhi. (2008).

2. **Anju T R**, C S Paulose. $GABA_A$ receptor functional regulation in the cerebral cortex of hypoxia induced neonatal rats: Effect of glucose and oxygen treatment. International Conference on Advances in Neuroscience & XXVI Annual Meeting of Indian Academy of Neurosciences. Cochin. (2008).

3. **Anju T R**, Jobin Mathew, Jayanarayanan S and C. S. Paulose. Enhanced $5HT_{2A}$ receptor function in the cerebellum of hypoxic neonatal rats: Role of glucose, oxygen, and epinephrine resuscitation. International

Conference on Neuroscience Updates, Annual meeting of Society for Neurochemistry. Cochin (2009).

4. **Anju T R**, Pretty Mary Abhraham and C. S. Paulose. Enhanced $5HT_{2A}$ Receptors in the Cerebral Cortex of Hypoxia Induced Neonatal Rats: Effect of Glucose and Oxygen Supplementation. Indian Journal of Clinical Biochemistry (Abstract). 36^{th} ACBICON 2009 National Conference of Association of Clinical Biochemists of India conducted by Amrita Institute of Medical Sciences, Cochin, (2009).

5. Sherin A, Jobin M, Peeyush K, **Anju T R**, C S Paulose. Insulin induced hypoglycemia mediated changes in cholinergic receptor expression in the cerebellum of diabetic rats. International Conference on Neuroscience Updates, Annual meeting of Society for Neurochemistry. Cochin (2009).

6. Chinthu Romeo, **Anju T R**, C S Paulose. Down regulation of muscarinic m1 receptor in the cerebellum of spinal cord injured rats: bone marrow cells and neurotransmitters supplementation. International Ageing Conference – 2010, Banaras Hindu University, Varanasi. (2010).

7. Roshni Baby Thomas, **Anju T R**, Chinthu Romeo, C S Paulose. Cortical muscarinic receptor subtypes down regulation and enhanced Bax expression in hypoxic neonatal rats and cognitive impairment. Annual meeting of society for Biotechnologists, India and National symposium on innovations in Biotechnology- 2010, SRM University, Chennai (2010).

Table-1
Body Weight and Blood Glucose level of Control and Experimental Groups of Neonatal Rats

Experimental groups	Body weight (g)		Blood Glucose levels (mg/dl)
	Initial day of Experiment	Final day of Experiment	
C	8.56 ± 0.29	12.16 ± 0.15	120.23 ± 1.74
Hx	7.35 ± 0.54	13.22 ± 0.55	123.52± 0.58
Hx+G	7.12 ± 0.25	13.34 ± 0.62	118.55 ± 1.15
Hx+G+O	7.85 ± 0.45	13.14 ± 0.32	123.21 ± 2.15
Hx+O	7.33 ± 0.18	13.11± 0.35	120.37 ± 1.38
Hx + E	7.12 ± 0.25	12.52 ± 0.15	119.05 ± 1.25
Hx+G+E+O	7.03 ± 0.15	13.02 ± 0.13	123.59 ± 1.03

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O , Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 1
Superoxide dismutase activity in the heart of Control and experimental rats.

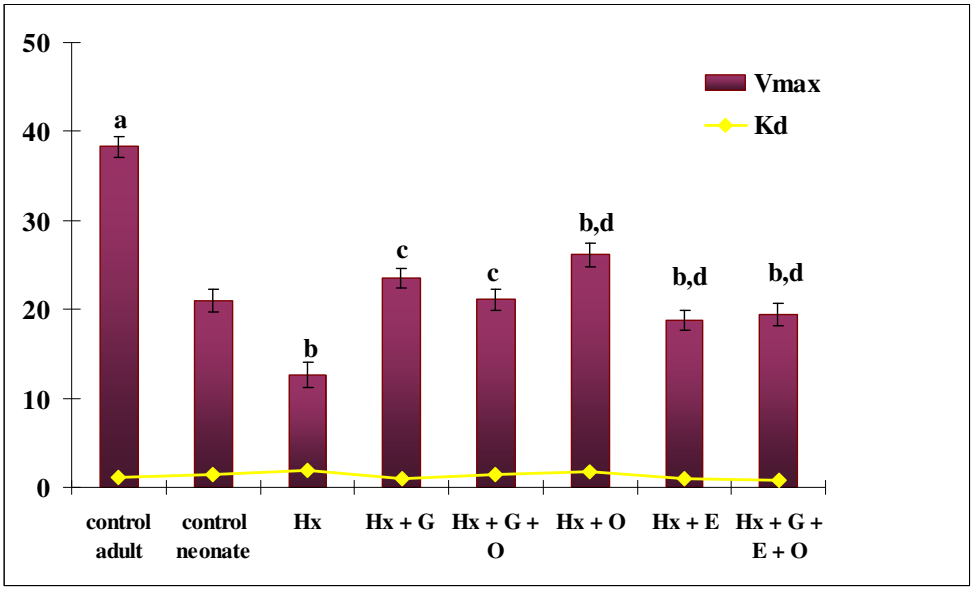


Table- 2
Superoxide dismutase activity in the heart of Control and experimental rats.

Experimental groups	V_{max}(U/mg protein)	K_m (µM)
Control adult	38.25 ± 1.12 [a]	1.15 ± 0.08 [a]
Control neonate	20.94 ± 1.19	1.40 ± 0.05
Hx	19.67 ± 1.05 [b]	1.85 ± 0.05 [b]
Hx + G	23.52 ± 1.10 [c]	1.00 ± 0.10 [c]
Hx + G + O	21.09 ± 1.05 [c]	1.40 ± 0.05 [c]
Hx + O	26.13 ± 1.10 [b,d]	1.75 ± 0.10 [b, d]
Hx + E	18.77 ± 1.05 [b, d]	0.99 ± 0.05 [b, d]
Hx + G + E + O	19.43 ± 1.07 [b, d]	0.80 ± 0.05 [b, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. [a] $p<0.001$, [b] $p<0.01$when compared with control. [c] $p<0.01$, [d] $p<0.001$ when compared with hypoxic group. [g] $p<0.01$ when compared with Hx + G + O
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O , Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure-2
Superoxide dismutase activity in the cerebral cortex of Control and experimental rats.

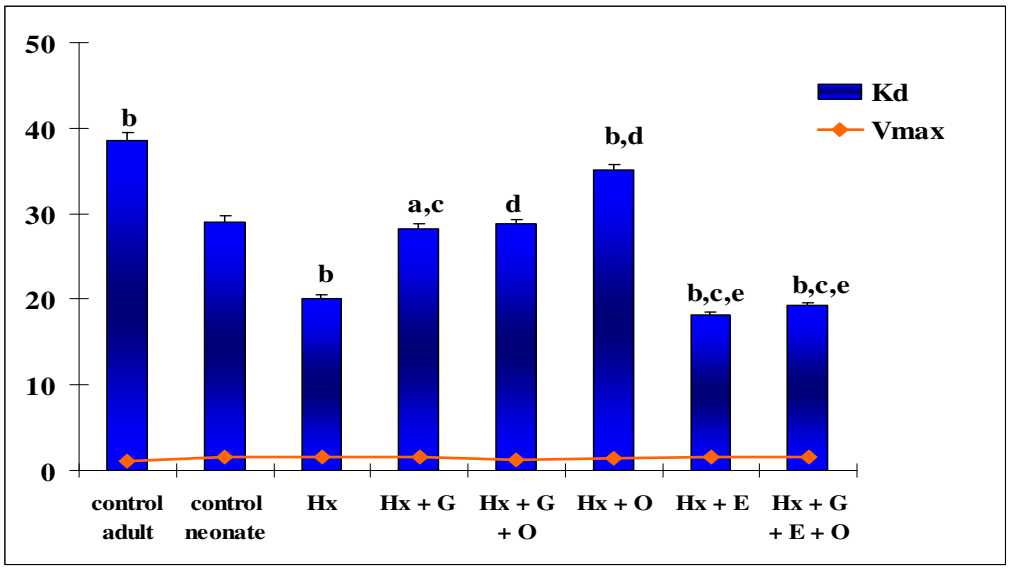


Table- 3
Superoxide dismutase activity in the cerebral cortex of Control and experimental rats.

Experimental groups	V_{max}(U/mg protein).	K_m (µM)
Control adult	38.56 ± 0.10 [b]	1.04 ± 0.15 [a]
Control neonate	29.00 ± 0.10	1.53 ± 0.05
Hx	20.05 ± 0.05 [b]	1.50 ± 0.30
Hx + G	28.25 ± 0.15 [a, c]	1.52 ± 0.02
Hx + G + O	28.80 ± 0.10 [d]	1.25 ± 0.02 [b, d]
Hx + O	35.15 ± 0.05 [b, d]	1.35 ± 0.15 [a, c]
Hx + E	18.22 ± 0.15 [b, c, e]	1.55 ± 0.06
Hx + G + E+ O	19.30 ± 0.10 [b, c, e]	1.50 ± 0.05

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] p<0.01, [b] p<0.001 when compared with control. [c] p<0.01, [d] p<0.001 when compared with hypoxic group. [e] p<0.001 when compared with Hx + G + O.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 3
Catalase activity in the heart of Control and experimental rats.

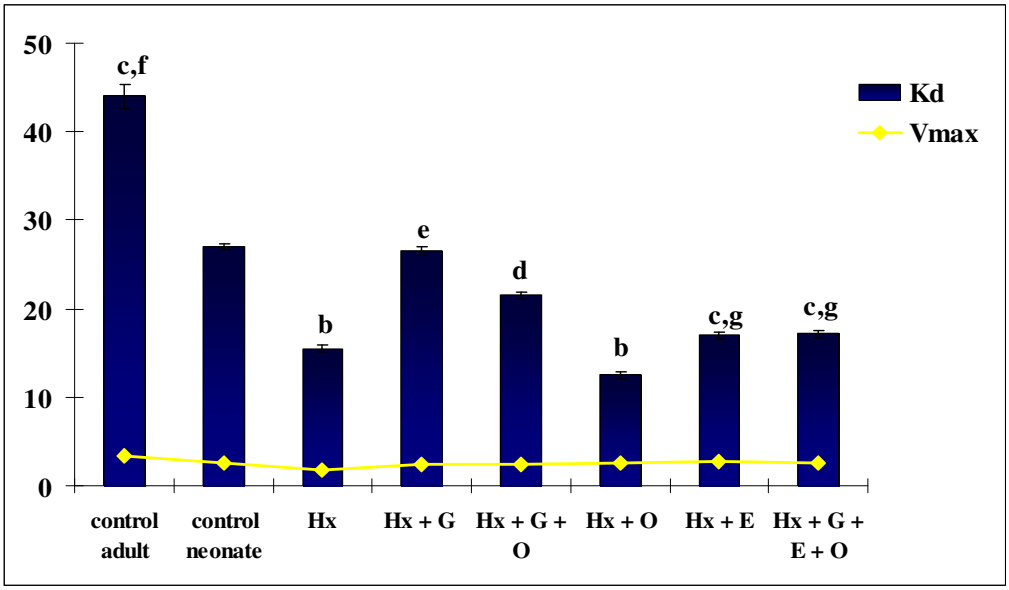


Table- 4
Catalase activity in the heart of Control and experimental rats.

Experimental groups	V_{max}(U/mg protein).	K_m (µM)
Control adult	44.00 ± 0.20 [c, f]	3.30 ± 0.01 [a, e]
Control neonate	27.00 ± 0.02	2.65 ± 0.15
Hx	15.50 ± 0.05 [b]	1.80 ± 0.01 [a]
Hx + G	26.50 ± 0.10 [e]	2.45 ± 0.25
Hx + G + O	21.50 ± 0.05 [d]	2.45 ± 0.01
Hx + O	12.50 ± 0.05 [b]	2.50 ± 0.10
Hx + E	16.98 ± 0.15 [c, g]	2.75 ± 0.02 [e, g]
Hx + G + E + O	17.15 ± 0.10 [c, g]	2.55 ± 0.01 [e, g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] p<0.05, [b] p<0.01, [c] p<0.001 when compared with control. [d] p<0.05, [e] p<0.01, [f] p<0.001 when compared with hypoxic group. [g] p<0.01 when compared with Hx + G + O.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 4
Catalase activity in the cerebral cortex of Control and experimental rats.

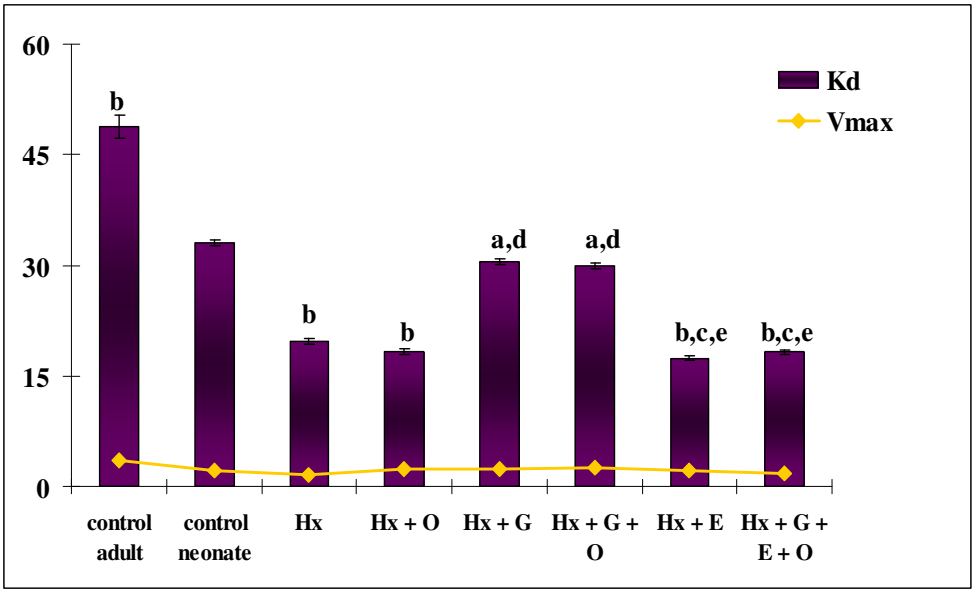


Table- 5
Catalase activity in the cerebral cortex of Control and experimental rats.

Experimental groups	V_{max}(U/mg protein).	K_m (µM)
Control adult	48.85 ± 0.15 [b]	3.50 ± 0.10 [b]
Control neonate	33.10 ± 0.09	2.20 ± 0.10
Hx	19.60 ± 0.04 [b]	1.55 ± 0.05 [a]
Hx + O	18.35 ± 0.03 [b]	2.35 ± 0.05 [c]
Hx + G	30.50 ± 0.05 [a, d]	2.40 ± 0.01 [b]
Hx + G + O	29.90 ± 0.10 [a, d]	2.50 ± 0.01 [c]
Hx + E	17.40 ± 0.10 [b,c,e]	2.12 ± 0.01 [e]
Hx + G + E+ O	18.20 ± 0.10 [b,c,e]	1.80 ± 0.01 [e]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] p<0.01, [b]p<0.001 when compared with control. [c] p<0.01, [d] p<0.001 when compared with hypoxic group. [e] p<0.001 when compared with Hx + G + O.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 5
T3 content in the heart of control and experimental neonatal rats.

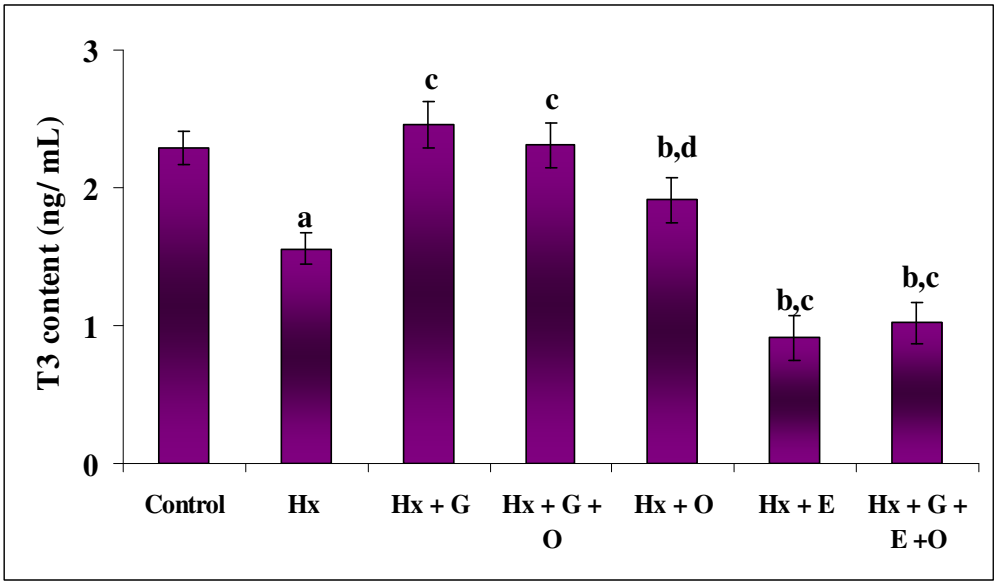


Table- 6
T3 content in the heart of control and experimental neonatal rats.

Experimental groups	Content of T3 in ng/mL
Control	2.29 ± 0.11
Hx	1.56 ± 0.12 [a]
Hx + G	2.46 ± 0.17 [c]
Hx + G + O	2.31 ± 0.16 [c]
Hx + O	1.91 ± 0.16 [b, d]
Hx + E	0.91 ± 0.16 [b,c]
Hx + G + E + O	1.02 ± 0.15 [b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] p<0.001, [b] p<0.01when compared with control
[c] p<0.001, [d] p<0.01 when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 6
T3 concentration in the serum of control and experimental neonatal rats.

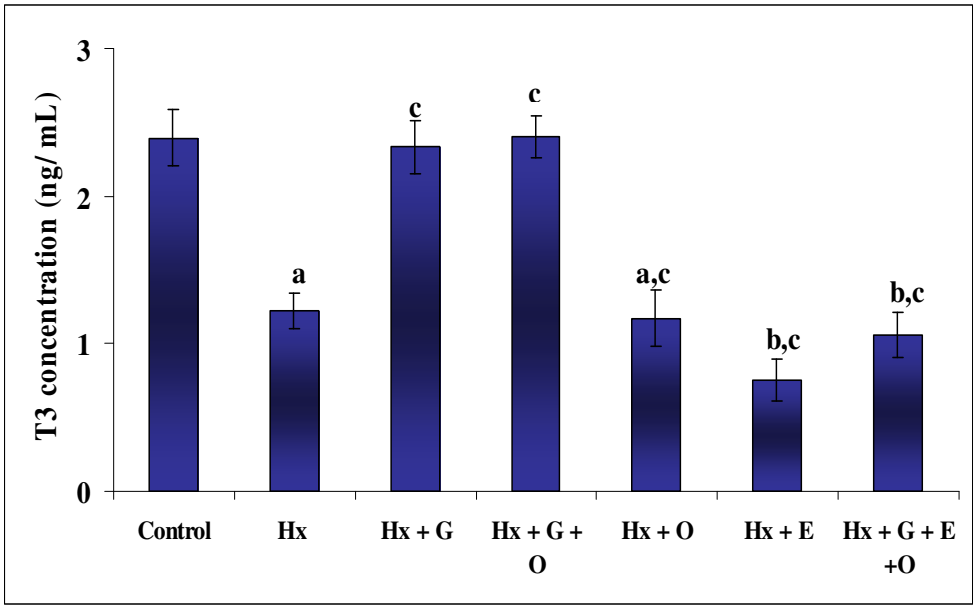


Table- 7
T3 concentration in the serum of control and experimental neonatal rats.

Experimental groups	Concentration of T3 in ng/mL
Control	2.39± 0.12
Hx	1.22± 0.19 [a]
Hx + G	2.33± 0.19 [c]
Hx + G + O	2.40± 0.15 [c]
Hx + O	1.17± 0.18 [a,c]
Hx + E	0.75± 0.14 [b,c]
Hx + G + E + O	1.06± 0.14 [b,c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.01$ when compared with control
[c] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 7
Circulating insulin level in the plasma of control and experimental neonatal rats.

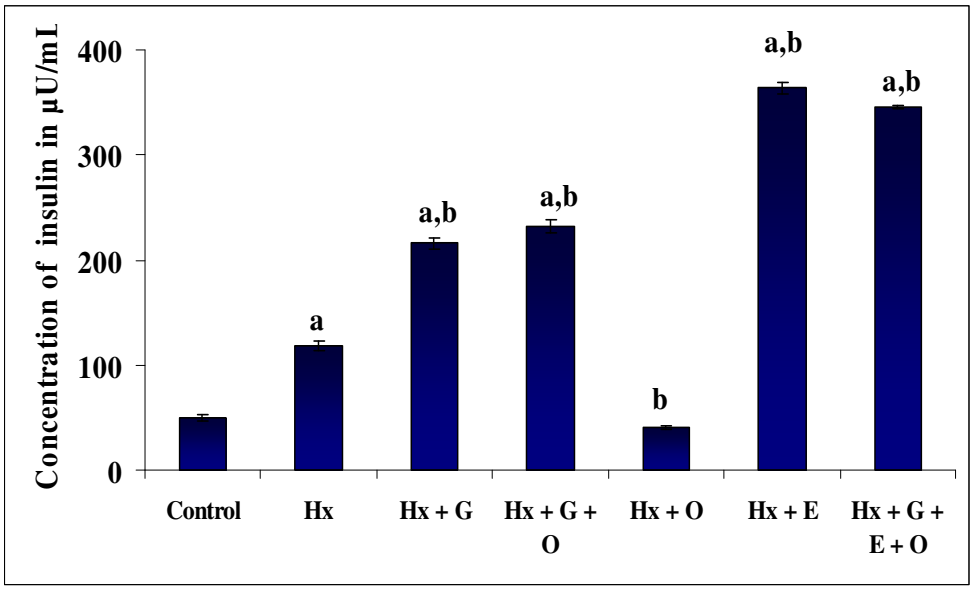


Table- 8
Circulating insulin level in the plasma of control and experimental groups of neonatal rats

Experimental groups	Concentration of insulin in µU/mL
Control	118.82 ± 1.18
Hx	149.48 ± 1.36 [a]
Hx + G	215.65 ± 1.32 [a, b]
Hx + G + O	232.44 ± 1.24 [a, b]
Hx + O	40.44 ± 1.23 [b]
Hx + E	364.05 ± 1.29 [a, b]
Hx + G + E + O	345.15 ± 1.32 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 8
Real Time PCR amplification of insulin receptor mRNA from the heart of Control and experimental neonatal rats

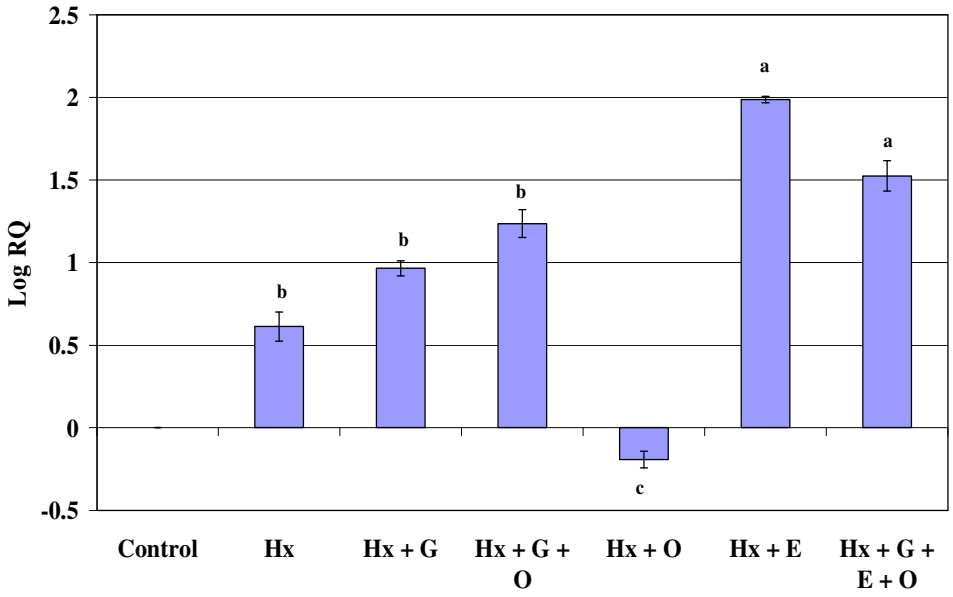


Table- 9
Real Time PCR amplification of insulin receptor mRNA from the heart of Control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	0.63 ± 0.03 [b]
Hx + G	0.97 ± 0.05 [b]
Hx + G + O	1.24 ± 0.08 [b]
Hx + O	-0.19 ± 0.01 [c]
Hx + E	1.99 ± 0.03 [a]
Hx + G + E + O	1.50 ± 0.05 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 9
Scatchard analysis of [^{125}I] triiodothyronine binding against triiodothyronine in the heart of control and experimental neonatal rats.

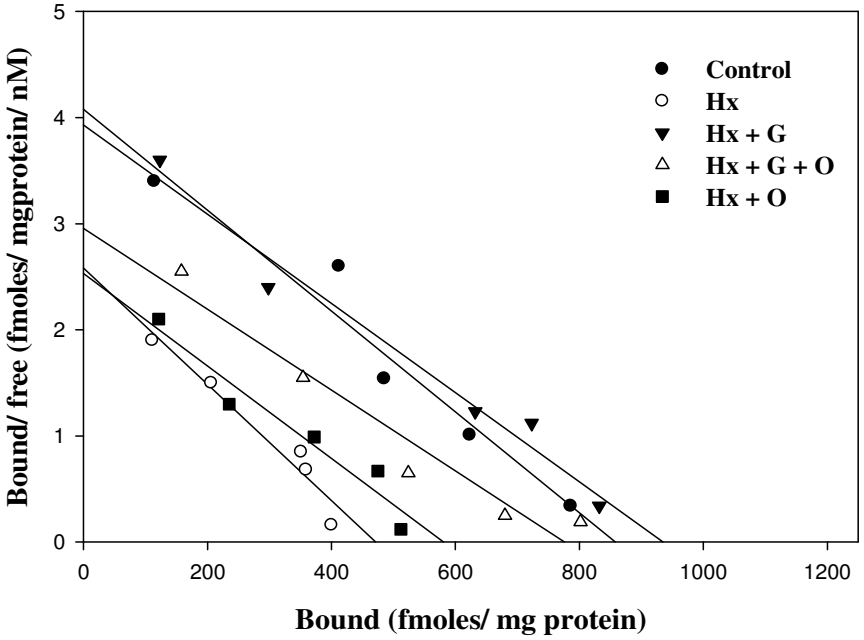


Table- 10
Scatchard analysis of [^{125}I] triiodothyronine binding against triiodothyronine in the heart of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	851.01 ± 3.60	2.01 ± 0.14
Hx	471.52 ± 3.82 [a]	2.50 ± 0.12 [b]
Hx + G	932.70 ± 2.90 [c]	1.73 ± 0.10 [c]
Hx + G + O	773.32 ± 3.81 [c]	1.31 ± 0.15 [c]
Hx + O	579.71 ± 2.52 [a, d]	1.92 ± 0.14 [b, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.05$ when compared with control
[c] $p<0.001$, [d] $p<0.05$ when compared with hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 10
Scatchard analysis of [^{125}I] triiodothyronine binding against triiodothyronine in the heart of control and experimental neonatal rats.

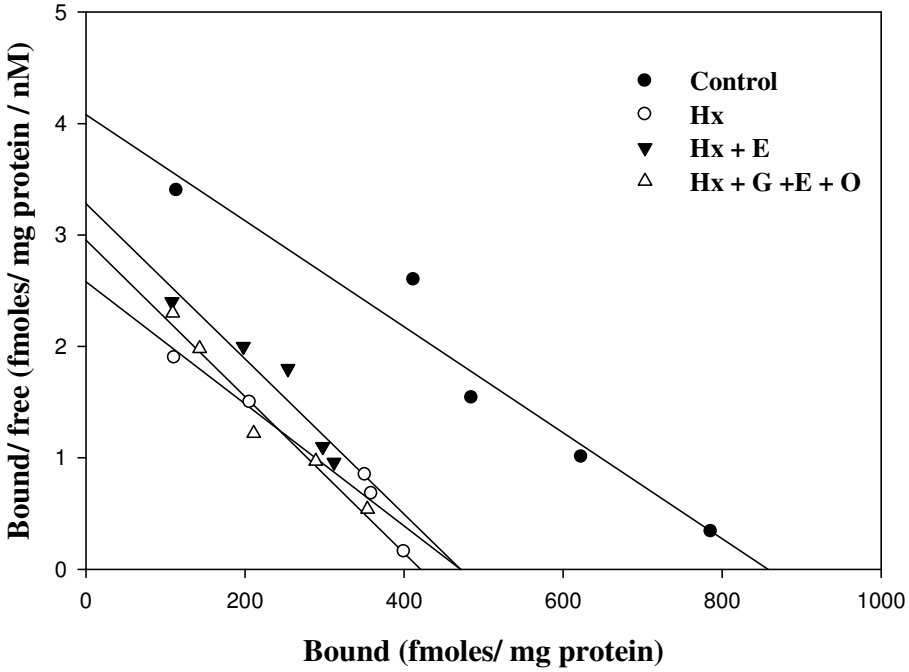


Table- 11
Scatchard analysis of [^{125}I] triiodothyronine binding against triiodothyronine in the heart of control and experimental neonatal rats

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	851.01± 3.60	2.01±0.14
Hx	471.52± 3.82 [a]	2.50± 0.12 [b]
Hx + E	466.40±2.60 [a]	1.90±0.10 [b, c]
Hx + G + E + O	414.41±4.50 [a, d]	1.84±0.12 [b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.05$ when compared with control
[c] $p<0.001$, [d] $p<0.05$ when compared with hypoxic group
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table-12
GABA Content (µmoles/g wet wt.) in the brain regions of Control and Experimental Groups of Neonatal Rats

Experimental groups	GABA Content (µmoles/g wet wt.)			
	Cerebral cortex	Cerebellum	Brain stem	Corpus striatum
Control	7.50±1.2	6.45±1.2	8.45±1.8	7.25±1.4
Hx	2.26± 1.5 [a]	2.02± 1.0[a]	4.06± 1.4[a]	3.25± 1.6[a]
Hx + G	7.25 ±1.4 [b]	6.25 ±1.4 [b]	9.85 ±2.2 [b]	6.55 ±1.5 [b]
Hx + G + O	9.76 ±1.1 [a, b]	6.60 ±1.4 [b]	8.66 ±1.4 [b]	7.05 ±1.4 [b]
Hx + O	7.05 ±1.1 [b]	3.55 ±1.8 [b]	6.01 ±1.5 [b]	4.05 ±1.2 [b]
Hx + E	4.35 ±1.5 [a, b]	3.05 ±1.2 [a]	4.55 ±1.6 [a]	3.05 ±1.6 [a]
Hx + G + E + O	5.42 ±1.2 [a, c]	3.12 ±1.1 [a]	5.02 ±1.4 [a]	3.12 ±1.5 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table-13
5- HT and 5- hydroxyindole acetic acid Content (nmoles/g wet wt.) in the cerebellum of Control and Experimental Groups of Neonatal Rats

Experimental groups	Cerebellum	
	5- HT	5- HIAA
Control	204.55 ± 3.19	198.32 ± 2.65
Hx	346.56 ± 2.05 [a]	170.24 ± 2.05 [a]
Hx + G	201.31 ± 2.50 [b]	182.12 ± 2.10 [b]
Hx + G + O	212.27 ± 2.05 [b]	192.96 ± 1.05 [b]
Hx + O	301.87 ± 2.10 [a]	97.91 ± 1.10 [a]
Hx + E	365.45 ± 1.07 [a]	32.34 ± 1.80 [a]
Hx + G + E + O	376.61 ± 1.76 [a]	42.68 ± 2.55 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to Hx
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table-14

5- HT and 5- hydroxyindole acetic acid Content (nmoles/g wet wt.) in the brain stem of Control and Experimental Groups of Neonatal Rats

Experimental groups	Brain stem	
	5- HT	5- HIAA
Control	149.38 ± 2.05	71.55 ± 2.01
Hx	481.79 ± 4.19 [a]	42.03 ± 2.05 [a]
Hx + G	126.11 ± 2.10 [b]	87.92 ± 1.55 [b]
Hx + G + O	138.67 ± 2.05 [b]	78.78 ± 1.85 [b]
Hx + O	242.92 ± 3.10 [a]	67.54 ± 1.10 [a]
Hx + E	450.55 ± 2.07 [a]	31.55 ± 2.02 [a]
Hx + G + E + O	457.97 ± 1.70 [a]	35.42 ± 3.15 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to Hx
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 11
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the cerebral cortex of control and experimental neonatal rats.

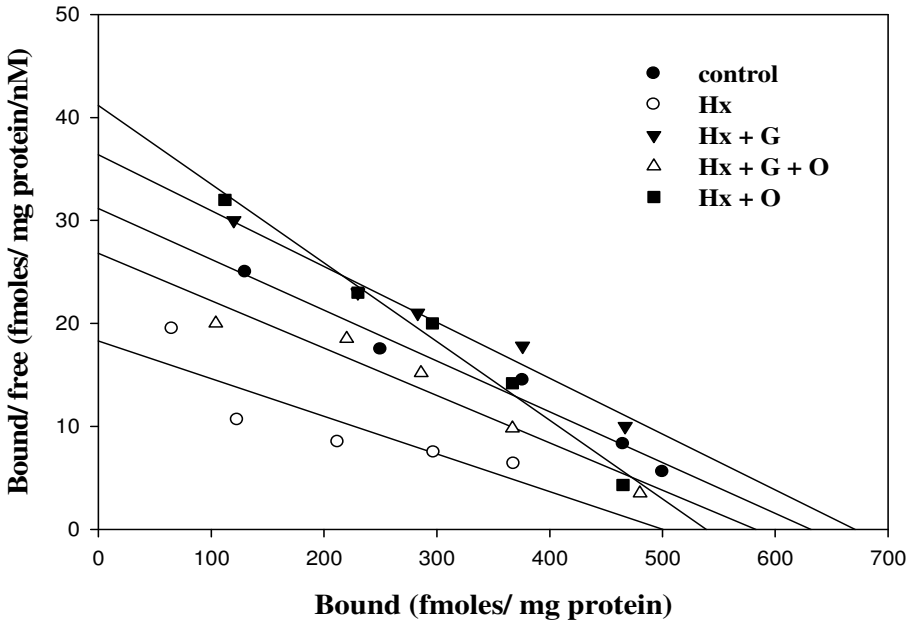


Table- 15
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	628.62 ± 4.22	20.20 ±2.05
Hx	499.80 ± 2.55 [a]	26.91 ± 3.12 [a]
Hx + G	669.81 ± 4.05 [b]	18.50 ± 2.00 [b]
Hx + G + O	583.11 ± 3.12 [b]	21.92 ± 2.50 [c]
Hx + O	533.92 ± 3.12 [a, c]	12.90 ± 1.45 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$, [c] $p<0.01$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 12
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the cerebral cortex of control and experimental neonatal rats

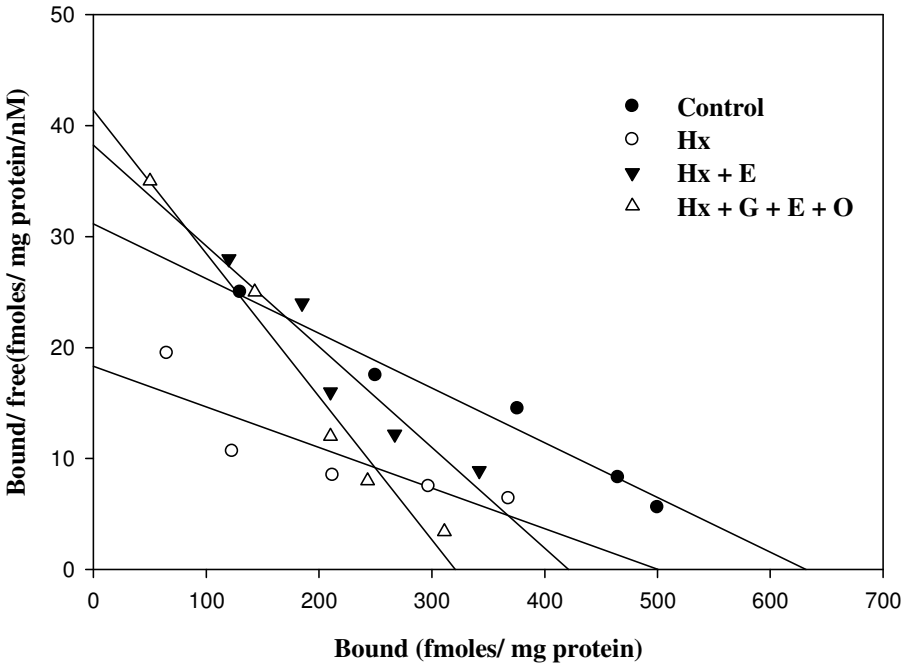


Table- 16
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	628.62 ± 4.22	20.20 ± 2.05
Hx	499.80 ± 2.55 [a]	26.91 ± 3.12 [a]
Hx + E	418.62 ± 3.55 [a, c]	10.90 ± 2.10 [b]
Hx + G + E + O	319.53 ± 2.50 [a, b]	7.83 ±1.80 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$, [c] $p<0.01$ when compared with hypoxic group
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure - 13

Binding parameters of [^{3}H] GABA against GABA in the cerebral cortex of control and experimental neonatal rats.

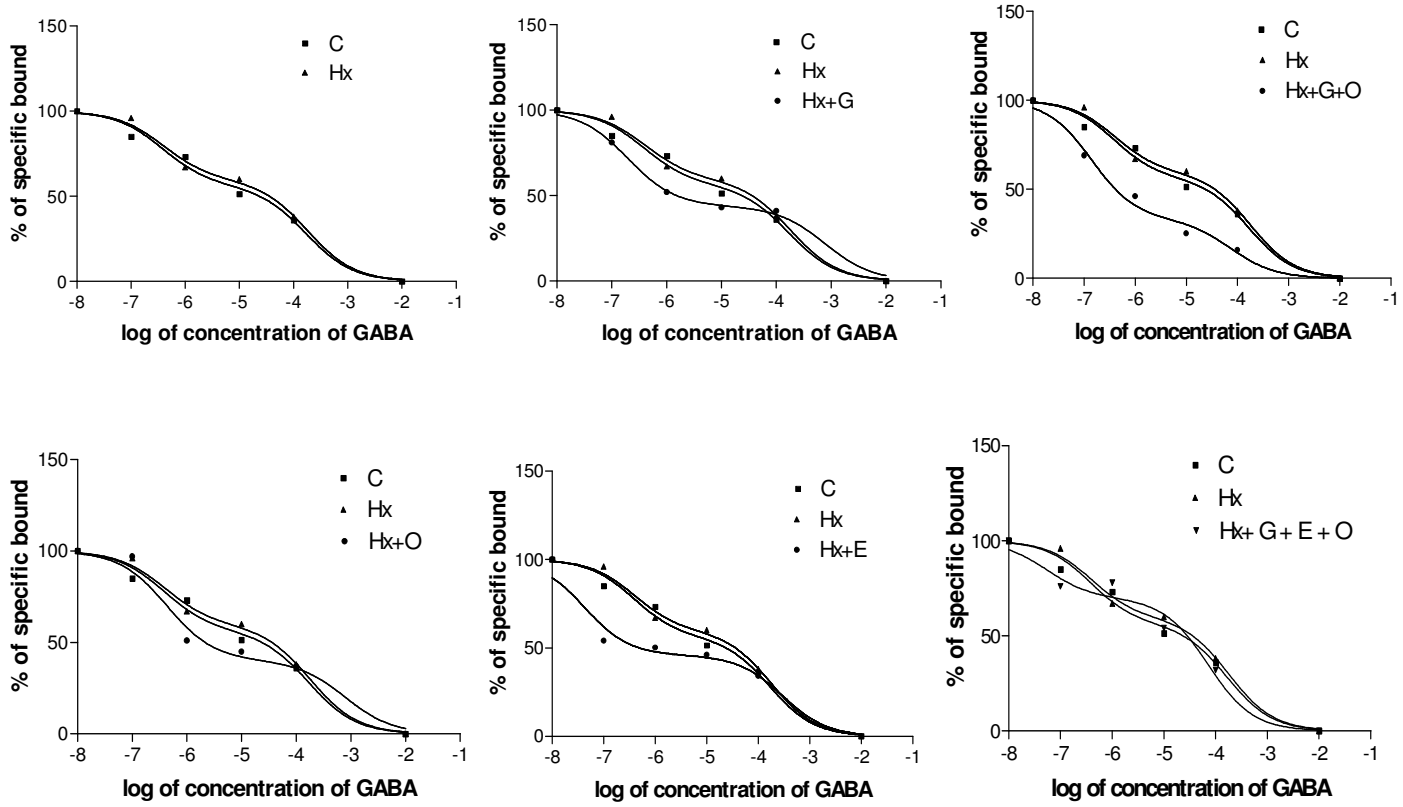


Table - 17

Binding parameters of [^{3}H] GABA against GABA in the cerebral cortex of control and experimental neonatal rats.

Group	Best-fit model	Log (EC_{50})-1	Log (EC_{50})-2	Ki $_{(H)}$	Ki $_{(L)}$	Hill slope
C	Two- site	-6.395	-3.643	1.4880 x 10^{-7}	6.6880 x 10^{-5}	-0.6888
Hx	Two- site	-6.419	-3.776	1.3080 x 10^{-7}	6.1960 x 10^{-5}	-0.6946
Hx + G	Two- site	-6.378	-3.112	1.4430 x 10^{-8}	6.854 x 10^{-4}	-0.4960
Hx + G + O	Two- site	-6.365	-3.105	5.2030 x 10^{-8}	6.7730 x 10^{-5}	- 0.6877
Hx + O	Two- site	-6.402	-3.725	1.3970 x 10^{-7}	5.903 x 10^{-4}	-0.5808
Hx + E	Two- site	-7.371	-3.727	1.5750 x 10^{-8}	7.099 x 10^{-4}	- 0.6658
Hx + G + E + O	Two- site	-7.327	-3.742	1.6723 x 10^{-8}	7.1124 x 10^{-4}	- 0.97543

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 14
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the cerebral cortex of control and experimental neonatal rats.

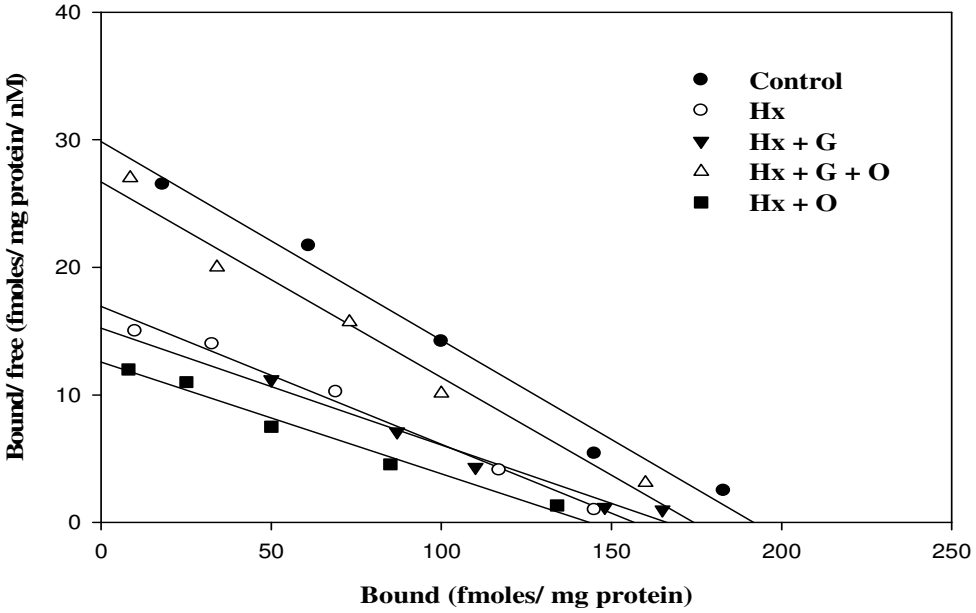


Table- 18
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	191.72 ± 1.88	6.40 ± 1.34
Hx	154.74 ± 2.05 [a]	9.21 ± 1.78 [a]
Hx + G	165.14 ± 2.66 [b, d]	10.70 ± 1.05 [a]
Hx + G + O	173.51 ± 2.90 [b, c]	6.52 ± 1.15 [c]
Hx + O	142.70 ± 2.45 [a, d]	11.23 ± 2.01 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.01$ when compared with control
[c] $p<0.001$, [d] $p<0.01$ when compared with hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 15
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the cerebral cortex of control and experimental neonatal rats.

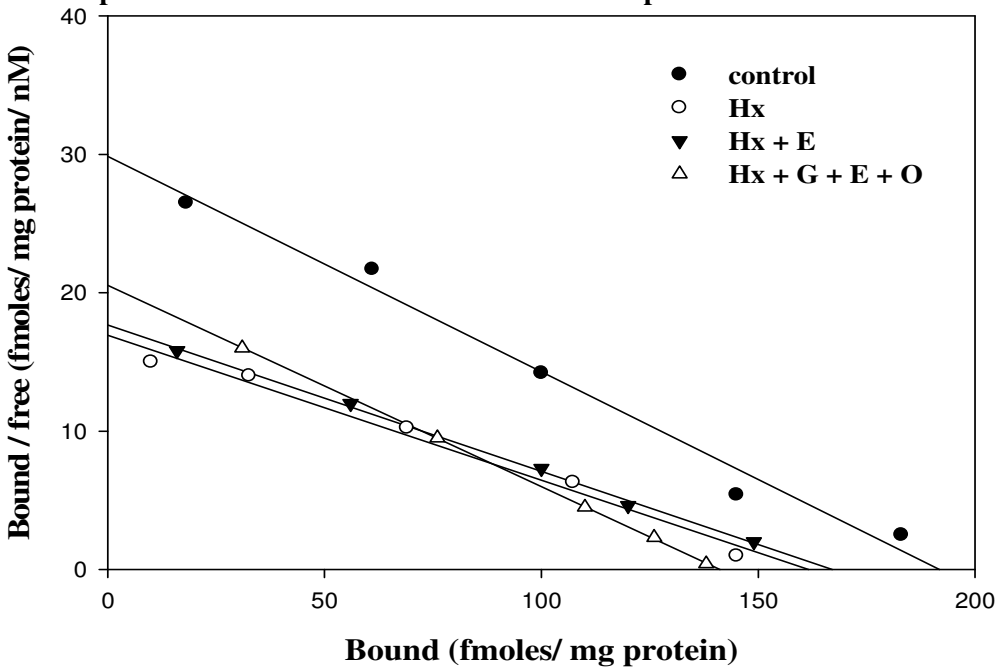


Table- 19
Scatchard analysis of [^{3}H] bicuculline binding bicuculline to $GABA_A$ receptor in the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	191.72 ± 1.88	6.40 ± 1.34
Hx	154.74 ± 2.05 [a]	9.21 ± 1.78 [a]
Hx + E	166.04 ± 2.12 [a]	9.38 ± 1.12 [a]
Hx + G + E + O	139.80 ± 2.15 [a]	6.79 ± 1.23 [c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.01$ when compared with control
[c] $p<0.001$, [d] $p<0.01$ when compared with hypoxic group
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 16
Scatchard analysis of [^{3}H] baclofen binding against baclofen to GABA $_B$ receptor in the cerebral cortex of control and experimental neonatal rats.

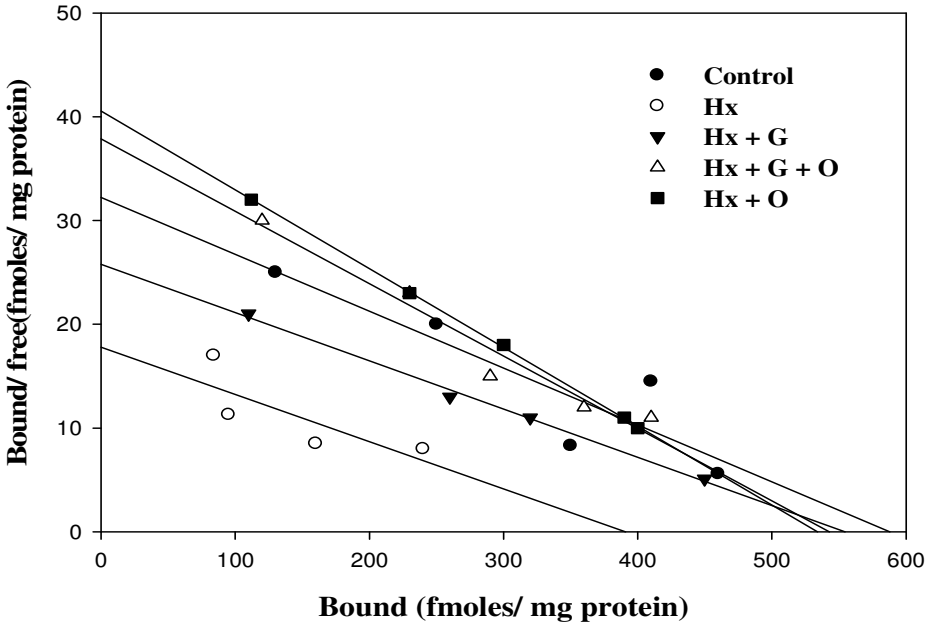


Table- 20
Scatchard analysis of [^{3}H] baclofen binding against baclofen to GABA $_B$ receptor in the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	586.70 ± 4.55	18.13 ±1.25
Hx	388.65 ± 3.05 [a]	21.14 ± 2.45 [a]
Hx + G	553.71 ± 3.35 [d]	21.49 ± 2.99 [a]
Hx + G + O	543.54 ± 3.55 [d]	14.16 ± 1.20 [d]
Hx + O	529.53 ± 4.06 [c, d]	13.12 ± 1.15 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared with control
[d] $p<0.001$ when compared with hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 17
Scatchard analysis of [^{3}H] baclofen binding against baclofen to GABA $_B$ receptor in the cerebral cortex of control and experimental neonatal rats.

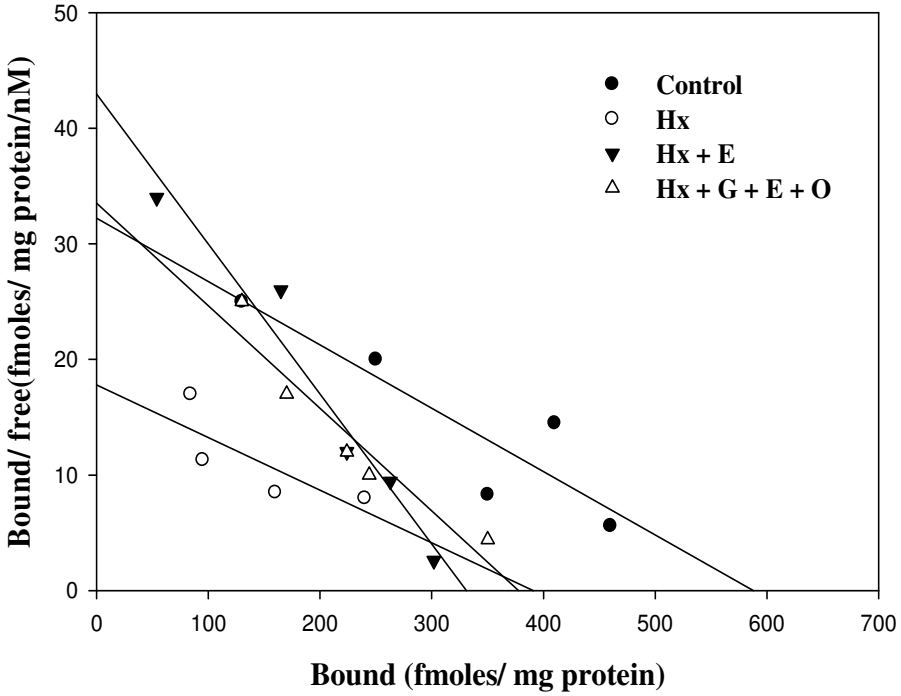


Table- 21
Scatchard analysis of [^{3}H] baclofen binding against baclofen to GABA $_B$ receptor in the cerebral cortex of control and experimental neonatal rats.

Experimental groups	B_{max}(fmoles/mg protein)	K_d (nM)
Control	586.70 ± 4.55	18.13 ±1.25
Hx	388.65 ± 3.05 [a]	21.14 ± 2.45 [a]
Hx + E	325.22 ± 2.45 [a, e]	7.42 ± 1.25 [d]
Hx + G + E + O	374.80 ± 3.12 [a]	11.20 ± 2.20 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared with control
[d] $p<0.001$, [e] $p<0.05$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 18
Real Time PCR amplification of $GABA_{A\acute{u}1}$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

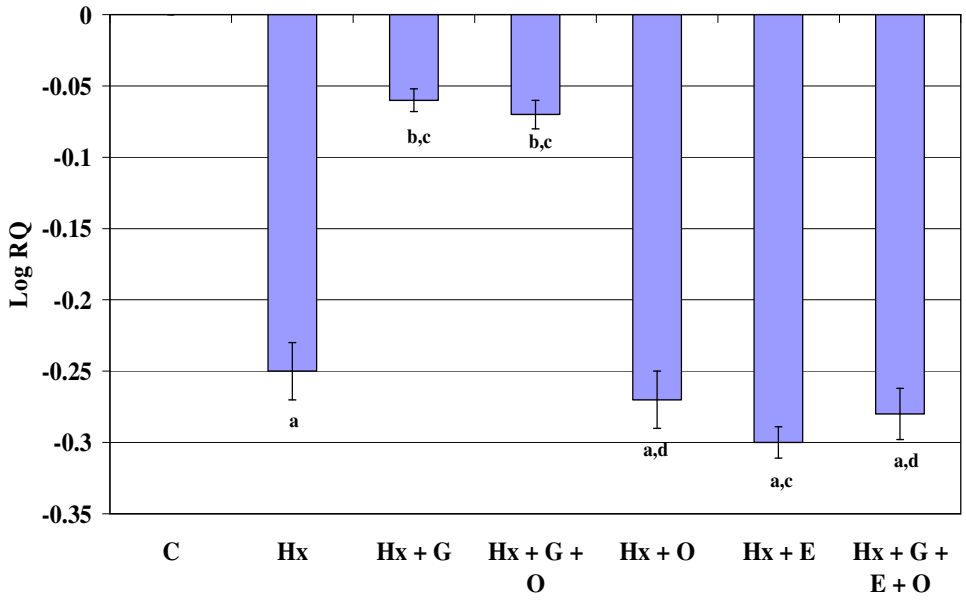


Table- 22
Real Time PCR amplification of $GABA_{A\acute{u}1}$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rat

Experimental groups	Log RQ
Control	0
Hx	-0.25 ± 0.02 [a]
Hx + G	-0.06 ± 0.008 [b, c]
Hx + G + O	-0.07 ± 0.01 [b, c]
Hx + O	-0.27 ± 0.02 [a, d]
Hx + E	-0.30 ± 0.01 [a, c]
Hx + G + E + O	-0.28 ± 0.02 [a, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. [a] $p<0.001$, [b] $p<0.05$ when compared to Control, [c] $p<0.001$, [d] $p<0.01$ when compared to Hx. Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 19
Real Time PCR amplification of $GABA_{A\acute{o}5}$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

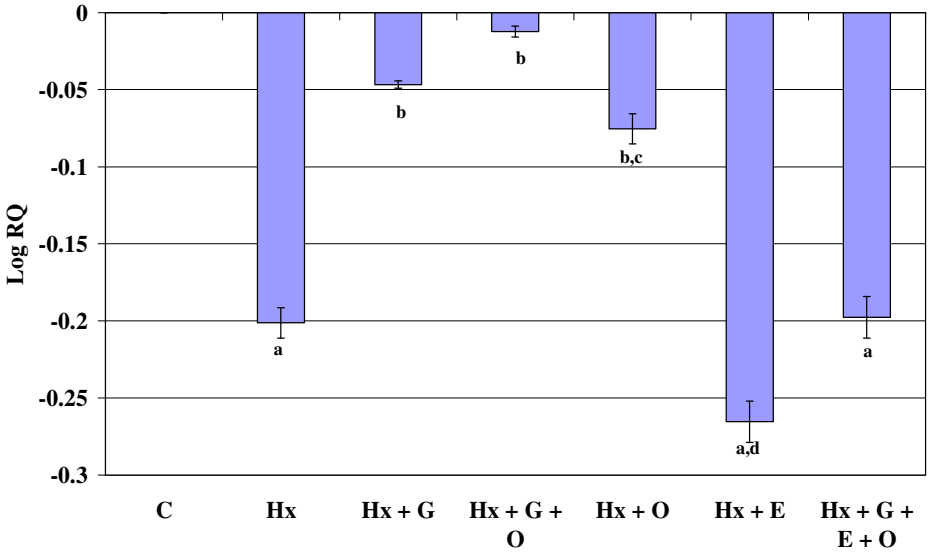


Table- 23
Real Time PCR amplification of $GABA_{A\acute{o}5}$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.20 ± 0.01^{a}
Hx + G	-0.05 ± 0.002^{b}
Hx + G + O	-0.01 ± 0.003^{b}
Hx + O	$-0.08 \pm 0.01^{b, c}$
Hx + E	$-0.27 \pm 0.01^{a, d}$
Hx + G + E + O	-0.19 ± 0.01^{a}

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.05$ when compared to Control, [b] $p<0.001$, [d] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 20
Real Time PCR amplification of $GABA_{A\gamma3}$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

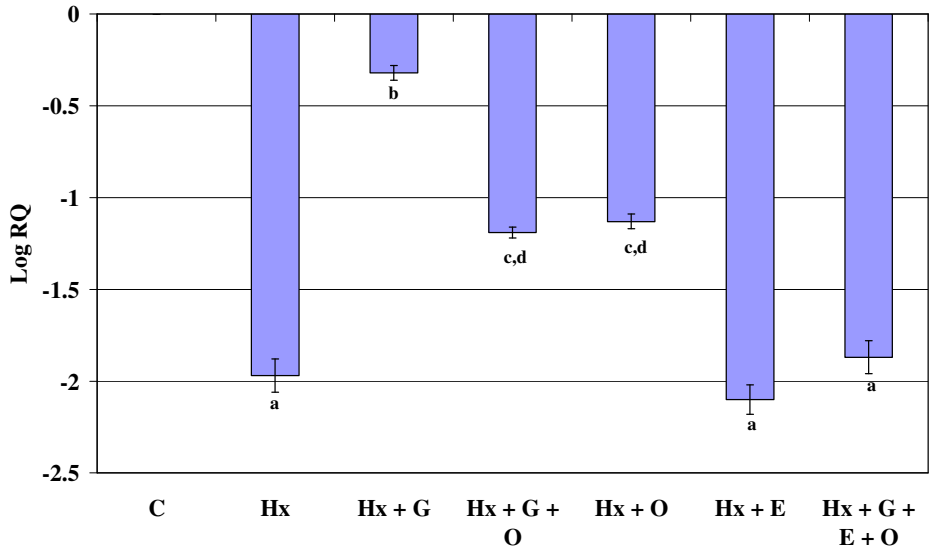


Table- 24
Real Time PCR amplification of $GABA_{A\gamma3}$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.97 ± 0.09[a]
Hx + G	-0.32 ± 0.04 [b]
Hx + G + O	-1.19 ± 0.03 [c, d]
Hx + O	-1.13 ± 0.04 [c, d]
Hx + E	-2.1 ± 0.08 [a]
Hx + G + E + O	-1.87 ± 0.09 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.01$ when compared to Control
[b] $p<0.001$, [d] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 21
Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

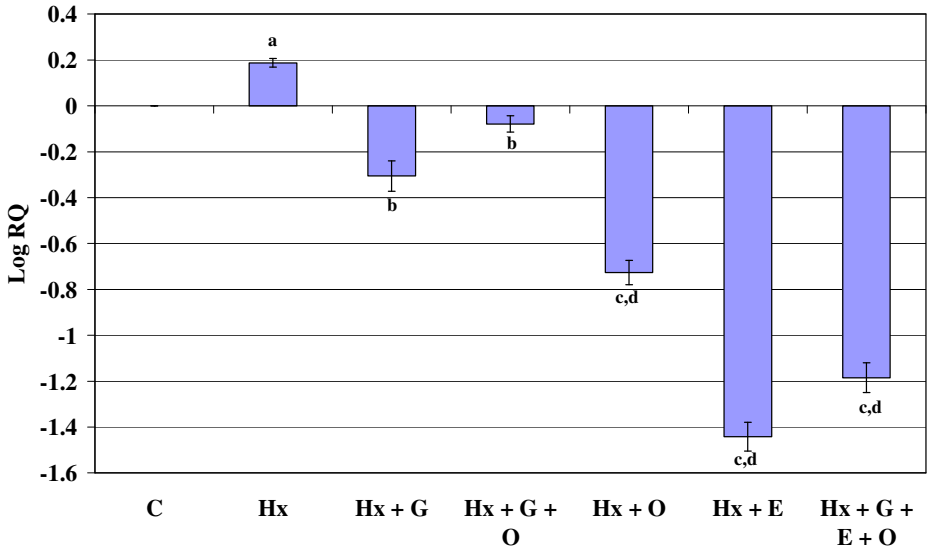


Table- 25
Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	0.19 ± 0.02 [a]
Hx + G	-0.31 ± 0.07 [b]
Hx + G + O	-0.08 ± 0.04 [b]
Hx + O	-0.73 ± 0.05 [c, d]
Hx + E	-1.44 ± 0.06 [c, d]
Hx + G + E + O	-1.18 ± 0.06 [c, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.05$, [c] $p<0.001$ when compared to Control
[b] $p<0.01$, [d] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 22
Real Time PCR amplification of $GABA_B$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

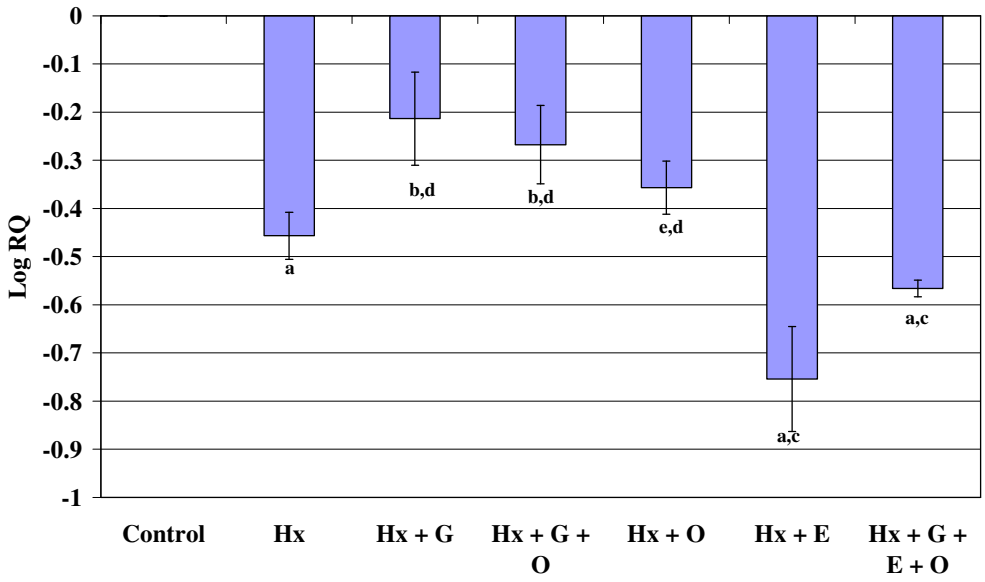


Table- 26
Real Time PCR amplification of $GABA_B$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.46 ± 0.05 [a]
Hx + G	-0.21 ± 0.10 [b, d]
Hx + G + O	-0.27 ± 0.08 [b, d]
Hx + O	-0.36 ± 0.05 [e, d]
Hx + E	-0.75 ± 0.10 [a, c]
Hx + G + E + O	-0.57 ± 0.02 [a, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [d] $p<0.05$ when compared to Control
[b] $p<0.001$, [c] $p<0.01$, [e] $p<0.05$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 23
Real Time PCR amplification of GAD mRNA from the cerebral cortex of control and experimental neonatal rats

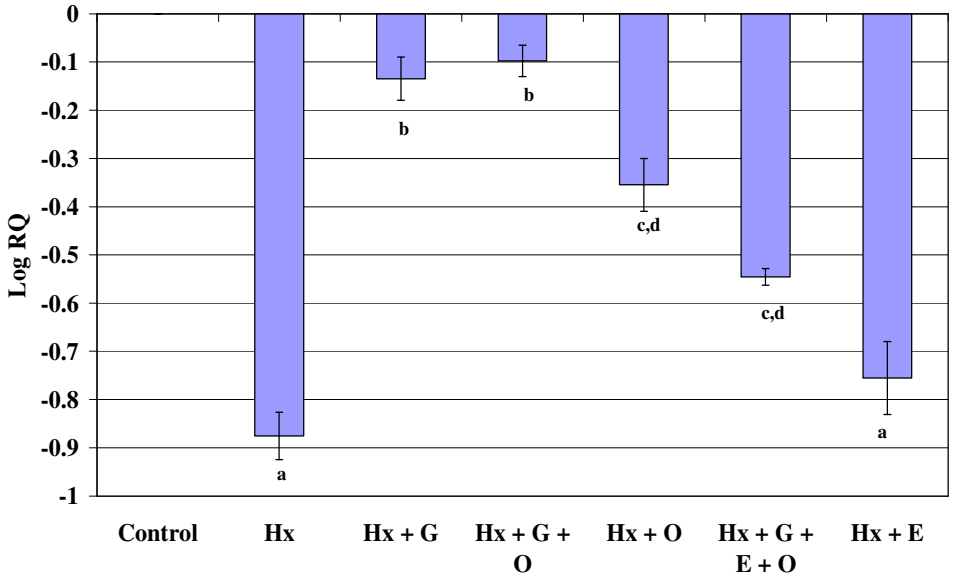


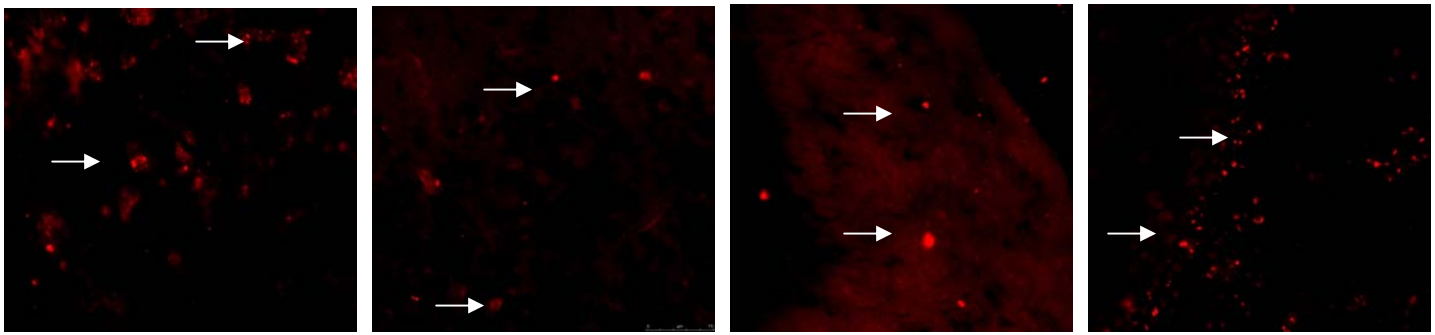
Table- 27
Real Time PCR amplification of GAD mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.87 ± 0.05 [a]
Hx + G	-0.13 ±0.05 [b]
Hx + G + O	-0.10 ±0.03 [b]
Hx + O	-0.35 ±0.06 [c, d]
Hx + E	-0.76 ±0.07 [c, d]
Hx + G + E + O	-0.55 ±0.02 [a]

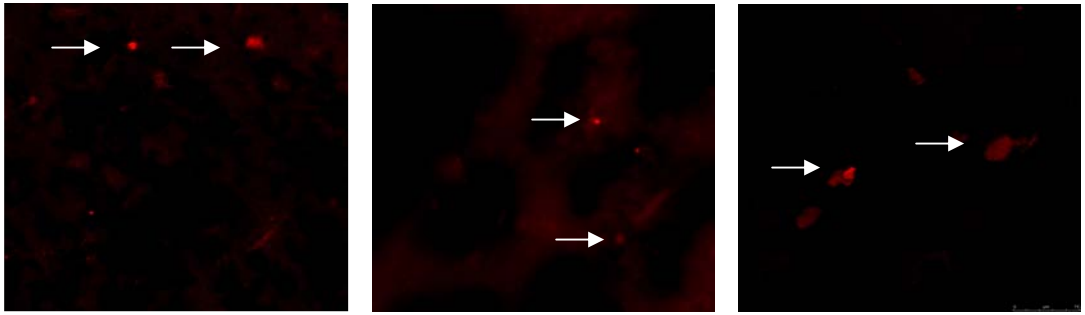
Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.01$ when compared to Control
[b] $p<0.001$, [d] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure-24

$GABA_{A\alpha1}$ receptor expression in the cerebral cortex of control and experimental neonatal rats



Control **Hx** **Hx + G** **Hx + G + O**



Hx + E **Hx + G + E + O**

Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table- 28

$GABA_{A\alpha1}$ receptor expression in the cerebral cortex of control and experimental neonatal rats

Experimental groups	Mean Pixel intensity
Control	32.13 ± 3.45
Hx	12.24 ± 2.20 [a]
Hx + G	28.94 ± 3.12 [b]
Hx + G + O	34.25 ± 4.01 [b]
Hx + O	16.80 ± 2.11[a]
Hx + E	8.94 ± 1.02[a]
Hx + G + E + O	9.95 ± 1.10[a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.

[a] $p<0.001$ when compared to Control

[b] $p<0.001$ when compared to hypoxic group

Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 25
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the cerebral cortex of control and experimental neonatal rats.

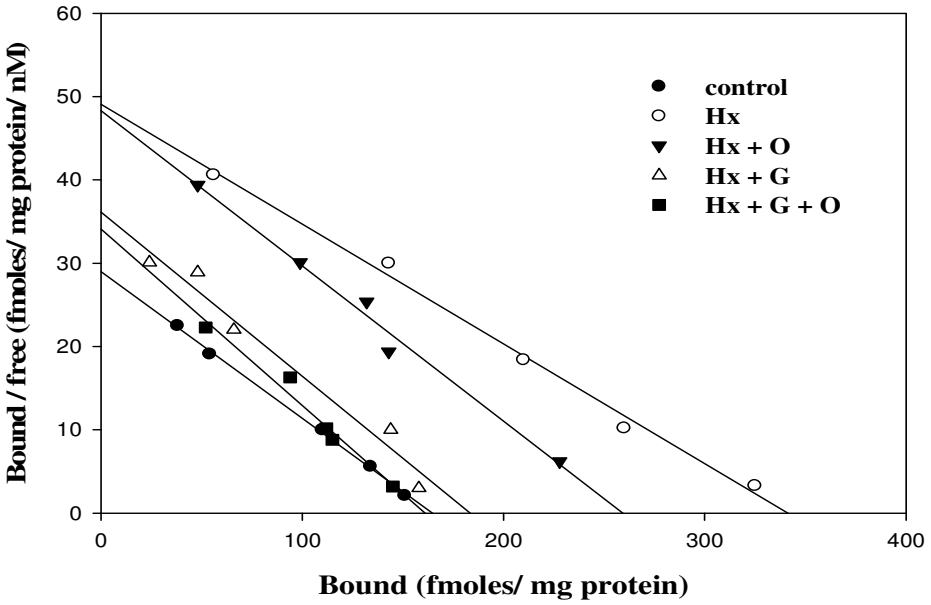


Table- 29
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	164.19 ± 2.55	5.63 ±1.00
Hx	341.15 ± 4.01 [a]	6.92 ± 1.12 [a]
Hx + G	184.52 ± 2.50 [d]	5.15 ± 1.01 [d]
Hx + G + O	165.00 ± 1.55 [d]	4.89 ± 1.00 [d]
Hx + O	258.16 ± 3.05 [a, e]	5.36 ± 1.04 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared with control
[d] $p<0.001$, [e] $p<0.01$ when compared with hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 26
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the cerebral cortex of control and experimental neonatal rats.

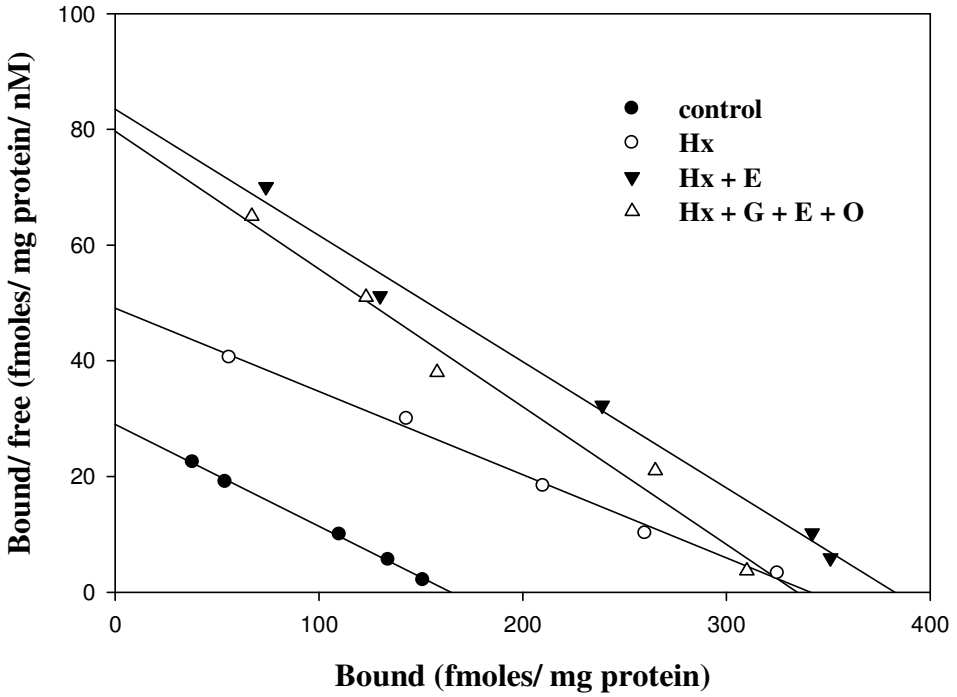


Table- 30
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	164.19 ± 2.55	5.63 ±1.00
Hx	341.15 ± 4.01 [a]	6.92 ± 1.12 [a]
Hx + E	383.49 ± 3.56 [a]	4.54 ± 1.10 [e]
Hx + G + E + O	332.70 ± 3.33 [a]	4.15 ± 1.02 [e]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] p<0.001, [b] p<0.01, [c] p<0.05 when compared with control
[d] p<0.001, [e]p<0.01 when compared with hypoxic group
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 27
Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT$_{2A}$ receptor in the cerebral cortex of control and experimental neonatal rats.

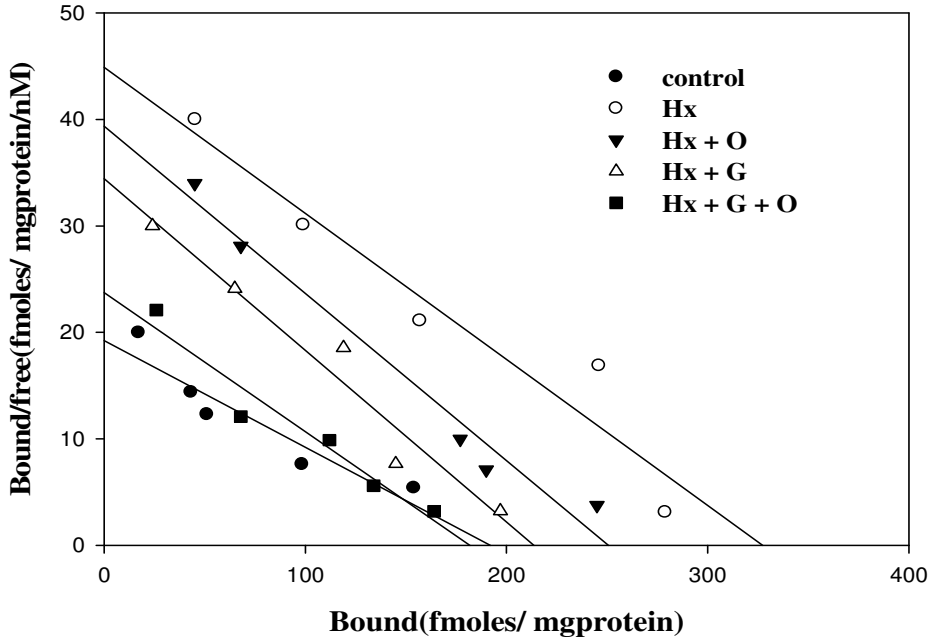


Table- 31
Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT$_{2A}$ receptor in the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	192.48 ± 1.45	9.99 ± 2.45
Hx	326.19 ± 2.34 [a]	7.28 ± 2.12 [a]
Hx + G	213.62 ± 2.66 [b, e]	6.15 ± 2.05 [a, e]
Hx + G + O	179.15 ± 2.30 [c, d]	7.55 ± 2.45 [a]
Hx + O	249.29 ± 2.50 [b, e]	6.30 ± 1.85 [a, e]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared with control
[d] $p<0.001$, [e] $p<0.01$ when compared with hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 28
Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the cerebral cortex of control and experimental neonatal rats.

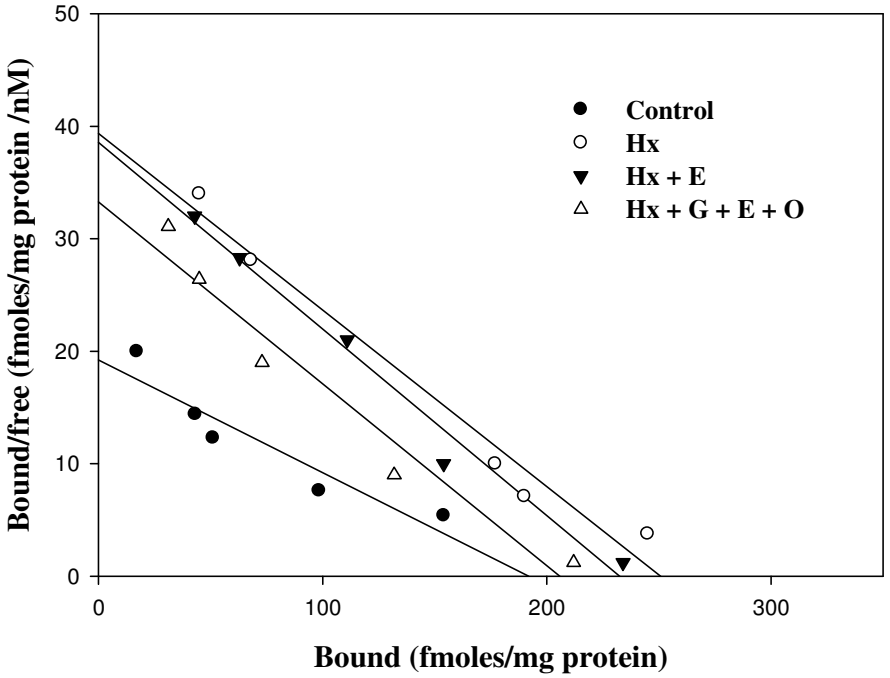


Table- 32
Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	192.48 ± 1.45	9.99 ± 2.45
Hx	326.19 ± 2.34 [a]	7.28 ± 2.12 [a]
Hx + E	232.58 ± 2.60 [b, d]	6.01 ± 2.10 [a]
Hx + G + E + O	205.12 ± 2.50 [b, d]	6.12 ± 3.12 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared with control
[d] $p<0.001$, [e] $p<0.01$ when compared with hypoxic group
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 29
Real Time PCR amplification of 5-HT_{2A} receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

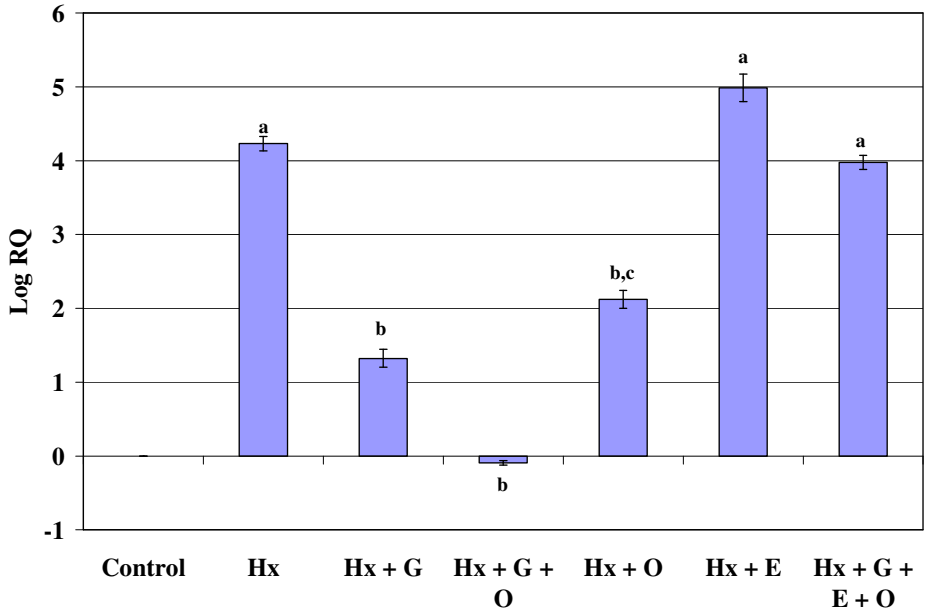


Table- 33
Real Time PCR amplification of 5-HT_{2A} receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	4.23 ± 0.10 [a]
Hx + G	1.32 ± 0.12 [b]
Hx + G + O	-0.09 ± 0.03 [b]
Hx + O	2.12 ± 0.12 [b, c]
Hx + E	4.99 ± 0.19 [a]
Hx + G + E + O	3.98 ± 0.10 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. [a] $p<0.001$ [c] $p< 0.05$ when compared to Control [b] $p<0.001$ when compared to hypoxic group Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 30
Real Time PCR amplification of 5-HT transporter mRNA from the cerebral cortex of control and experimental neonatal rats

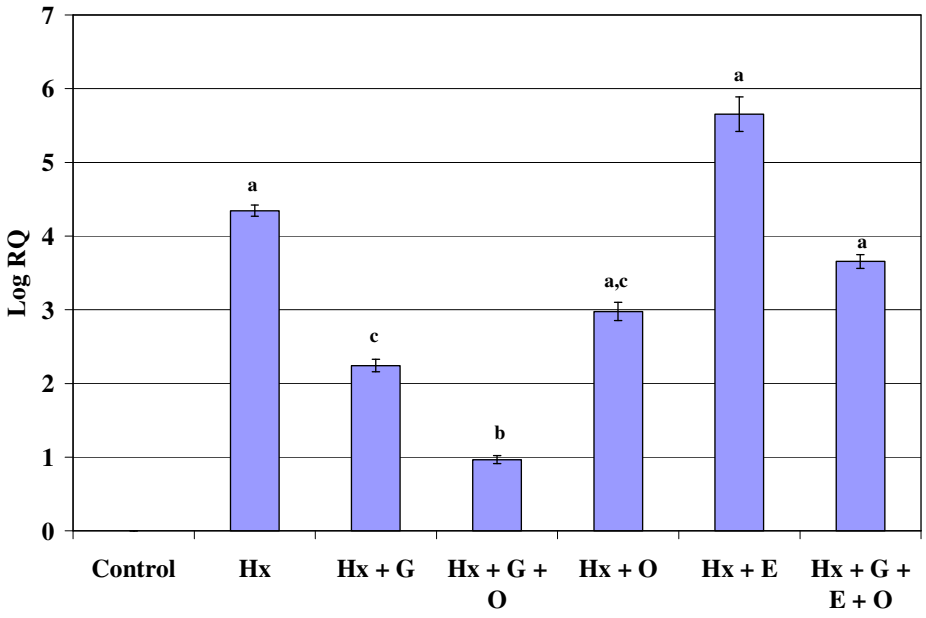


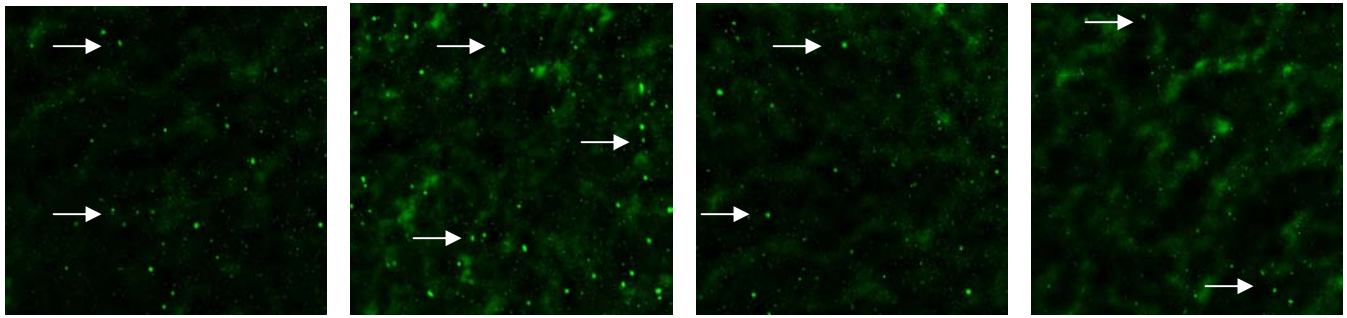
Table- 34
Real Time PCR amplification of 5-HT transporter mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	4.34 ± 0.08 [a]
Hx + G	2.24 ± 0.09 [c]
Hx + G + O	0.97 ± 0.05 [b]
Hx + O	2.98 ± 0.12 [a, c]
Hx + E	5.65 ± 0.24 [a]
Hx + G + E + O	3.66 ± 0.10 [a]

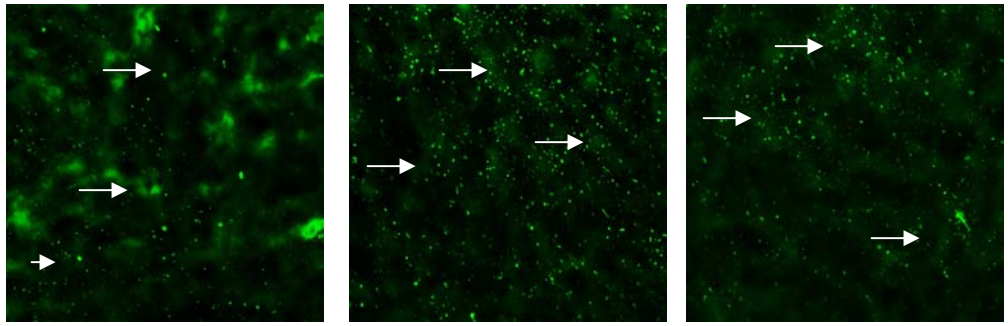
Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. [a] $p<0.001$ when compared to Control [b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 31

5-HT_{2A} receptor expression in the cerebral cortex of control and experimental neonatal rats



Control **Hx** **Hx + G** **Hx + G + O**



Hx + O **Hx + E** **Hx + G + E + O**

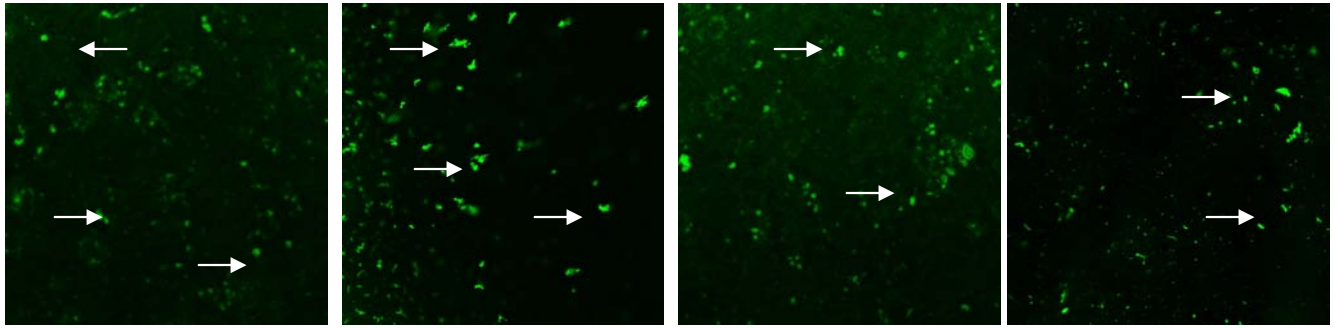
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table- 35
5-HT_{2A} receptor expression in the cerebral cortex of control and experimental neonatal rats

Experimental groups	Mean Pixel intensity
Control	22.45 ± 3.50
Hx	42.32 ± 4.20 [a]
Hx + G	24.50 ± 3.05 [b]
Hx + G + O	21.25 ± 2.05 [b]
Hx + O	36.80 ± 3.46 [a]
Hx + E	44.50 ± 5.05 [a]
Hx + G + E + O	42.85 ± 4.10 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 32
5-HT transporter expression in the cerebral cortex of control and experimental neonatal rats



Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table-36
5-HT transporter expression in the cerebral cortex of control and experimental neonatal rats

Experimental groups	Mean Pixel intensity
Control	32.50 ± 3.50
Hx	79.20 ± 3.00 [a]
Hx + G	34.45 ± 2.50 [b]
Hx + G + O	31.25 ± 3.05 [b]
Hx + O	47.54 ± 2.55
Hx + E	72.65 ± 5.00 [a]
Hx + G + E + O	83.45 ± 5.50 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 33
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the cerebral cortex of control and experimental neonatal rats.

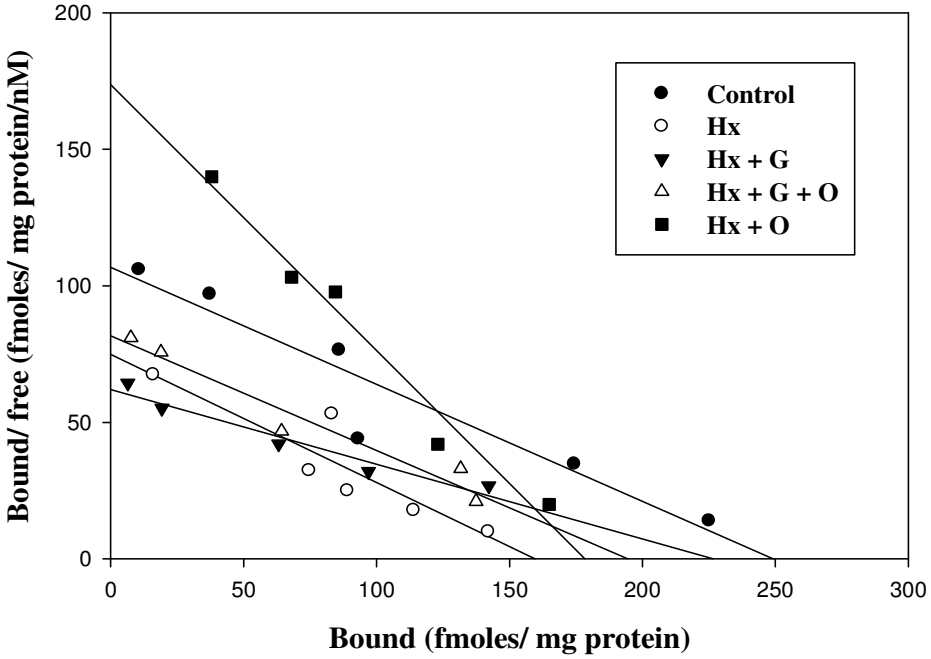


Table- 37
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	248.70 ± 4.20	2.32 ± 1.02
Hx	158.11 ± 2.15 [a]	2.09 ± 1.12
Hx + G	225.64 ± 2.02 [b]	3.58 ± 2.10 [a, b]
Hx + G + O	194.34 ± 1.23 [b]	2.32 ± 2.15 [a, b]
Hx + O	177.48 ± 1.85 [a, b]	1.01 ± 1.00 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 34
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the cerebral cortex of control and experimental neonatal rats.

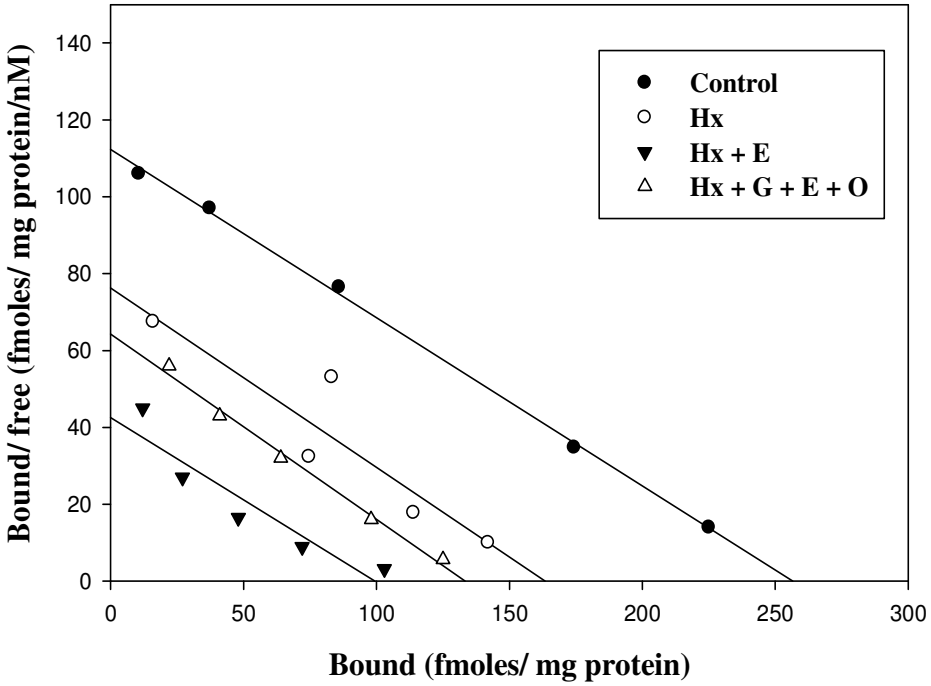


Table- 38
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	248.70 ± 3.6	2.32 ±0.14
Hx	158.11 ± 3.8 [a]	2.09 ± 0.12
Hx + E	98.73 ± 2.6 [a, b]	2.31 ±0.10 [b]
Hx + G + E + O	131.82 ± 4.5 [a, b]	2.03 ±0.12 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 35
Real Time PCR amplification of Muscarinic M_1 receptor mRNA from the cerebral cortex of control and experimental neonatal rats

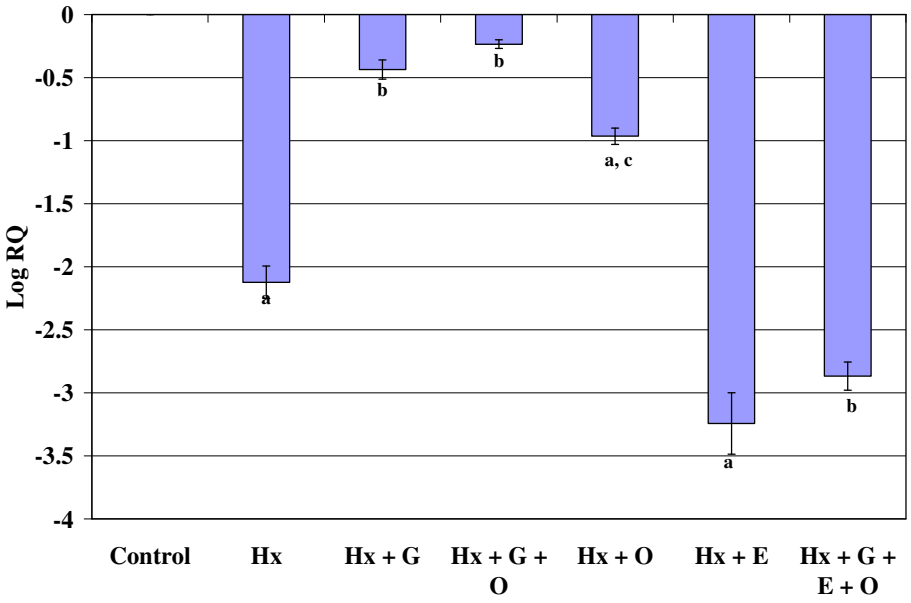


Table- 39
Real Time PCR amplification of Muscarinic M_1 receptor mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-2.12 ± 0.13 [a]
Hx + G	-0.44 ± 0.08 [b]
Hx + G + O	-0.23 ± 0.03 [b]
Hx + O	-0.97 ± 0.07 [a, c]
Hx + E	-3.24 ± 0.24 [a]
Hx + G + E + O	-2.87 ± 0.11 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. [a] $p<0.001$ when compared to Control [b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 36
Real Time PCR amplification of Muscarinic M_2 receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

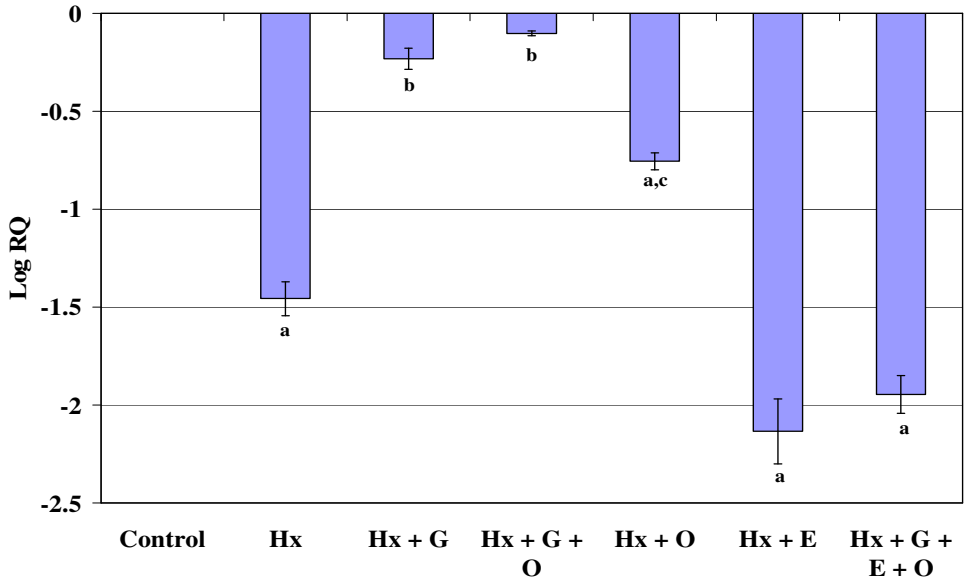


Table- 40
Real Time PCR amplification of Muscarinic M_2 receptor mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.46 ± 0.09 [a]
Hx + G	-0.23 ± 0.05 [b]
Hx + G + O	-0.10 ± 0.01 [b]
Hx + O	-0.76 ± 0.04 [a, c]
Hx + E	-2.13 ± 0.17 [a]
Hx + G + E + O	-1.95 ± 0.10 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. [a] $p<0.001$ when compared to Control [b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 37
Real Time PCR amplification of Muscarinic M_3 receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

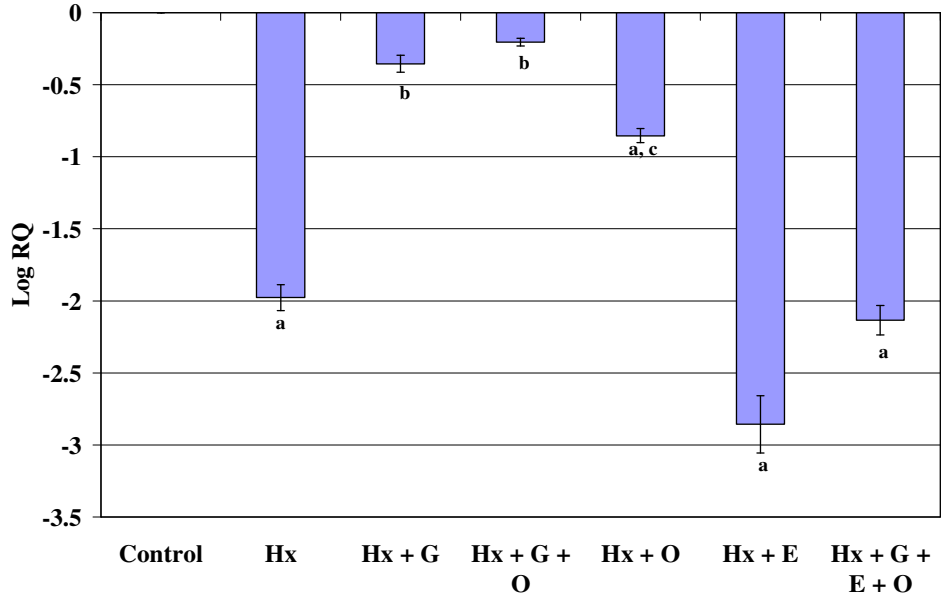


Table- 41
Real Time PCR amplification of Muscarinic M_3 receptor mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.46 ± 0.09 [a]
Hx + G	-0.23 ± 0.05 [b]
Hx + G + O	-0.10 ± 0.01 [b]
Hx + O	-0.76 ± 0.04 [a, c]
Hx + E	-2.13 ± 0.17 [a]
Hx + G + E + O	-1.95 ± 0.10 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. [a] $p<0.001$ when compared to Control [b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 38

Real Time PCR amplification of choline acetyl transferase mRNA from the cerebral cortex of control and experimental neonatal rats.

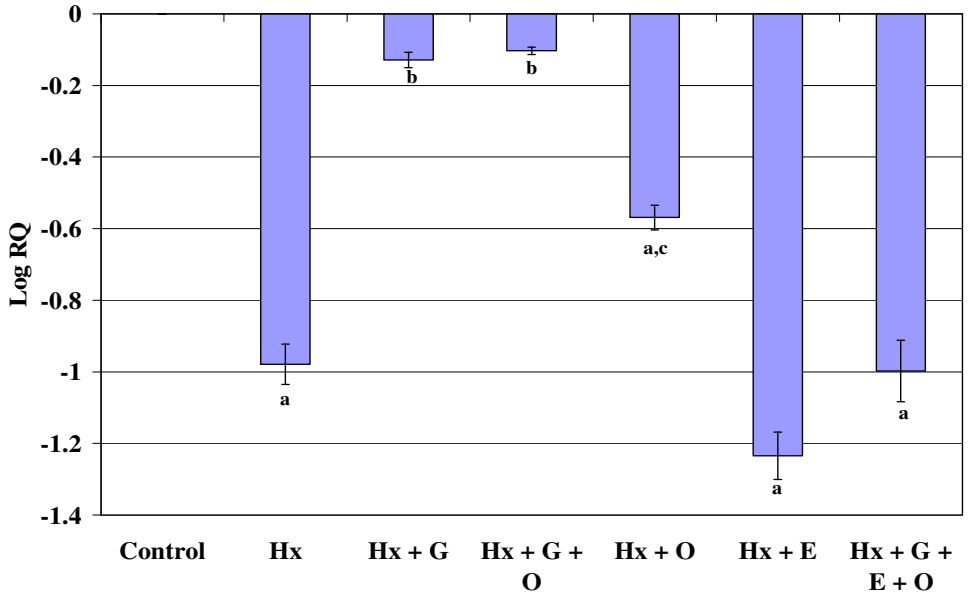


Table- 42

Real Time PCR amplification of choline acetyl transferase mRNA from the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Log RQ
Control	0
Hx	-0.98 ± 0.06 [a]
Hx + G	-0.13 ± 0.02 [b]
Hx + G + O	-0.10 ± 0.01 [b]
Hx + O	-0.57 ± 0.03 [a, c]
Hx + E	-1.23 ± 0.07 [a]
Hx + G + E + O	-0.99 ± 0.09 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. [a] $p<0.001$ when compared to Control [b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 39
Real Time PCR amplification of acetyl choline esterase mRNA from the cerebral cortex of control and experimental neonatal rats.

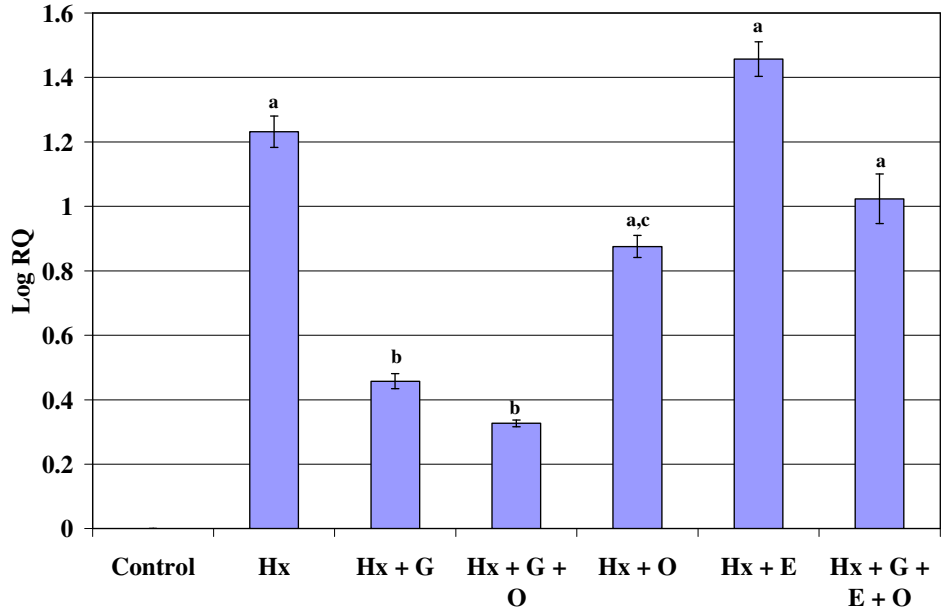


Table- 43
Real Time PCR amplification of acetyl choline esterase mRNA from the cerebral cortex of control and experimental neonatal rats.

Experimental groups	Log RQ
Control	0
Hx	1.23 ± 0.05 [a]
Hx + G	0.46 ± 0.02 [b]
Hx + G + O	0.33 ± 0.01 [b]
Hx + O	0.88 ± 0.03 [a, c]
Hx + E	1.46 ± 0.05 [a]
Hx + G + E + O	1.02 ± 0.08 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. [a] $p<0.001$ when compared to Control [b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 40
Real Time PCR amplification of Hif 1 mRNA from the cerebral cortex of control and experimental neonatal rats.

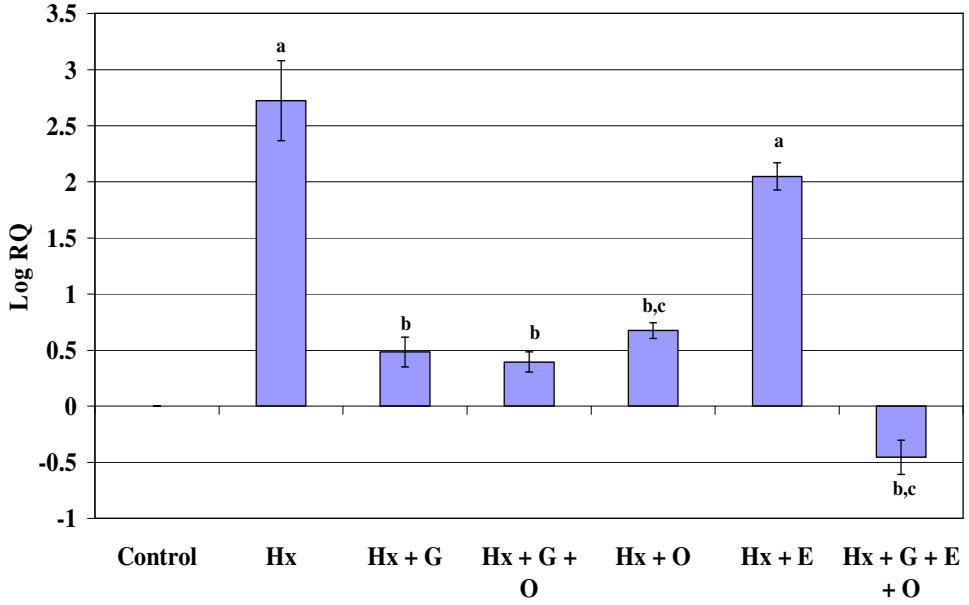


Table- 44
Real Time PCR amplification of Hif 1 mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	2.72 ± 0.36 [a]
Hx + G	0.48 ± 0.13 [b]
Hx + G + O	0.39 ± 0.09 [b]
Hx + O	0.67 ± 0.07 [b, c]
Hx + E	2.05 ± 0.15 [b, c]
Hx + G + E + O	-0.46 ± 0.12 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.05$ when compared to Control [b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 41
Real Time PCR amplification of SOD mRNA from the cerebral cortex of control and experimental neonatal rats.

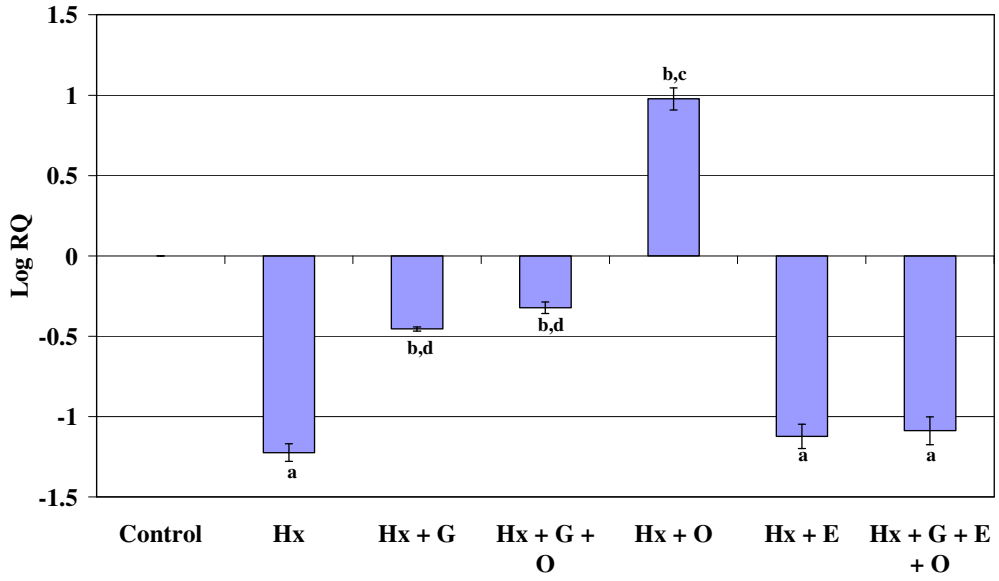


Table- 45
Real Time PCR amplification of SOD mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.22 ± 0.05 [a]
Hx + G	-0.45 ± 0.01 [b, c]
Hx + G + O	-0.32 ± 0.04 [b, c]
Hx + O	0.98 ± 0.07 [a, d]
Hx + E	-1.12 ± 0.08 [a, c]
Hx + G + E + O	-1.09 ± 0.09 [a, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. [a] $p<0.001$, [c] $p<0.01$, [d] $p<0.05$ when compared to Control [b] $p<0.001$ when compared to Hx. Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 42
Real Time PCR amplification of GPx mRNA from the cerebral cortex of control and experimental neonatal rats.

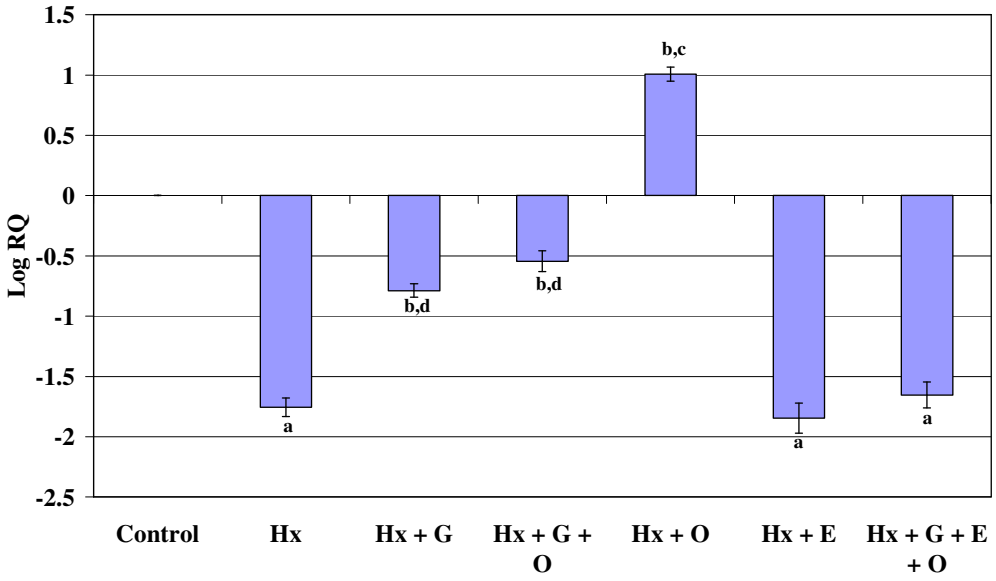


Table- 46
Real Time PCR amplification of GPx mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.76 ± 0.08 [a]
Hx + G	-0.79 ± 0.06 [b, d]
Hx + G + O	-0.55 ± 0.09 [b, d]
Hx + O	1.01 ± 0.06 [b, c]
Hx + E	-1.85 ± 0.12 [a]
Hx + G + E + O	-1.65 ± 0.11 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. [a] $p<0.001$, [c] $p<0.01$, [d] $p<0.05$ when compared to Control [b] $p<0.001$ when compared to Hx. Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 43
Real Time PCR amplification of BAX mRNA from the cerebral cortex of control and experimental neonatal rats.

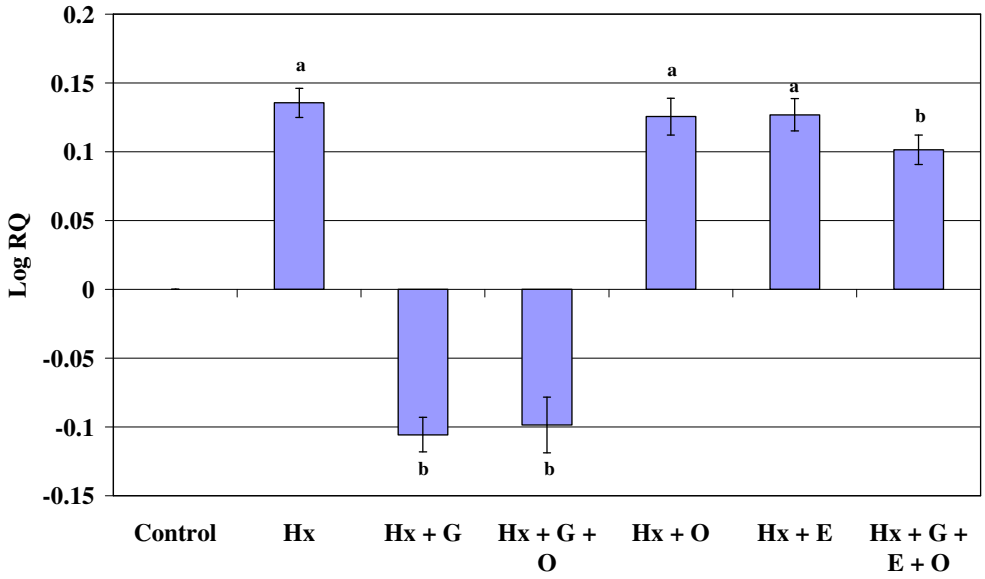


Table- 47
Real Time PCR amplification of BAX mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	0.14 ± 0.01 [a]
Hx + G	-0.11 ± 0.01 [b]
Hx + G + O	-0.09 ± 0.02 [b]
Hx + O	0.13 ± 0.01 [b]
Hx + E	0.13 ± 0.01 [a]
Hx + G + E + O	0.10 ± 0.01 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control [b] $p<0.001$ when compared to Hx.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 44
Real Time PCR amplification of CREB mRNA from the cerebral cortex of control and experimental neonatal rats.

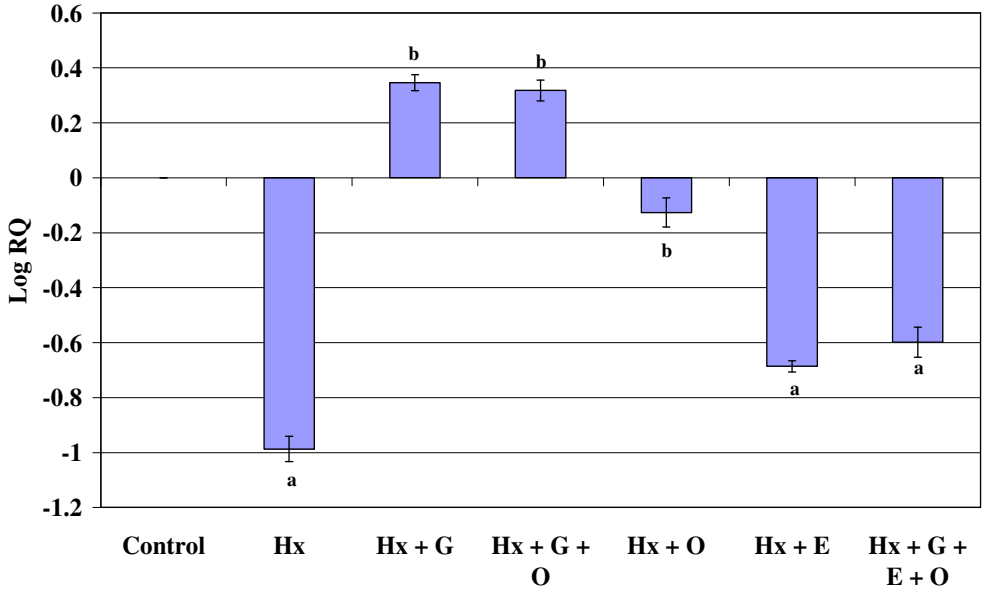


Table- 48
Real Time PCR amplification of CREB mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.99 ± 0.01 [a]
Hx + G	0.35 ± 0.02 [b]
Hx + G + O	0.32 ± 0.01 [b]
Hx + O	-0.13 ± 0.01 [b]
Hx + E	-0.69 ± 0.01 [a]
Hx + G + E + O	-0.59 ± 0.01 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control [b] $p<0.001$ when compared to Hx.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 45
Real Time PCR amplification of phospholipase C mRNA from the cerebral cortex of control and experimental neonatal rats.

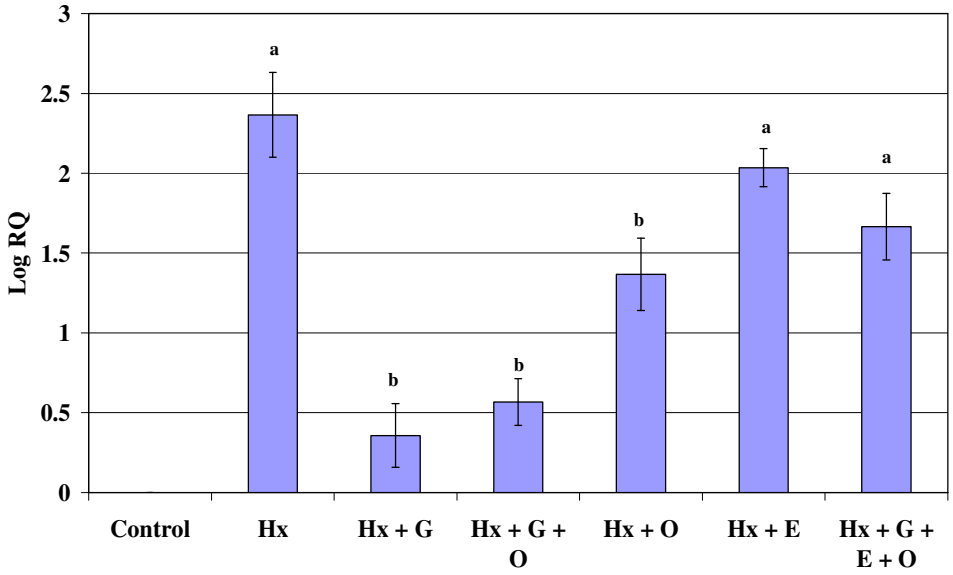


Table- 49
Real Time PCR amplification of phospholipase C mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	2.37 ± 0.01 [a]
Hx + G	0.36 ± 0.02 [b]
Hx + G + O	0.57 ± 0.01 [b]
Hx + O	1.37 ± 0.01 [b]
Hx + E	2.03 ± 0.01 [a]
Hx + G + E + O	1.67 ± 0.01 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control [b] $p<0.001$ when compared to Hx.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 46
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the cerebellum of control and experimental neonatal rats.

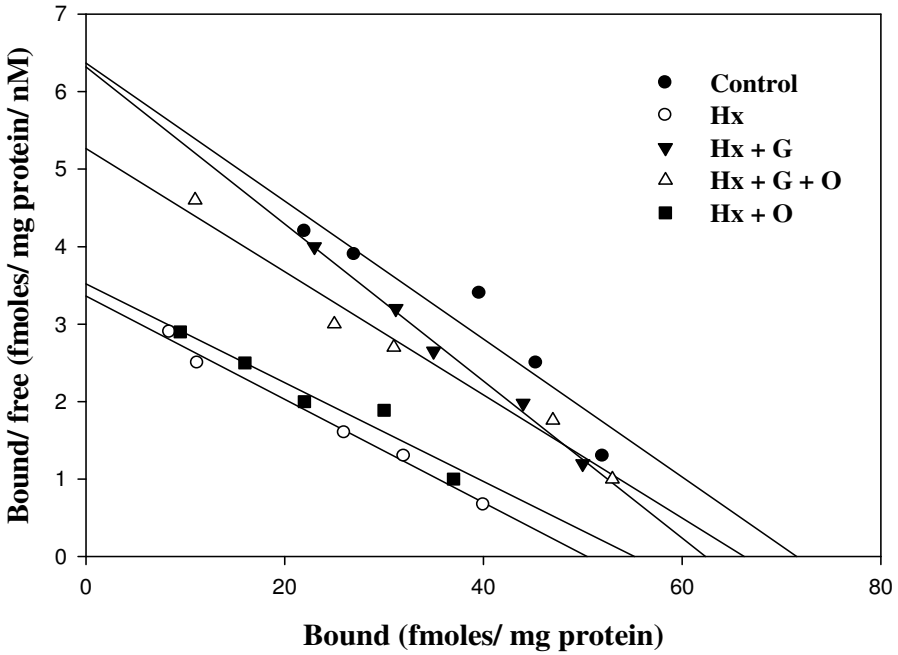


Table- 50
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the cerebellum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	71.50 ± 2.41	11.11 ± 0.95
Hx	50.01 ± 1.80 [a]	14.82 ± 0.82 [a]
Hx + G	62.18 ± 1.50 [b]	9.85 ± 0.36 [b]
Hx + G + O	66.33 ± 2.00 [b]	12.54 ± 0.42
Hx + O	55.34 ± 2.50 [a]	15.72 ± 0.54 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 47
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the cerebellum of control and experimental neonatal rats.

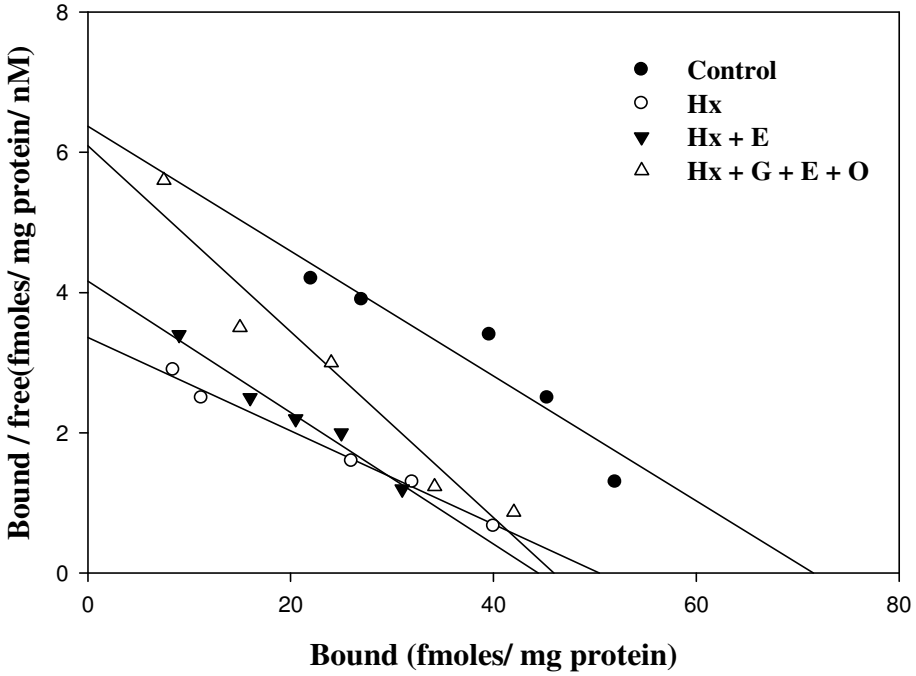


Table- 51
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the cerebellum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	71.50 ± 2.41	11.11 ± 0.95
Hx	50.01 ± 1.80 [a]	14.82 ± 0.82 [a]
Hx + E	44.02 ± 3.20 [a]	10.46 ± 0.10 [b]
Hx + G + E + O	45.50 ± 2.50 [a]	7.46 ± 0.11 [a, b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure - 48

Binding parameters of [^{3}H] GABA against GABA in the cerebellum of control and experimental neonatal rats.

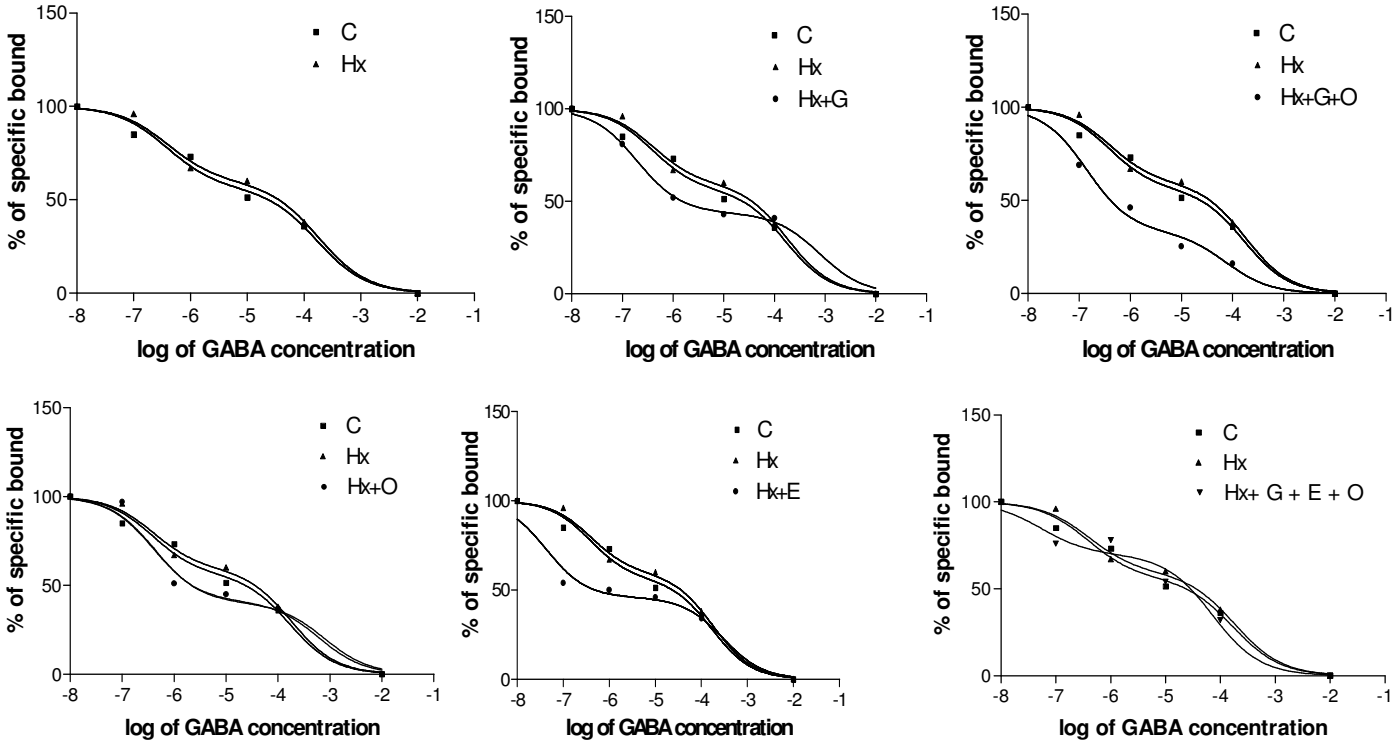


Table - 52

Binding parameters of [^{3}H] GABA against GABA in the cerebellum of control and experimental neonatal rats.

Group	Best-fit model	Log (EC_{50})-1	Log (EC_{50})-2	Ki $_{(H)}$	Ki $_{(L)}$	Hill slope
C	Two- site	-8.153	-4.545	3.6430 x 10^{-9}	2.3086x 10^{-5}	- 0.6832
Hx	Two- site	-9.243	-5.398	4.2340x 10^{-10}	1.0560 x 10^{-5}	- 0.6944
Hx + G	Two- site	-8.406	-4.580	4.0410 x 10^{-12}	5.1230 x 10^{-4}	- 0.6522
Hx + G + O	Two- site	-8.053	-4.567	4.2740 x 10^{-11}	9.1930 x 10^{-4}	- 0.5831
Hx + O	Two- site	-9.027	-4.706	5.0880 x 10^{-9}	1.3380 x 10^{-5}	- 0.7926
Hx + E	Two- site	-9.332	-5.508	5.7180 x 10^{-9}	8.0440 x 10^{-6}	- 0.6957
Hx + G + E + O	Two- site	-9.554	-5.554	5.0987 x 10^{-9}	9.0023 x 10^{-6}	- 0.7964

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 49
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the cerebellum of control and experimental neonatal rats.

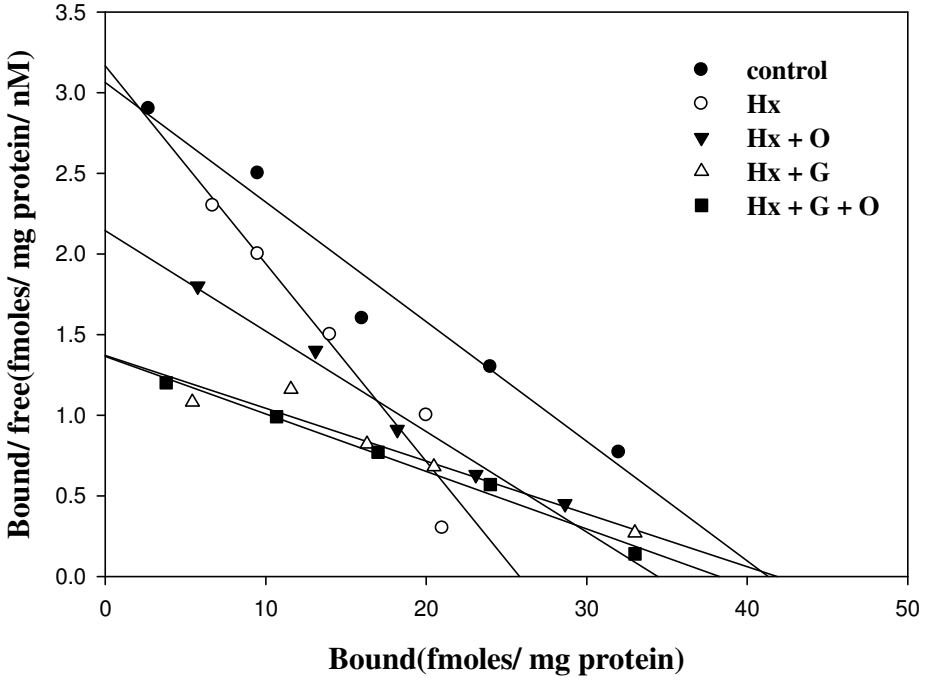


Table- 53
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the cerebellum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	41.04 ± 3.20	13.50 ± 0.53
Hx	25.83 ± 1.25 [a]	8.10 ± 0.44 [a]
Hx + G	41.87 ± 2.80 [b]	29.55 ± 0.62 [a, b]
Hx + G + O	38.23 ± 2.15 [b]	27.31 ± 0.45 [a, b]
Hx + O	34.06 ± 1.65 [a, c]	15.72 ± 0.24 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control. [b] $p<0.001$, [c] $p<0.01$ when compared with Hx.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 50
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the cerebellum of control and experimental neonatal rats.

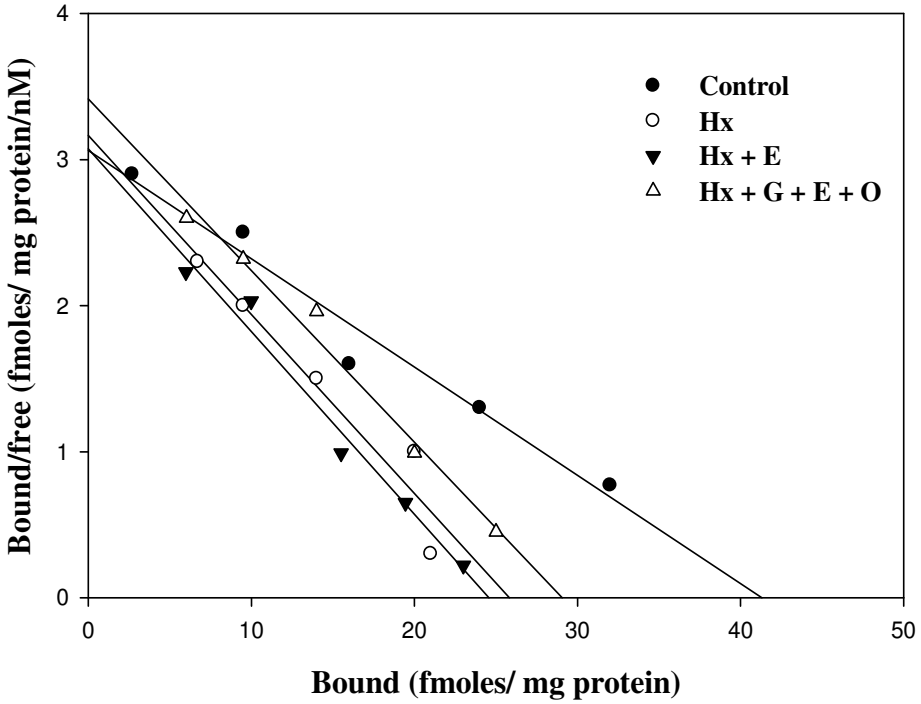


Table- 54
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the cerebellum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	41.04 ± 3.2	13.50 ± 0.53
Hx	25.83 ± 1.2 [a]	8.10 ± 0.44 [a]
Hx + E	24.38 ± 2.6 [a]	7.86 ± 0.10 [a]
Hx + G + E + O	29.07 ± 4.5 [a]	8.31 ± 0.12 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Fgure- 51
Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the cerebellum of control and experimental neonatal rats.

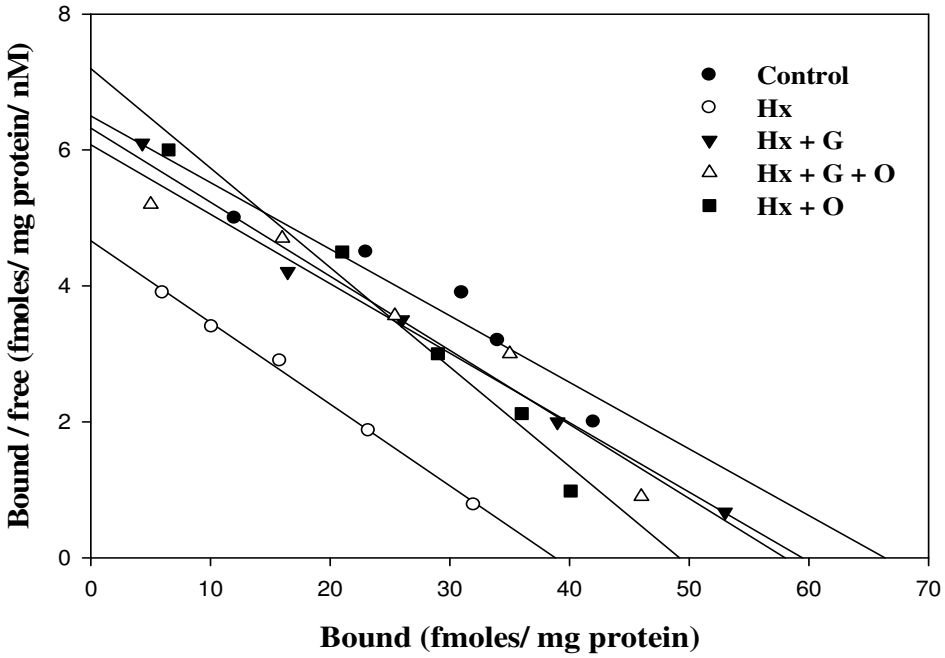


Table- 55
Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the cerebellum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	K_d (nM)
Control	66.07 ± 3.0	10.13 ± 1.5
Hx	38.65 ± 1.6 [a]	8.22 ± 1.00 [b]
Hx + G	57.90 ± 2.4 [d, c]	9.19 ± 0.95 [c]
Hx + G + O	59.50 ± 3.1 [d, c]	9.75 ± 1.2 [c]
Hx + O	48.86 ± 2.1 [a, e]	6.69 ± 0.89 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared with control
[d] $p<0.001$, [e] $p<0.01$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 52
Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the cerebellum of control and experimental neonatal rats.

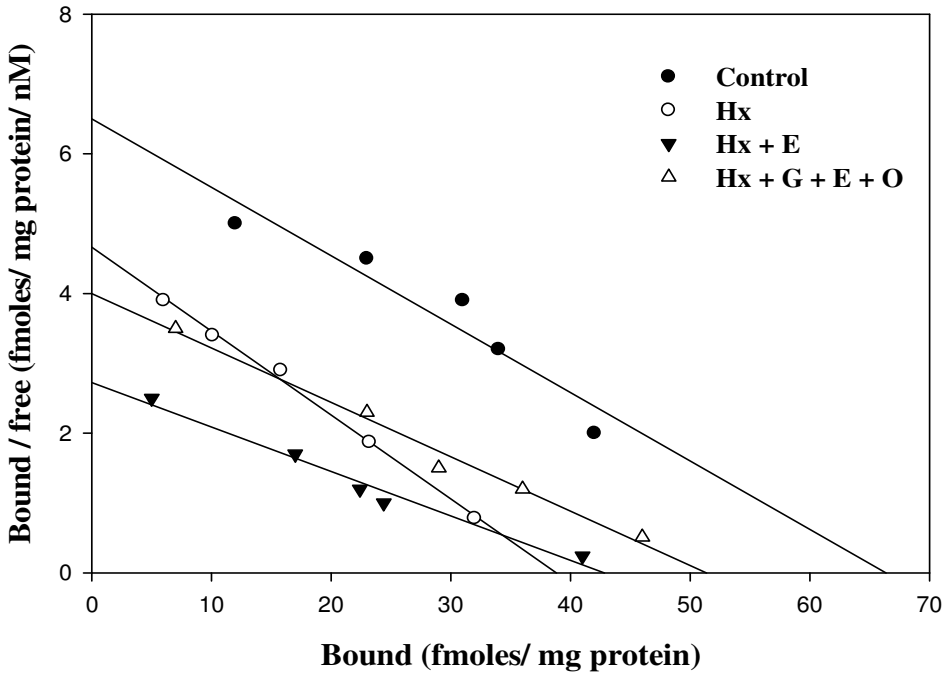


Table- 56
Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the cerebellum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	K_d (nM)
Control	66.07 ± 3.0	10.13 ± 1.5
Hx	38.65 ± 1.6 [a]	8.22 ± 1.00 [b]
Hx + E	42.29 ± 1.5 [a, e]	15.32 ± 0.99 [b, d]
Hx + G + E + O	51.19 ± 1.5 [a, e]	12.80 ± 0.99 [c, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared with control
[d] $p<0.001$, [e] $p<0.05$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 53
Real Time PCR amplification of $GABA_{A\acute{u}1}$ receptor subunit mRNA from the cerebellum of control and experimental neonatal rats

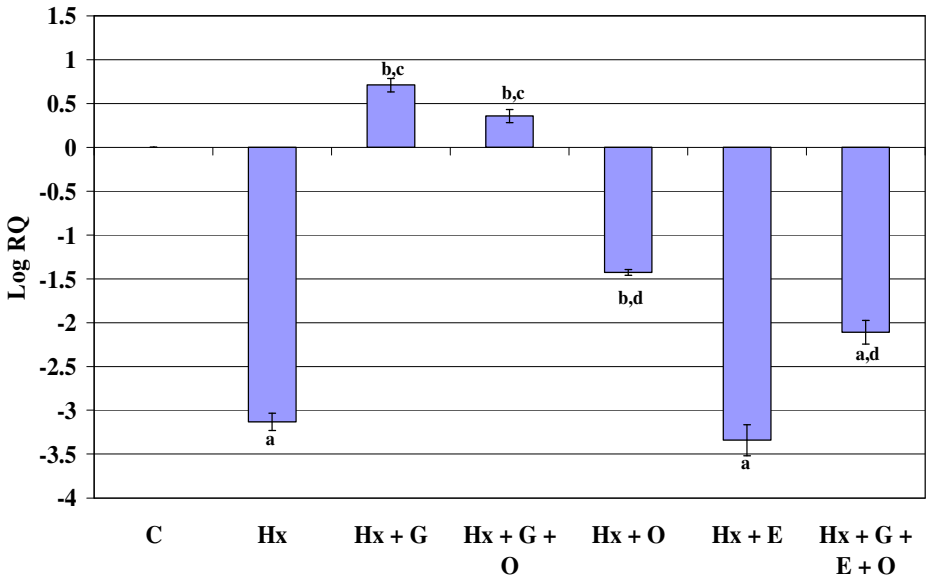


Table- 57
Real Time PCR amplification of $GABA_{A\acute{u}1}$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-3.13 ± 0.10 [a]
Hx + G	0.71 ± 0.08 [b, c]
Hx + G + O	0.35 ± 0.08 [b, c]
Hx + O	-1.43 ± 0.03 [b, d]
Hx + E	-3.34 ± 0.18 [a]
Hx + G + E + O	-2.11 ± 0.13 [a, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats. [a] $p<0.001$, [b] $p<0.05$ when compared to Control. [c] $p<0.001$, [d] $p<0.01$ when compared to Hx. Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 54
Real Time PCR amplification of $GABA_{A\acute{o}5}$ receptor subunit mRNA from the cerebellum of control and experimental neonatal rats

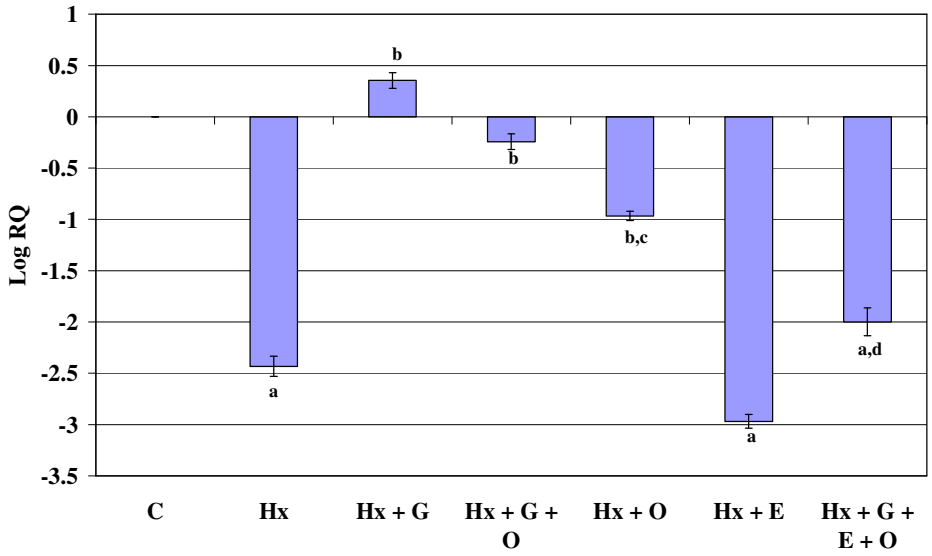


Table- 58
Real Time PCR amplification of $GABA_{A\acute{o}5}$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-2.43 ± 0.10 [a]
Hx + G	0.35 ± 0.08 [b]
Hx + G + O	-0.24 ± 0.08 [b]
Hx + O	-0.97 ± 0.05 [b, c]
Hx + E	-2.97 ± 0.07 [a]
Hx + G + E + O	-1.99 ± 0.13 [a, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.05$ when compared to Control
[b] $p<0.001$, [d] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 55
Real Time PCR amplification of $GABA_{A\gamma3}$ receptor subunit mRNA from the cerebellum of control and experimental neonatal rats

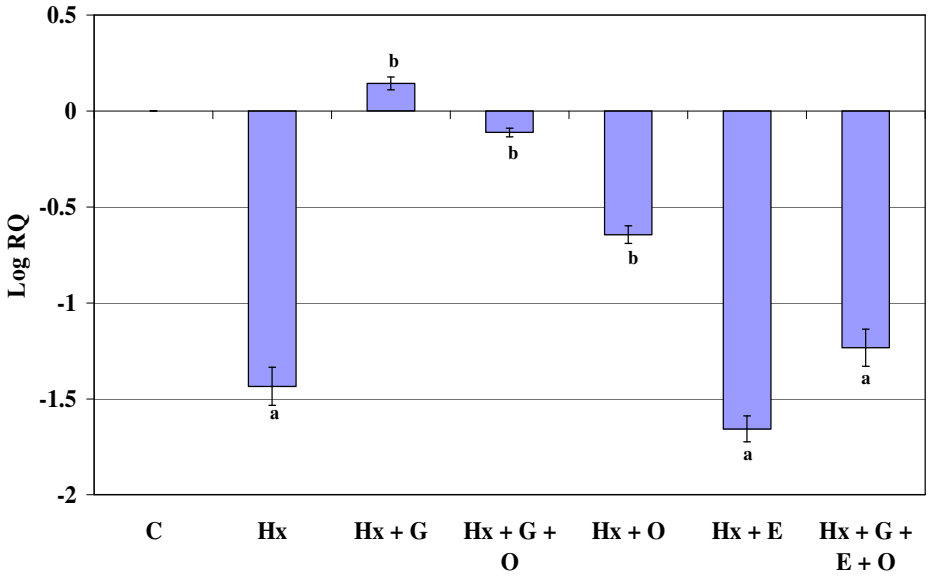


Table- 59
Real Time PCR amplification of $GABA_{A\ \gamma3}$ receptor subunit mRNA from the cerebral cortex of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.44 ± 0.10 [a]
Hx + G	0.14 ± 0.03 [b]
Hx + G + O	-0.11 ± 0.02 [b]
Hx + O	-0.64 ± 0.04 [b]
Hx + E	-1.66 ± 0.07 [a]
Hx + G + E + O	-1.23 ± 0.09 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 56
Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the cerebellum of control and experimental neonatal rats

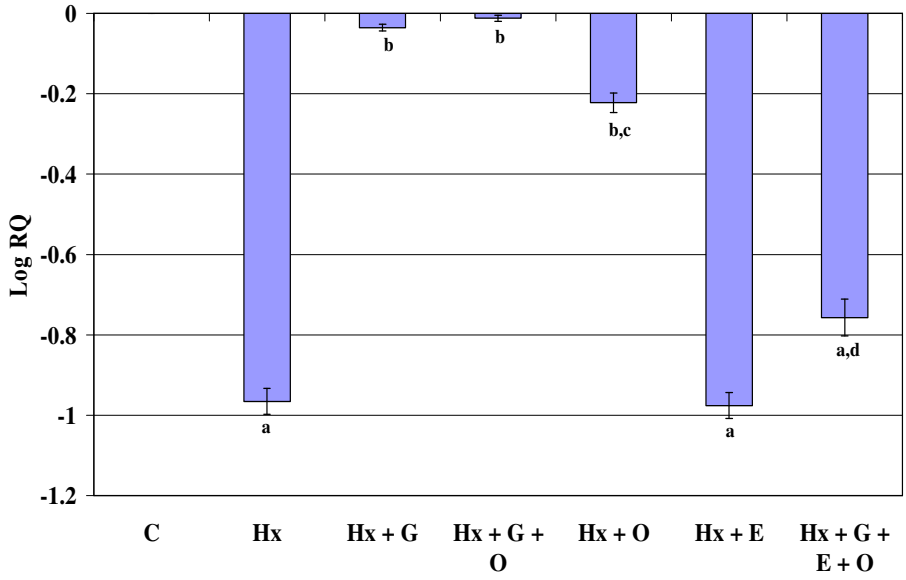


Table- 60
Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.97 ± 0.03 [a]
Hx + G	-0.04 ± 0.01 [b]
Hx + G + O	-0.01 ± 0.01 [b]
Hx + O	-0.22 ± 0.02 [b, c]
Hx + E	-0.98 ± 0.03 [a]
Hx + G + E + O	-0.76 ± 0.05 [a, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.05$ when compared to Control
[b] $p<0.001$, [d] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 57
Real Time PCR amplification of $GABA_B$ receptor subunit mRNA from the cerebellum of control and experimental neonatal rats

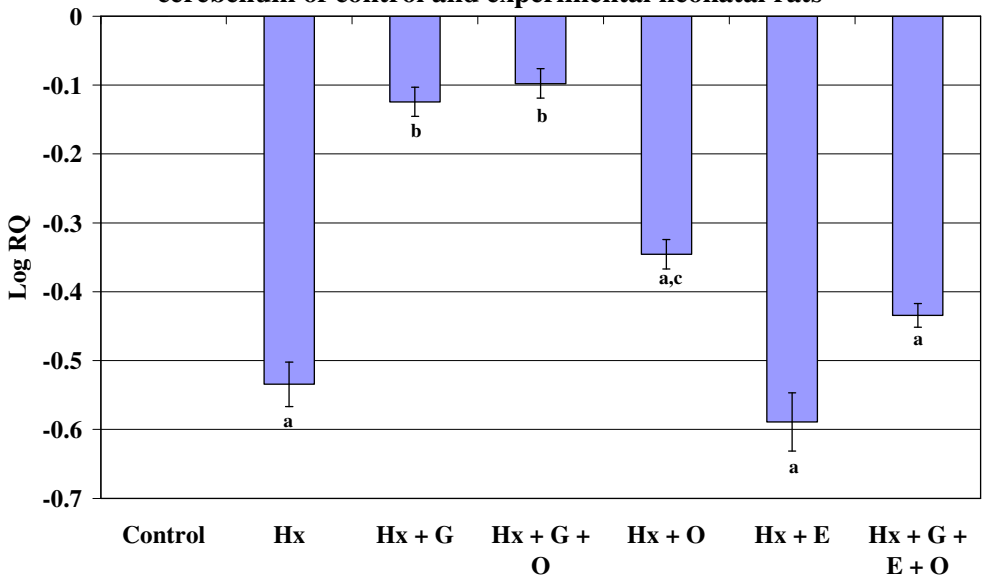


Table- 61
Real Time PCR amplification of $GABA_B$ receptor subunit mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.46 ± 0.05 [a]
Hx + G	-0.21 ± 0.10 [b]
Hx + G + O	-0.27 ± 0.08 [b]
Hx + O	-0.36 ± 0.05 [a, c]
Hx + E	-0.75 ± 0.10 [a]
Hx + G + E + O	-0.57 ± 0.02 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.05$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 58
Real Time PCR amplification of GAD mRNA from the cerebellum of control and experimental neonatal rats

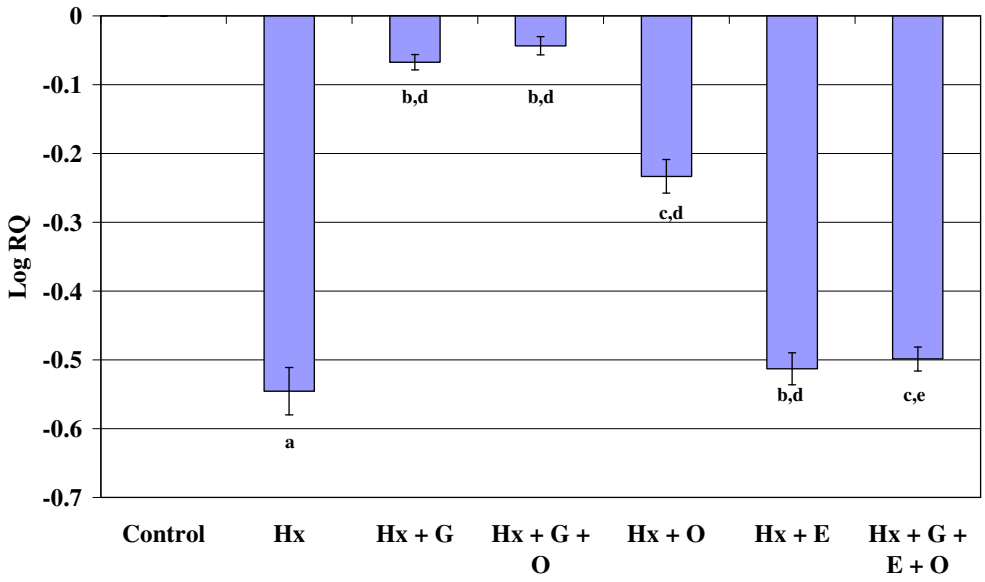


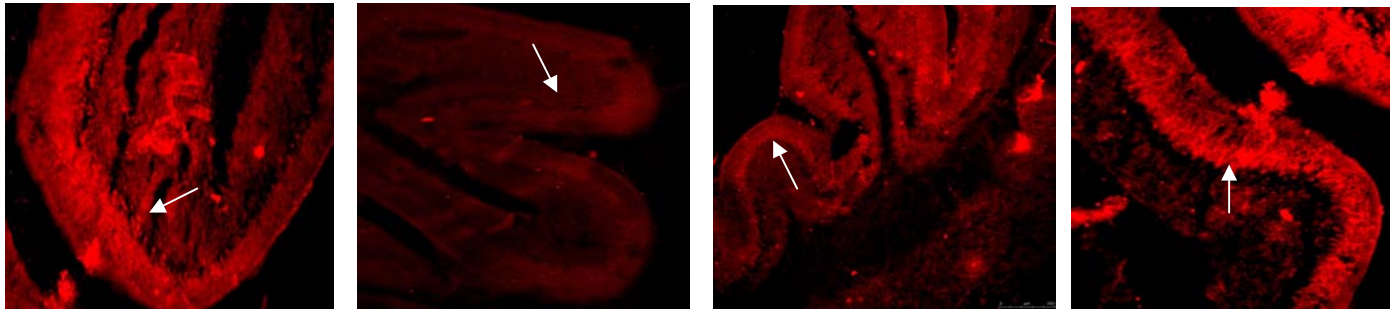
Table- 62
Real Time PCR amplification of GAD mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.55 ± 0.03 [a]
Hx + G	-0.07 ± 0.01 [b, d]
Hx + G + O	-0.04 ± 0.01 [b, d]
Hx + O	-0.23 ± 0.02 [c, d]
Hx + E	-0.51 ± 0.02 [c, e]
Hx + G + E + O	-0.51 ± 0.02 [b, d]

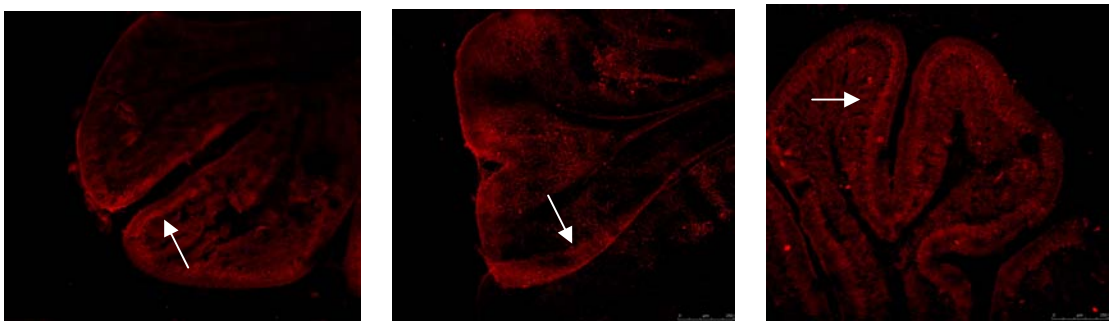
Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.01$ when compared to Control
[b] $p<0.001$, [d] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure-59

$GABA_{A\alpha1}$ receptor expression in the cerebellum of control and experimental neonatal rats



Control **Hx** **Hx + G** **Hx + G + O**



Hx + O **Hx + E** **Hx + G + E + O**

Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table- 63

$GABA_{A\alpha1}$ receptor expression in the cerebellum of control and experimental neonatal rats

Experimental groups	Mean Pixel intensity
Control	55.45 ± 6.25
Hx	25.60 ± 4.35 [a]
Hx + G	50.25 ± 5.45 [b]
Hx + G + O	52.18 ± 6.01 [b]
Hx + O	38.55 ± 5.05 [a]
Hx + E	32.15 ± 3.82 [a]
Hx + G + E + O	37.24 ± 2.98 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.

[a] $p<0.001$ when compared to Control

[b] $p<0.001$ when compared to hypoxic group

Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 60
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the cerebellum of control and experimental neonatal rats.

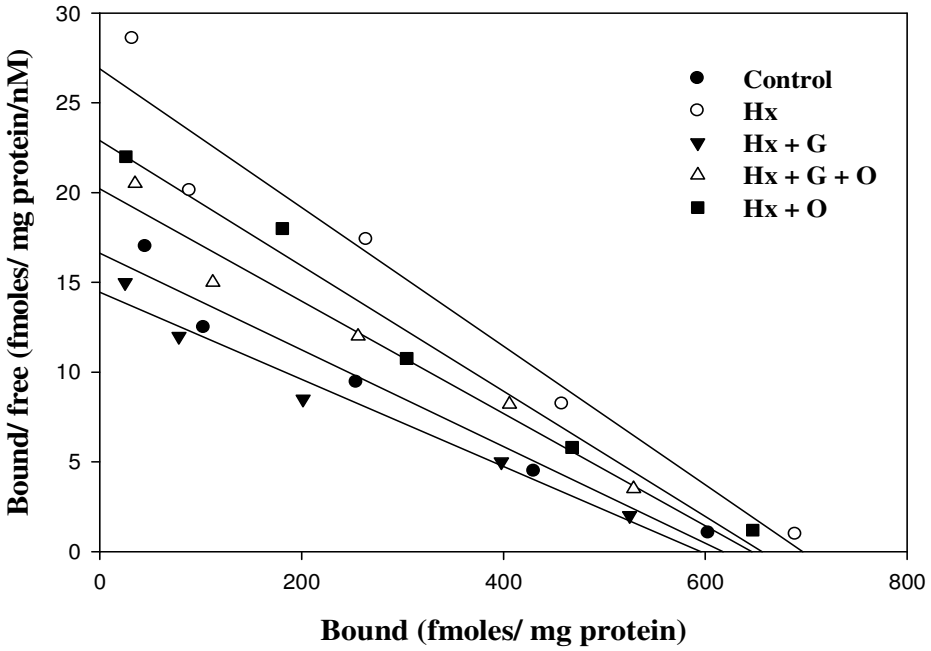


Table- 64
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the cerebellum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	616.62 ± 4.1	37.55 ± 1.14
Hx	696.61 ± 5.5 [a]	26.01 ± 1.04 [a]
Hx + G	593.41 ± 3.8 [b]	40.53 ± 1.10 [a, b]
Hx + G + O	636.50 ± 4.2 [b]	31.42 ± 1.15 [a, b]
Hx + O	653.42 ± 4.3 [a, b]	28.47 ± 1.24 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 61
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the cerebellum of control and experimental neonatal rats.

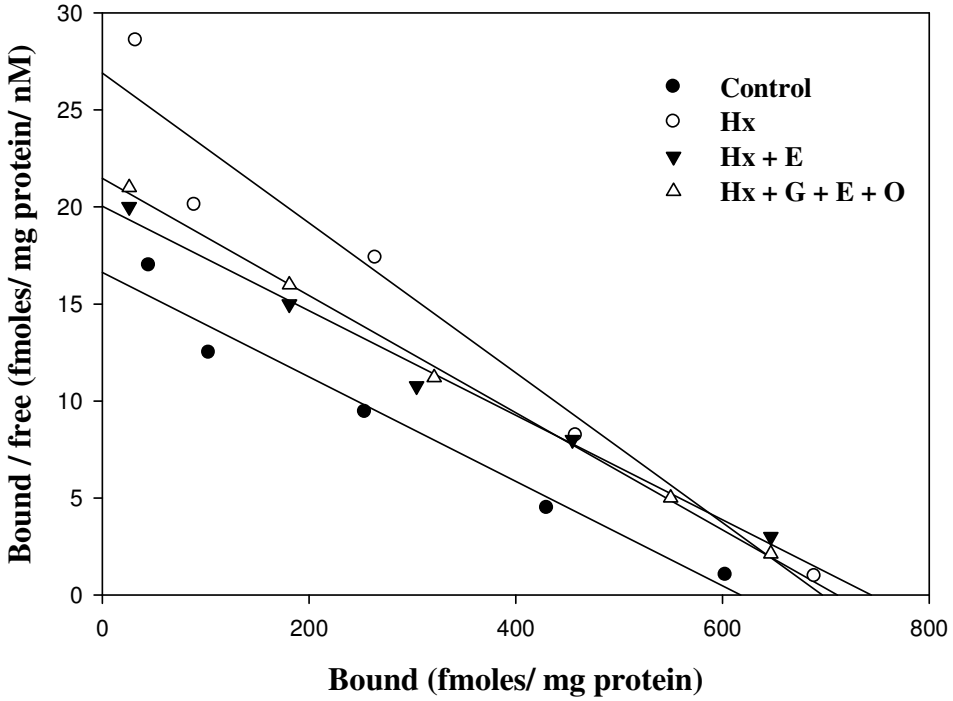


Table- 65
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the cerebellum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	616.62 ± 4.1	37.55 ± 1.14
Hx	696.61 ± 5.5 [a]	26.01 ± 1.04 [a]
Hx + E	743.36 ± 6.5 [a, b]	37.17 ± 1.10 [b]
Hx + G + E + O	708.32 ± 5.8 [a, b]	33.05 ±1.12 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 62

Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the cerebellum of control and experimental neonatal rats.

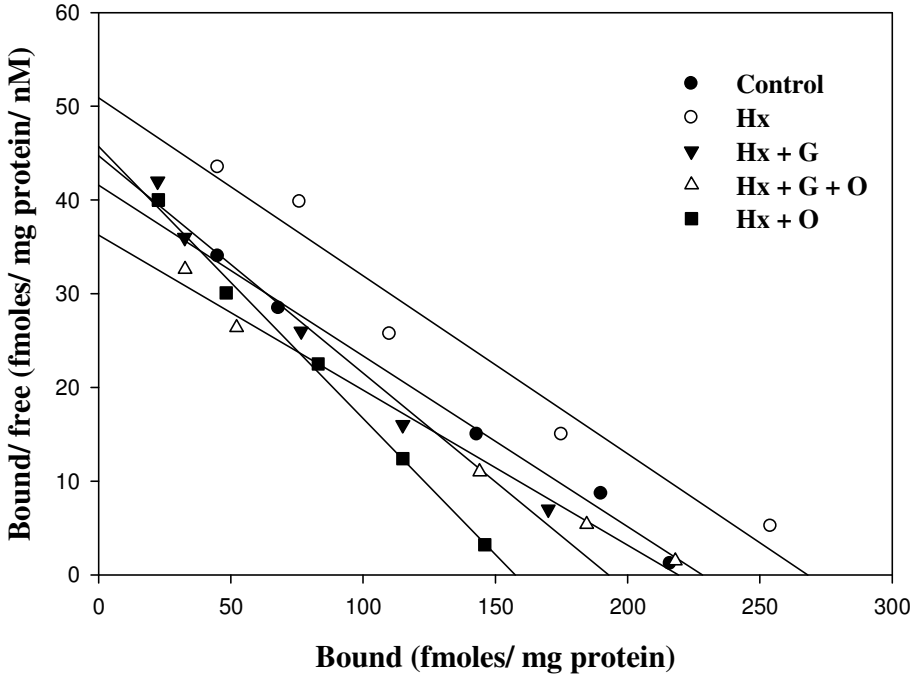


Table- 66

Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the cerebellum of control and experimental neonatal rats.

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	220.04±1.50	0.86±0.01
Hx	264.90± 1.67 [a]	0.56±0.05 [a]
Hx + G	196.45±1.25 [b]	0.55±0.05 [a]
Hx + G + O	220.84±.1.74 [b]	0.64±0.04 [a]
Hx + O	153.04±1.55 [a]	0.94±0.03 [b]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats.
[a] $p<0.001$ when compared with control.
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 63

Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the cerebellum of control and experimental neonatal rats.

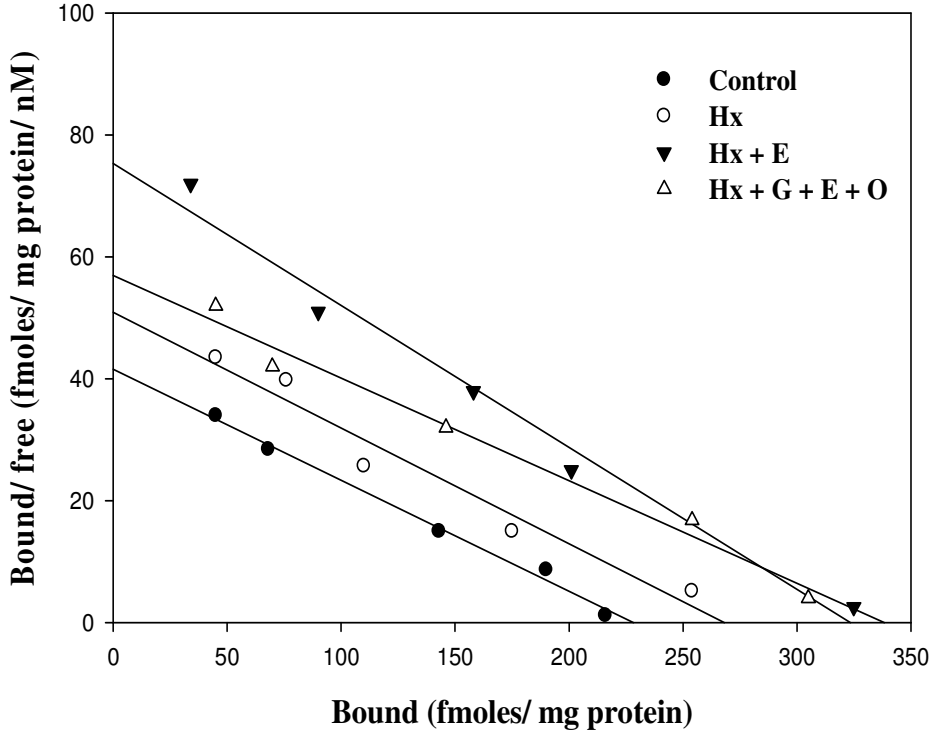


Table- 67

Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the cerebellum of control and experimental neonatal rats.

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	220.04±1.50	0.86±0.01
Hx	264.90± 1.67 [a]	0.56±0.05 [a]
Hx + E	328.30±1.55 [a]	0.80±0.05 [b]
Hx + G + E + O	313.20±1.60 [a]	0.63±0.06 [c]

Values are mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats.
[a] $p<0.001$ when compared with control.
[b]$p<0.001$, [c] $p<0.05$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 64
Real Time PCR amplification of 5-HT_{2A} receptor subunit mRNA from the cerebellum of control and experimental neonatal rats

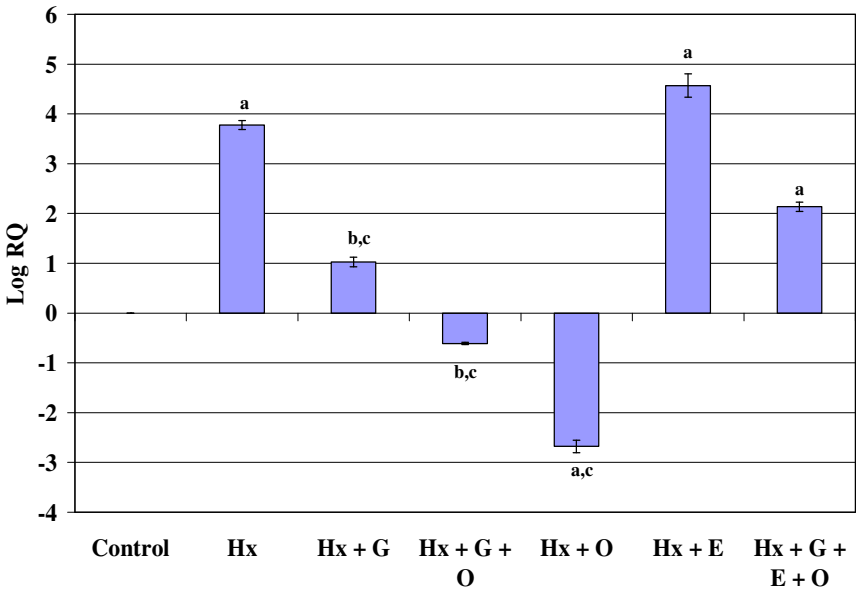


Table- 68
Real Time PCR amplification of 5-HT_{2A} receptor subunit mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	3.78 ± 0.09 [a]
Hx + G	1.02 ± 0.10 [b, c]
Hx + G + O	-0.61 ± 0.02 [b, c]
Hx + O	-2.68 ± 0.12 [a, c]
Hx + E	4.57 ± 0.24 [a]
Hx + G + E + O	2.13 ± 0.10 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.05$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 65
Real Time PCR amplification of 5-HT transporter mRNA from the cerebellum of control and experimental neonatal rats

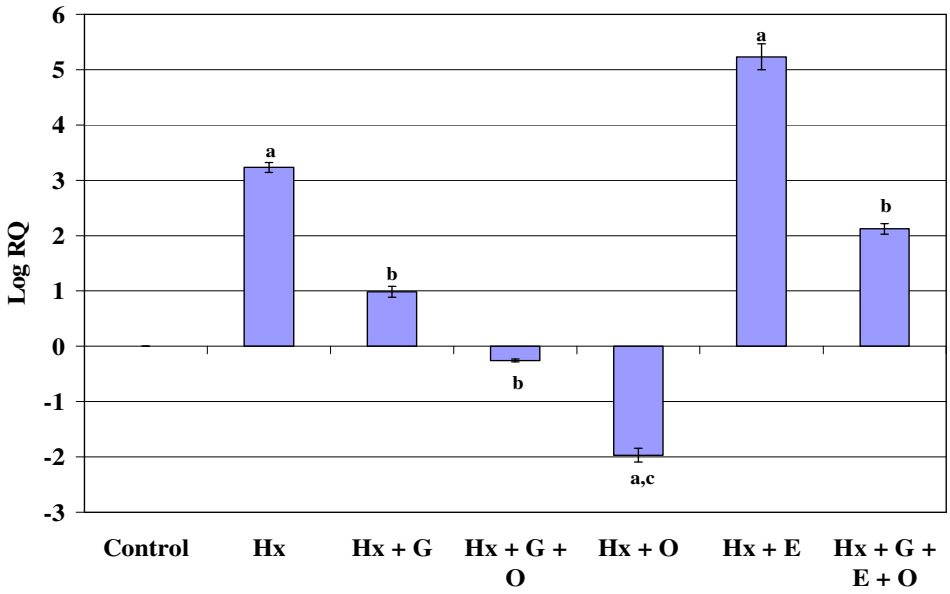
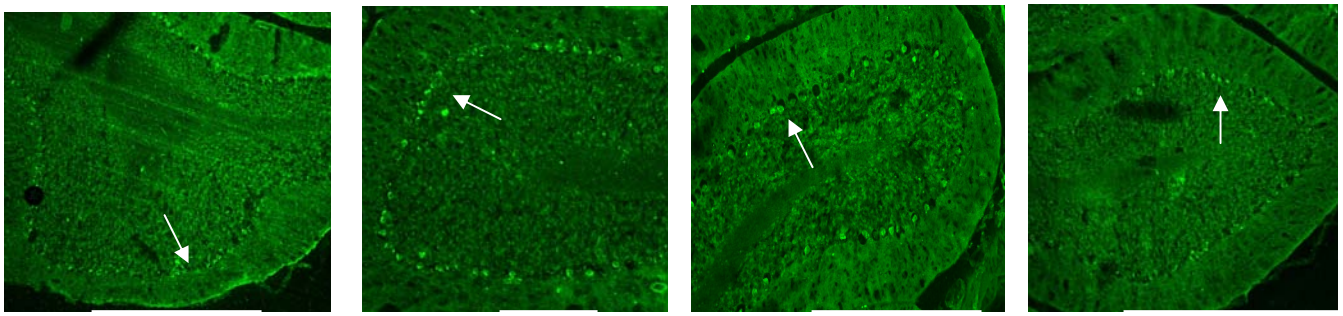


Table- 69
Real Time PCR amplification of 5-HT transporter mRNA from the cerebellum of control and experimental neonatal rats

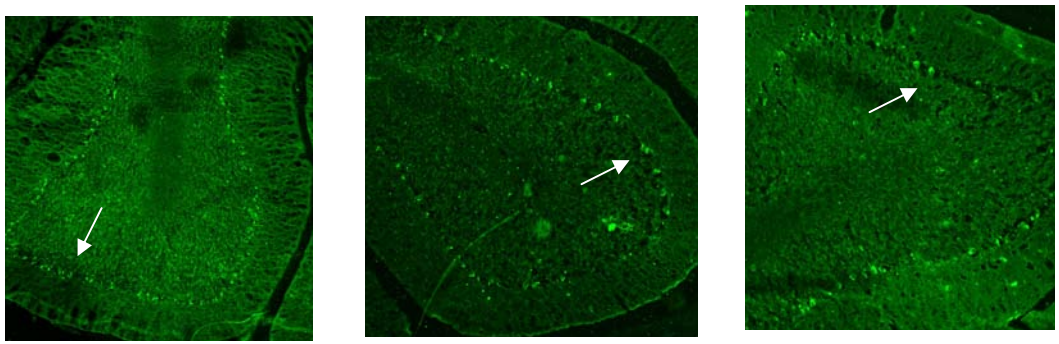
Experimental groups	Log RQ
Control	0
Hx	3.23 ± 0.09 [a]
Hx + G	0.99 ± 0.10 [b]
Hx + G + O	-0.26 ± 0.02 [b]
Hx + O	-1.97 ± 0.12 [a, c]
Hx + E	5.23 ± 0.24 [a]
Hx + G + E + O	2.12 ± 0.10 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ [c] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 66
5-HT_{2A} receptor expression in the cerebellum of control and experimental neonatal rats



Control **Hx** **Hx + G** **Hx + G + O**



Hx + O **Hx + E** **Hx + G + E + O**

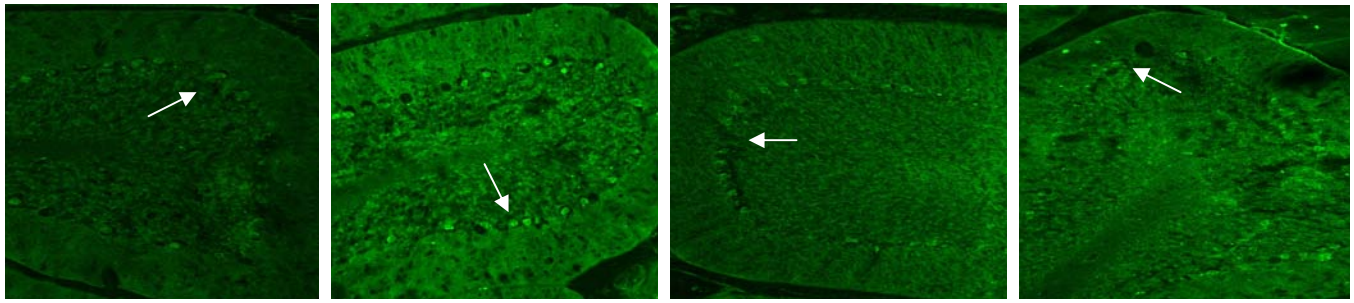
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table- 70
5-HT_{2A} receptor expression in the cerebellum of control and experimental neonatal rats

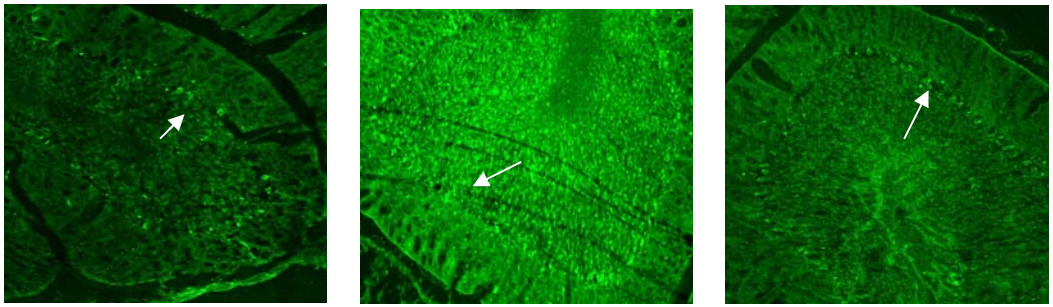
Experimental groups	Mean Pixel intensity
Control	46.78 ± 5.50
Hx	68.24 ± 8.25 [a]
Hx + G	40.35 ± 4.55 [b]
Hx + G + O	44.25 ± 5.05 [b]
Hx + O	58.25 ± 6.20
Hx + E	65.43 ± 8.50 [a]
Hx + G + E + O	62.01 ± 9.01 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 67
5-HT transporter expression in the cerebellum of control and experimental neonatal rats



Control **Hx** **Hx + G** **Hx + G + O**



Hx + O **Hx + E** **Hx + G + E + O**

Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table- 71
5-HT transporter expression in the cerebellum of control and experimental neonatal rats

Experimental groups	Mean Pixel intensity
Control	20.05 ± 2.55
Hx	42.13 ± 3.05 [a]
Hx + G	22.50 ± 2.00 [b]
Hx + G + O	20.55 ± 3.12 [b]
Hx + O	49.04 ± 3.25
Hx + E	50.20 ± 3.80 [a]
Hx + G + E + O	43.11 ± 3.50 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 68
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the cerebellum of control and experimental neonatal rats.

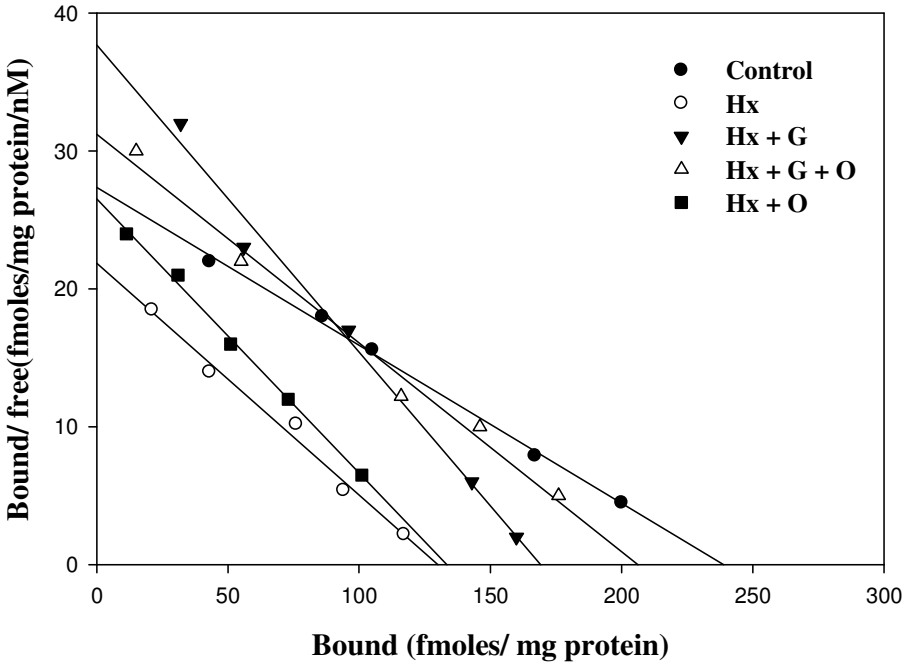


Table- 72
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the cerebellum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	237.54 ± 5.5	8.67 ± 1.20
Hx	130.20 ± 2.5 [a]	5.89 ± 1.01 [a]
Hx + G	169.38 ± 3.0 [b]	4.46 ± 1.20 [a, b]
Hx + G + O	206.28 ± 4.0 [b]	6.54 ± 2.0 [a, b]
Hx + O	133.14 ± 2.0 [a, b]	5.02 ± 1.10 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 69
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the cerebellum of control and experimental neonatal rats.

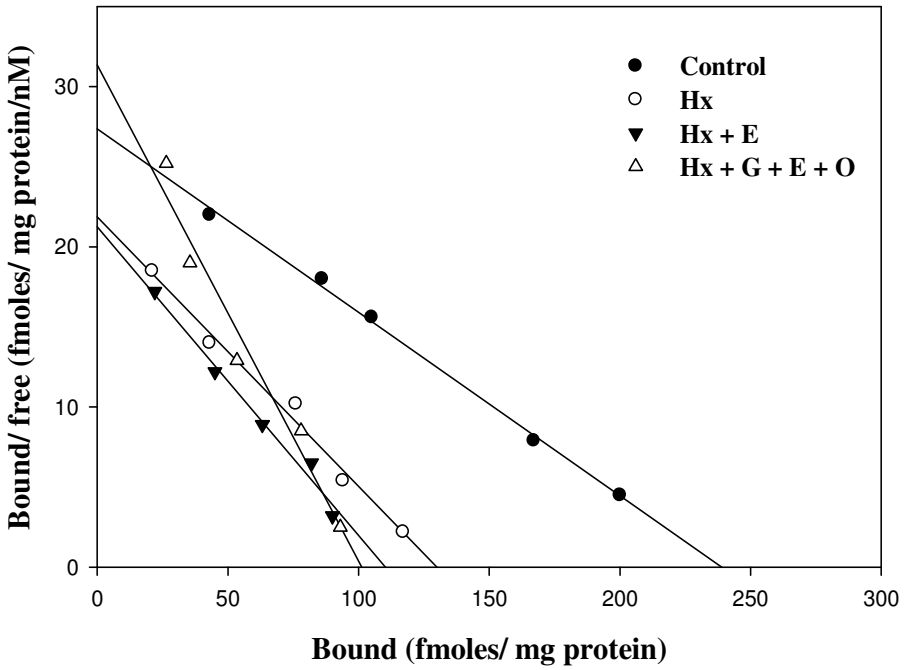


Table- 73
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the cerebellum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	237.54 ± 5.5	8.67 ± 1.20
Hx	130.20 ± 2.5 [a]	5.89 ± 1.01 [a]
Hx + E	108.12 ± 2.6 [a, b]	5.09 ± 1.10 [b]
Hx + G + E + O	100.62 ± 3.5 [a, b]	3.19 ± 1.12 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 70
Real Time PCR amplification of Muscarinic M_1 receptor mRNA from the cerebellum of control and experimental neonatal rats

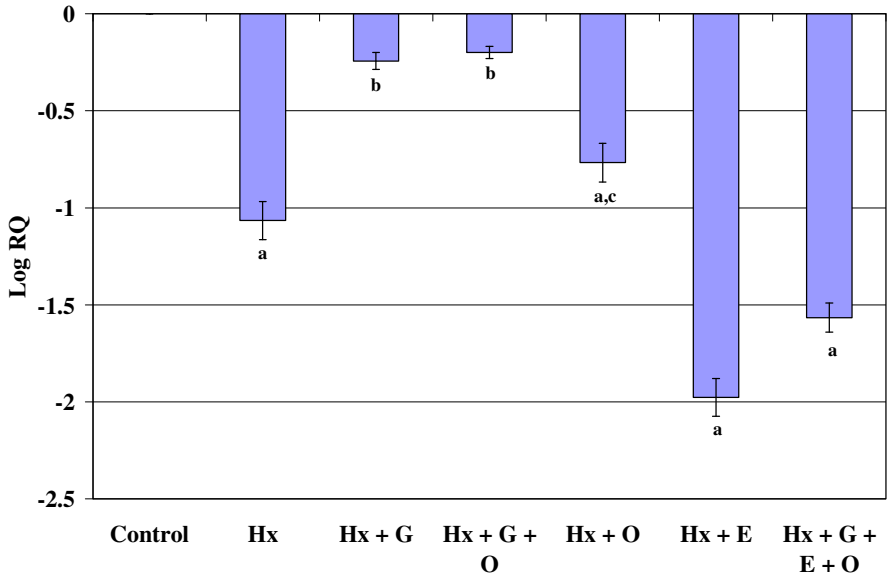


Table- 74
Real Time PCR amplification of Muscarinic M_1 receptor mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.07 ± 0.10 [a]
Hx + G	-0.24 ± 0.04 [b]
Hx + G + O	-0.19 ± 0.03 [b]
Hx + O	-0.77 ± 0.10 [a, c]
Hx + E	-1.98 ± 0.10 [a]
Hx + G + E + O	-1.57 ± 0.08 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.05$ when compared to hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 71
Real Time PCR amplification of Muscarinic M_2 receptor mRNA from the cerebellum of control and experimental neonatal rats

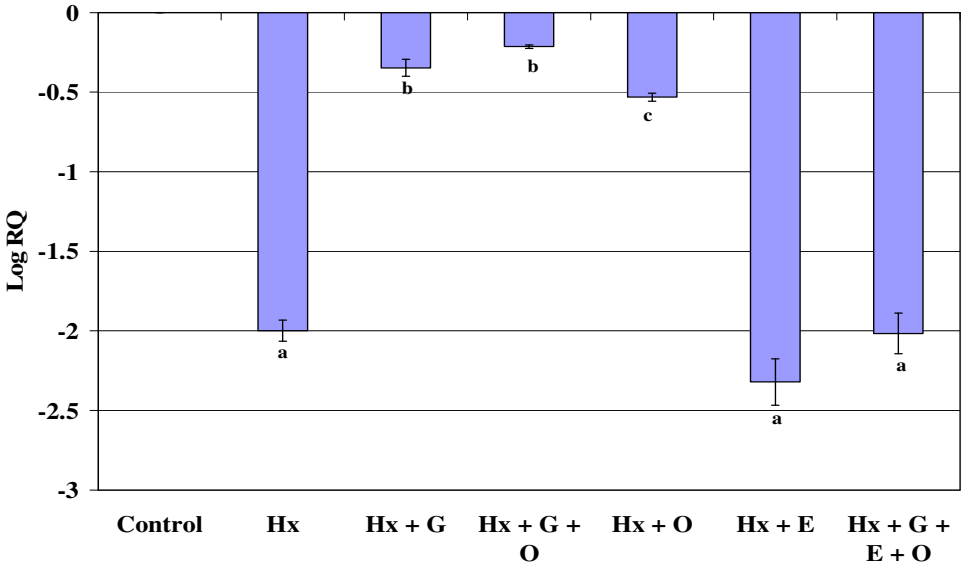


Table- 75
Real Time PCR amplification of Muscarinic M_2 receptor mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.99 ± 0.07 [a]
Hx + G	-0.35 ± 0.05 [b]
Hx + G + O	-0.21 ± 0.01 [b]
Hx + O	-0.53 ± 0.02 [c]
Hx + E	-2.32 ± 0.15 [a]
Hx + G + E + O	-2.02 ± 0.13 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$when compared to hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 72
Real Time PCR amplification of Muscarinic M_3 receptor mRNA from the cerebellum of control and experimental neonatal rats

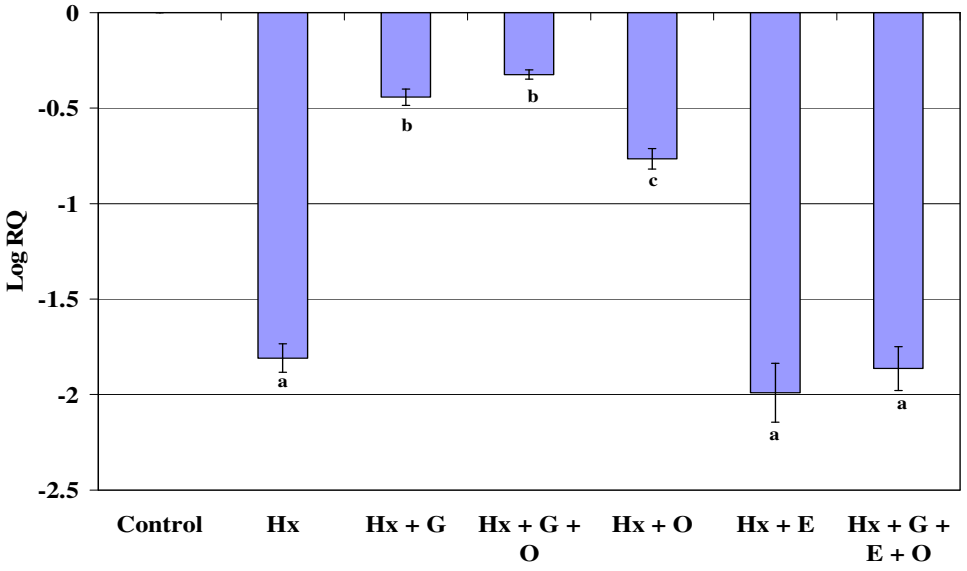


Table- 76
Real Time PCR amplification of Muscarinic M_2 receptor mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.81 ± 0.07 [a]
Hx + G	-0.44 ± 0.04 [b]
Hx + G + O	-0.32 ± 0.02 [b]
Hx + O	-0.77 ± 0.05 [c]
Hx + E	-1.99 ± 0.15 [a]
Hx + G + E + O	-1.86 ± 0.11 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 73
Real Time PCR amplification of acetylcholine esterase mRNA from the cerebellum of control and experimental neonatal rats.

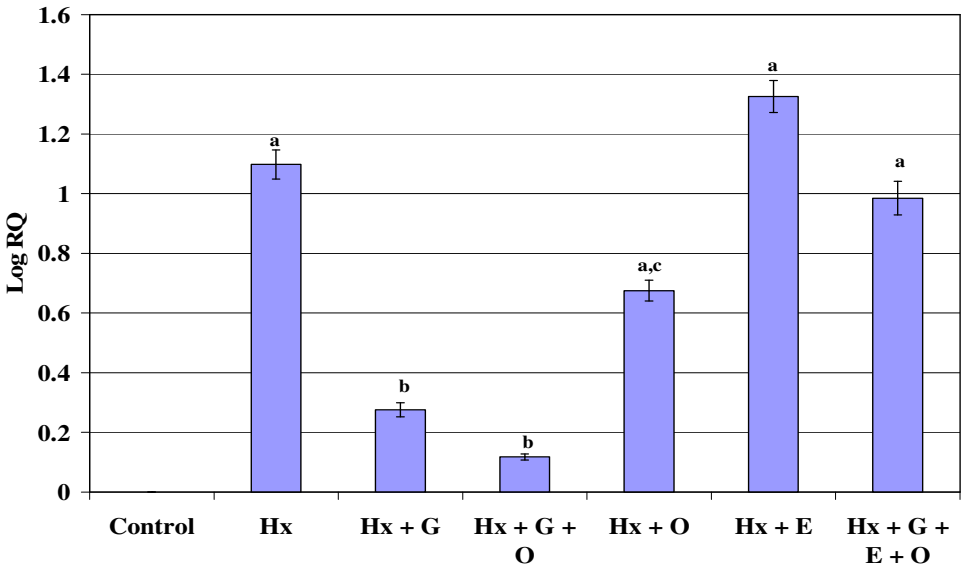


Table- 77
Real Time PCR amplification of acetylcholine esterase mRNA from the cerebellum of control and experimental neonatal rats.

Experimental groups	Log RQ
Control	0
Hx	1.09 ± 0.05 [a]
Hx + G	0.28 ± 0.02 [b]
Hx + G + O	0.12 ± 0.01 [b]
Hx + O	0.67 ± 0.03 [a, c]
Hx + E	1.33 ± 0.05 [a]
Hx + G + E + O	0.98 ± 0.06 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.05$ when compared to hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 74

Real Time PCR amplification of choline acetyl transferase mRNA from the cerebellum of control and experimental neonatal rats.

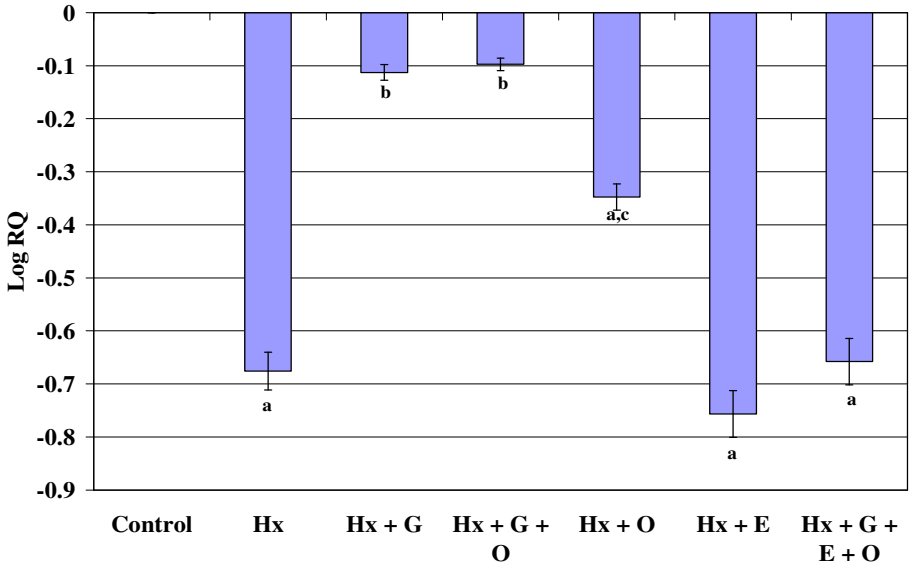


Table- 78

Real Time PCR amplification of acetylcholine esterase mRNA from the cerebellum of control and experimental neonatal rats.

Experimental groups	Log RQ
Control	0
Hx	-0.68 ± 0.05 [a]
Hx + G	-0.11 ± 0.02 [b]
Hx + G + O	-0.10 ± 0.01 [b]
Hx + O	-0.35 ± 0.03 [a, c]
Hx + E	-0.76 ± 0.05 [a]
Hx + G + E + O	-0.66 ± 0.06 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.05$ when compared to hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 75
Real Time PCR amplification of Hif 1 mRNA from the cerebellum of control and experimental neonatal rats.

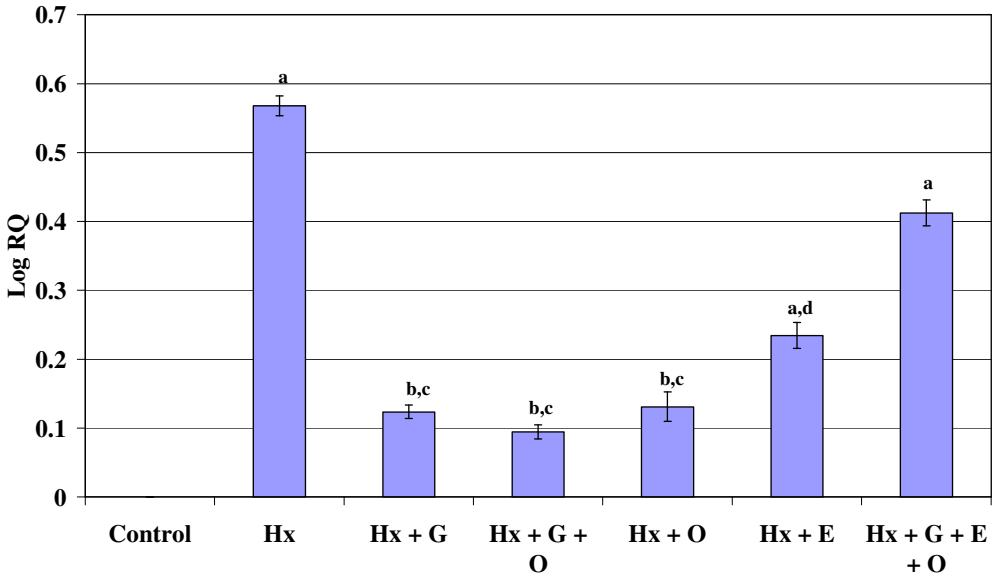


Table- 79
Real Time PCR amplification of Hif 1 mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	0.57 ± 0.01 [a]
Hx + G	0.12 ± 0.01 [b, c]
Hx + G + O	0.09 ± 0.01 [b, c]
Hx + O	0.13 ± 0.02 [b, c]
Hx + E	0.23 ± 0.02 [a]
Hx + G + E + O	0.41 ± 0.02 [a, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.05$ when compared to Control
[b] $p<0.001$, [d] $p<0.05$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 76
Real Time PCR amplification of SOD mRNA from the cerebellum of control and experimental neonatal rats.

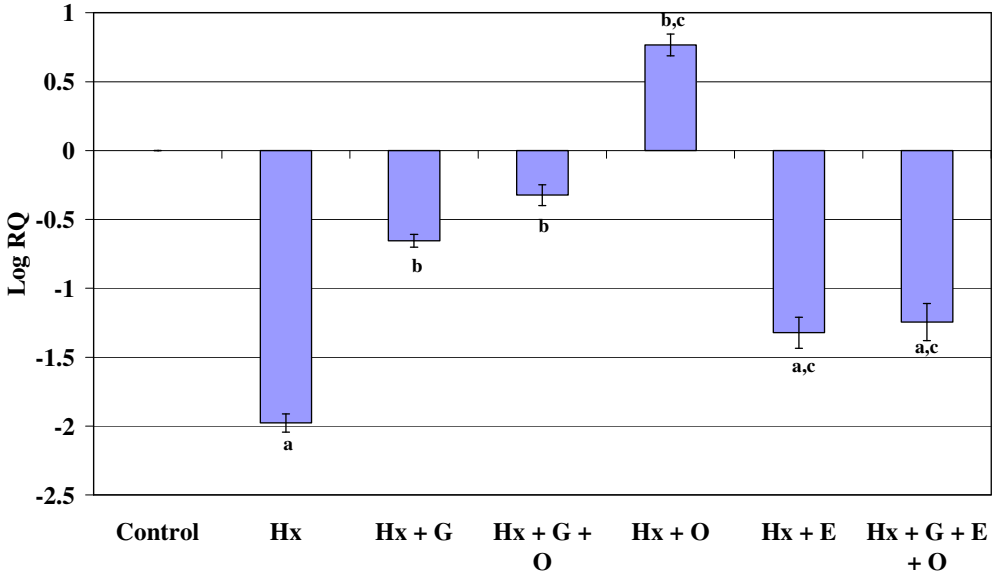


Table- 80
Real Time PCR amplification of SOD mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.98 ± 0.07 [a]
Hx + G	-0.66 ± 0.05 [b]
Hx + G + O	-0.32 ± 0.08 [b]
Hx + O	0.77 ± 0.08 [b, c]
Hx + E	-1.32 ± 0.11 [a, c]
Hx + G + E + O	-1.25 ± 0.13 [a, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.01$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 77
Real Time PCR amplification of GPx mRNA from the cerebellum of control and experimental neonatal rats.

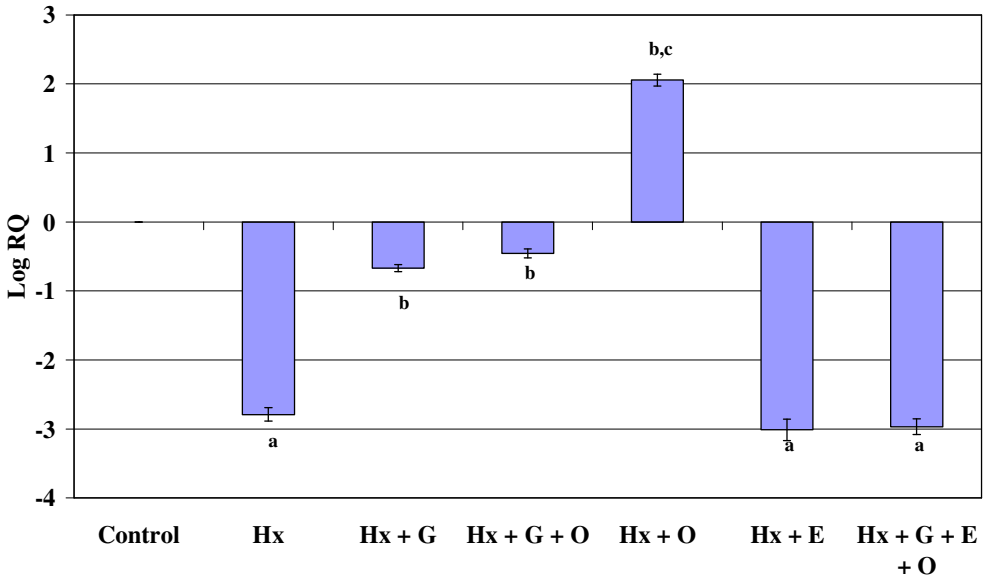


Table- 81
Real Time PCR amplification of GPx mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-2.79 ± 0.10 [a]
Hx + G	-0.67 ± 0.05 [b]
Hx + G + O	-0.46 ± 0.07 [b]
Hx + O	2.06 ± 0.09 [b, c]
Hx + E	-3.01 ± 0.15 [a]
Hx + G + E + O	-2.97 ± 0.11 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.01$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 78
Real Time PCR amplification of BAX mRNA from the cerebellum of control and experimental neonatal rats

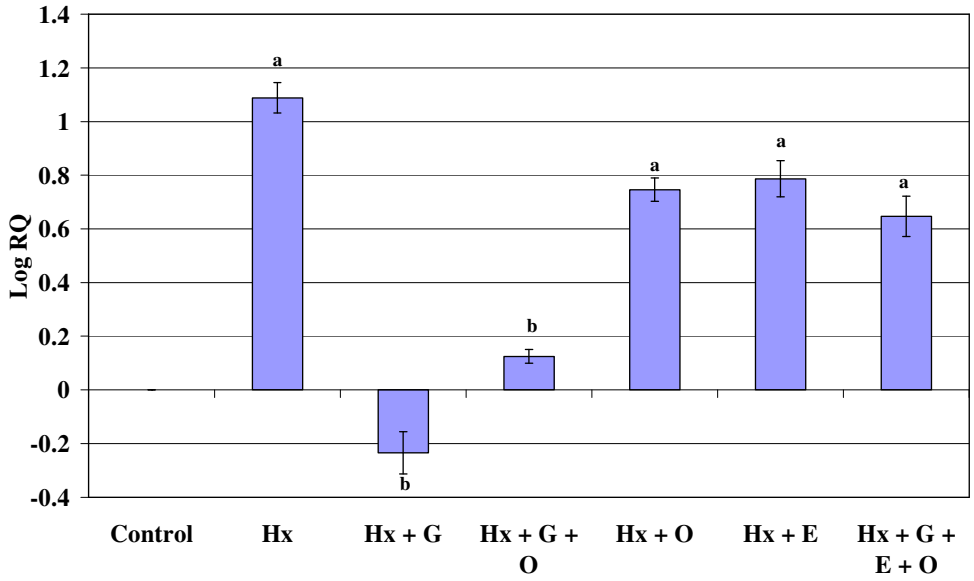


Table- 82
Real Time PCR amplification of BAX mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	1.09 ± 0.06 [a]
Hx + G	-0.23 ± 0.08 [b]
Hx + G + O	0.12 ± 0.03 [b]
Hx + O	0.75 ± 0.04 [a]
Hx + E	0.79 ± 0.07 [a]
Hx + G + E + O	0.65 ± 0.08 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 79
Real Time PCR amplification of CREB mRNA from the cerebellum of control and experimental neonatal rats

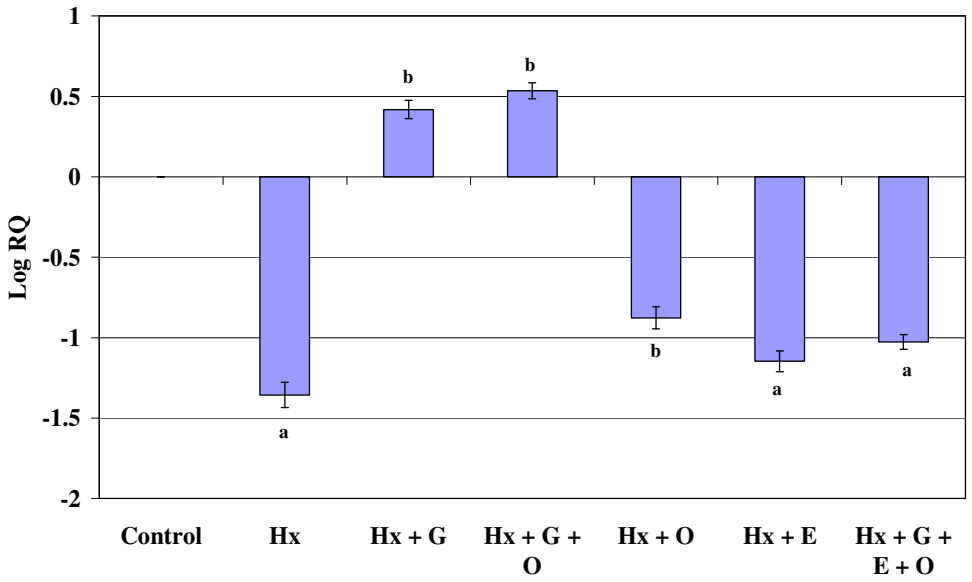


Table- 83
Real Time PCR amplification of CREB mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.36 ± 0.06 [a]
Hx + G	0.42 ± 0.08 [b]
Hx + G + O	0.53 ± 0.03 [b]
Hx + O	-0.88 ± 0.04 [b]
Hx + E	-1.15 ± 0.07 [a]
Hx + G + E + O	-1.03 ± 0.08 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 80
Real Time PCR amplification of phospholipase C mRNA from the cerebellum of control and experimental neonatal rats.

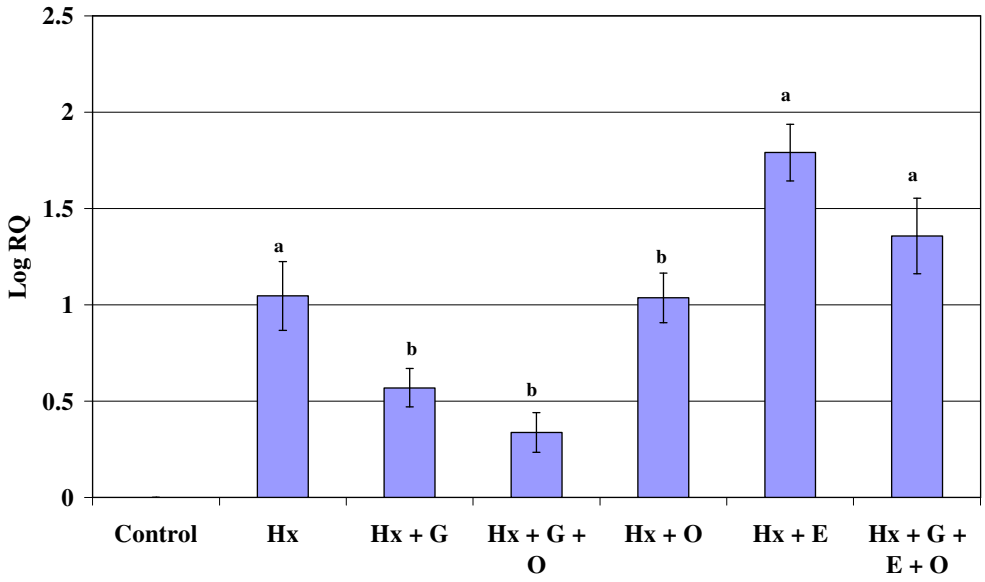


Table- 84
Real Time PCR amplification of phospholipase C mRNA from the cerebellum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	1.05 ± 0.18 [a]
Hx + G	0.57 ± 0.10 [b]
Hx + G + O	0.34 ± 0.10 [b]
Hx + O	1.04 ± 0.13 [b]
Hx + E	1.79 ± 0.15 [a]
Hx + G + E + O	1.36 ± 0.20 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 81
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the brain stem of control and experimental neonatal rats.

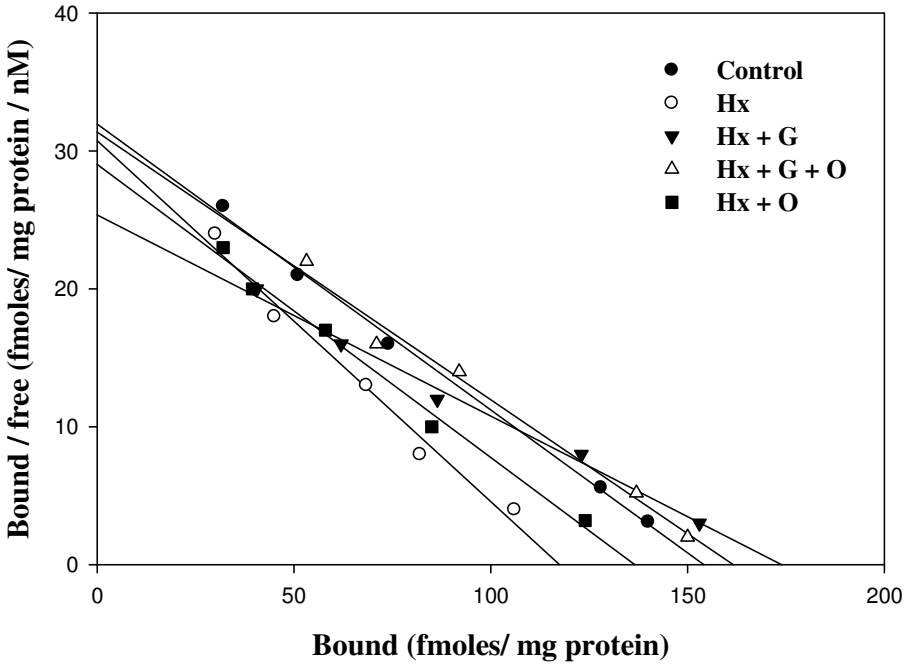


Table- 85
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the brain stem of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	153.36 ± 3.7	4.77 ± 0.44
Hx	116.68 ± 2.8 [a]	3.77 ± 0.22 [a]
Hx + G	173.36 ± 2.5 [b]	6.78 ± 0.35 [a, b]
Hx + G + O	160.84 ± 3.4 [b]	5.01 ± 0.26 [a, b]
Hx + O	136.68 ± 2.3 [a, b]	4.73 ± 0.29 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 82
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the brain stem of control and experimental neonatal rats.

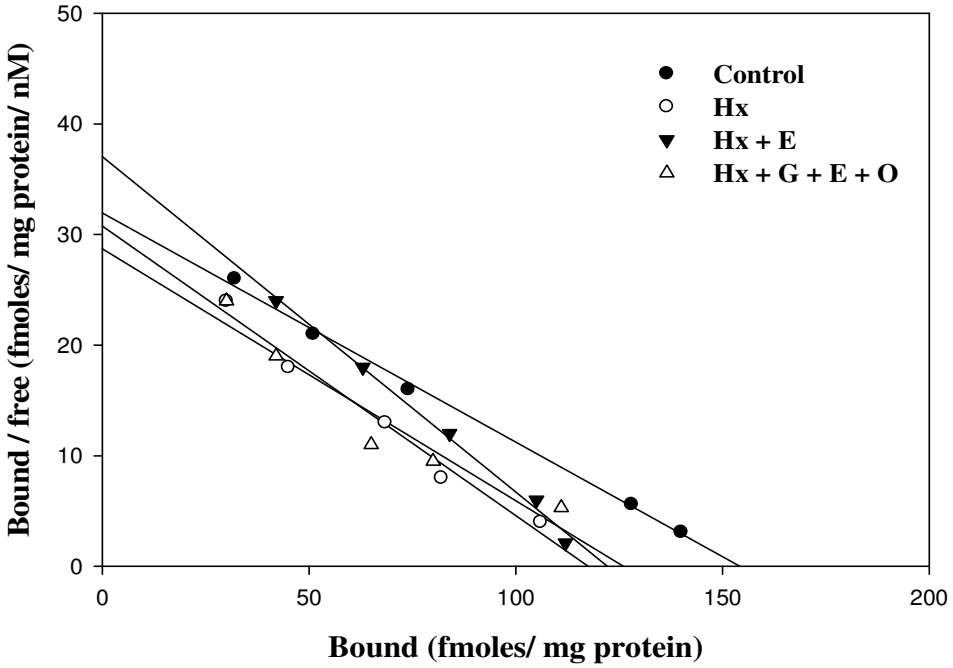


Table- 86
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the brain stem of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	153 .36 ± 3.7	4.77 ± 0.44
Hx	116.68 ± 2.8 [a]	3.77 ± 0.22 [a]
Hx + E	122.08 ± 2.6 [a]	3.30 ± 0.14 [a]
Hx + G + E + O	125.84 ± 4.5 [a]	4.10 ± 0.22 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure - 83

Binding parameters of [^{3}H] GABA against GABA in the brain stem of control and experimental neonatal rats.

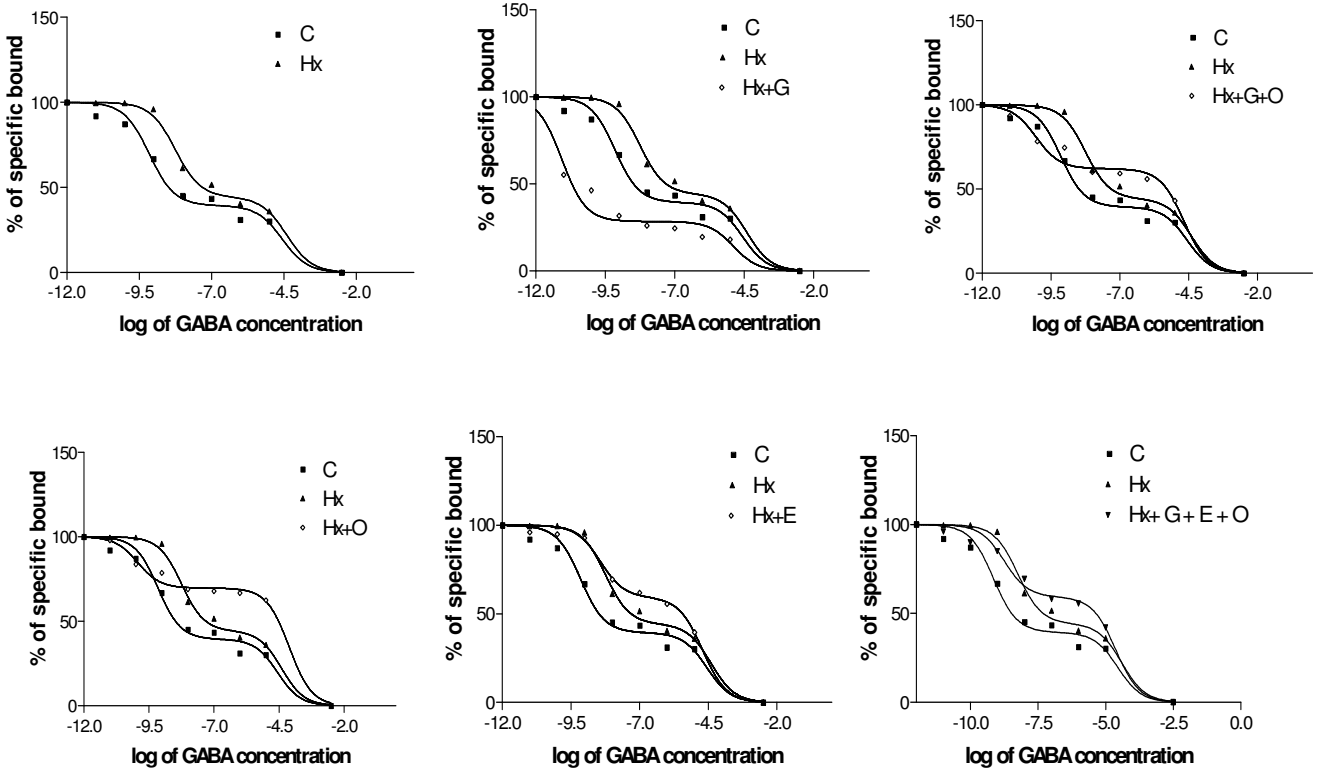


Table - 87

Binding parameters of [^{3}H] GABA against GABA in the brain stem of control and experimental neonatal rats.

Group	Best-fit model	Log (EC_{50})-1	Log (EC_{50})-2	Ki $_{(H)}$	Ki $_{(L)}$	Hill slope
C	Two- site	-9.147	-4.550	3.3090x 10^{-9}	1.3080x 10^{-5}	- 0.8832
Hx	Two- site	-10.243	-5.398	2.6540 x 10^{-10}	1.8560 x 10^{-6}	- 0.7944
Hx + O	Two- site	-9.927	-4.906	4.4880 x 10^{-9}	3.6380 x 10^{-5}	- 0.6926
Hx + G	Two- site	-9.06	-4.780	4.0210 x 10^{-12}	6.1230 x 10^{-5}	- 0.6522
Hx + G + O	Two- site	-9.05	-4.667	4.1740 x 10^{-11}	9.9930 x 10^{-5}	- 0.7831
Hx + E	Two- site	-11.432	-5.708	1.7180 x 10^{-10}	9.0940 x 10^{-6}	- 0.6957
Hx + G + E + O	Two- site	-11.564	-5.654	1.7286 x 10^{-10}	9.0223 x 10^{-6}	- 0.6964

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 84
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the brain stem of control and experimental neonatal rats.

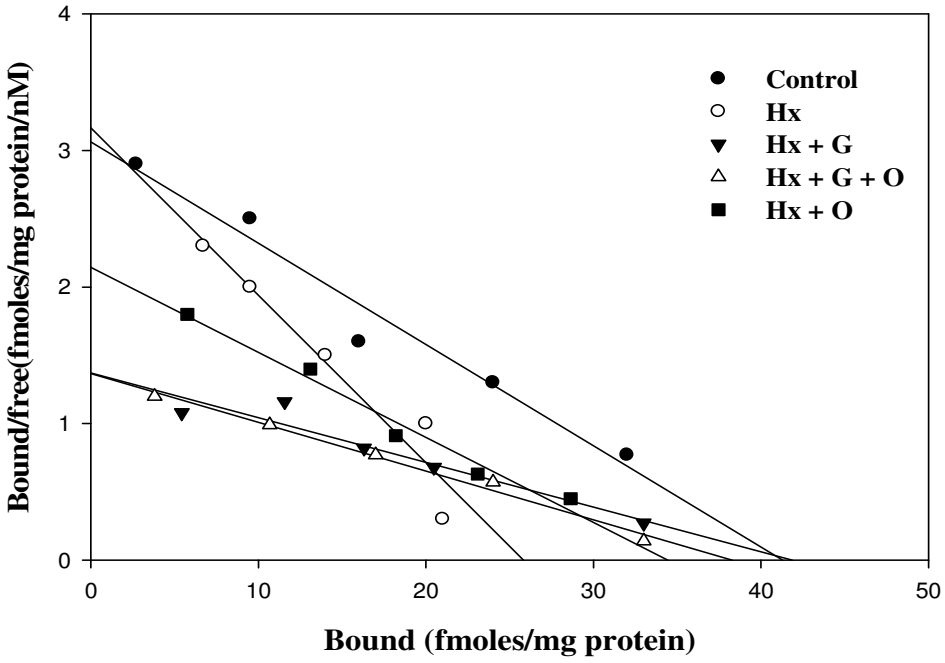


Table- 88
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the brain stem of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	40.94 ± 2.6	13.47 ±1.20
Hx	25.73 ± 1.5 [a]	8.07 ± 1.02 [a]
Hx + G	41.77 ± 2.6 [b]	30.27 ± 2.12 [a, b]
Hx + G + O	38.13 ± 3.0 [b]	27.24 ± 2.00 [a, b]
Hx + O	34.27 ± 2.0 [a, b]	15.94 ± 1.34 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 85
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the brain stem of control and experimental neonatal rats.

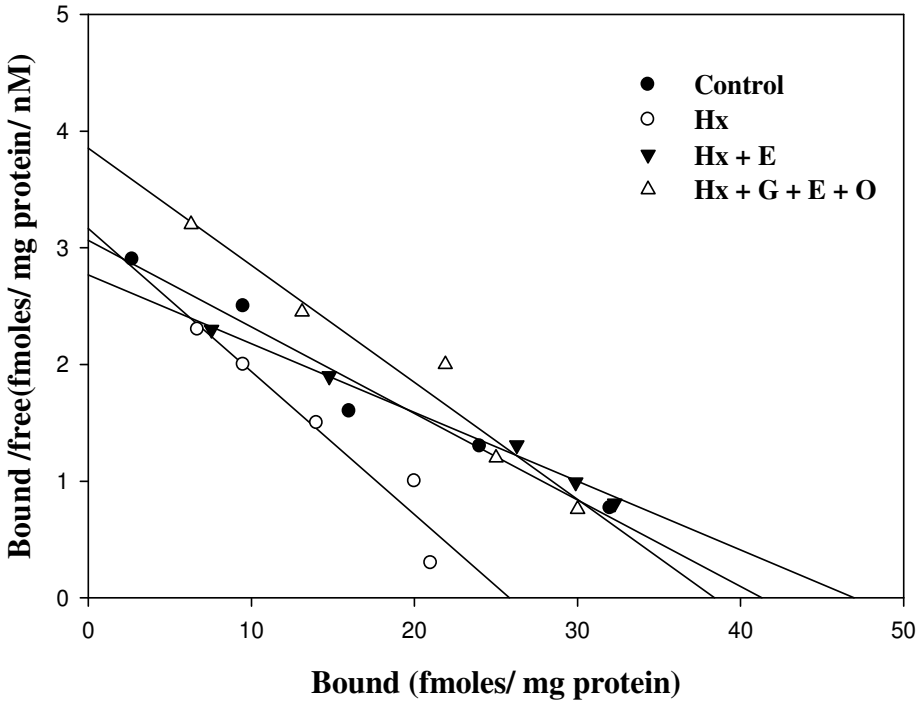


Table- 89
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the brain stem of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	40.94 ± 2.6	13.47 ±1.20
Hx	25.73 ± 1.5 [a]	8.07± 1.02 [a]
Hx + E	46.67 ± 2.6 [a, b]	16.85±1.85 [b]
Hx + G + E + O	38.23 ± 4.5 [a, b]	9.85±1.20 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 86
Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the brain stem of control and experimental neonatal rats.

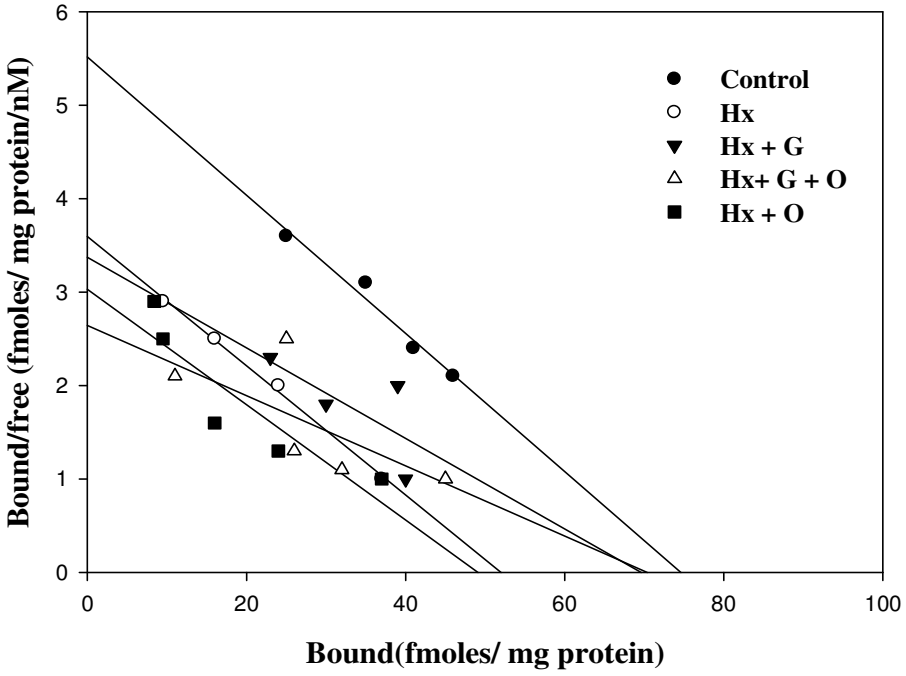


Table- 90
Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the brain stem of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	74.27 ±1.20	13.31 ±1.00
Hx	51.84 ± 1.50 [a]	14.44 ±0.99 [b]
Hx + G	69.41 ±1.40 [b]	20.47 ±0.99 [a]
Hx + G + O	70.47 ±1.10 [c]	26.10 ±1.20 [a]
Hx + O	49.10 ±1.10 [a]	16.36 ±1.50 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.05$ when compared with control
[d] $p<0.001$when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 87
Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the brain stem of control and experimental neonatal rats.

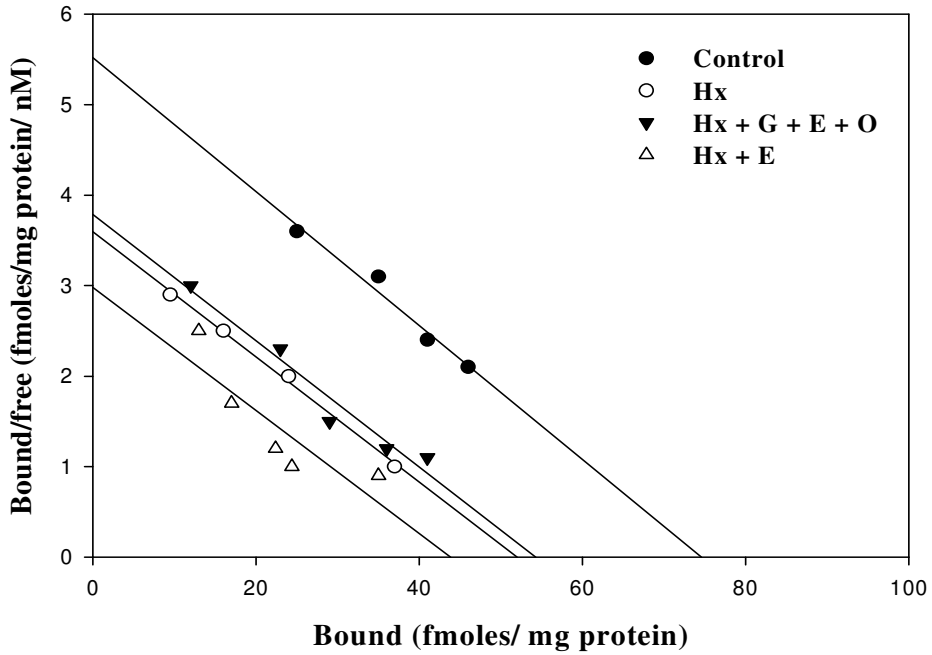


Table- 91
Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the brain stem of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	74.27 ±1.2	13.31 ±1.0
Hx	51.84 ± 1.5 [a]	14.44 ± 0.99 [b]
Hx + E	43.59 ±1.5 [a]	14.53 ± 0.99 [b]
Hx + G + E + O	53.95 ±1.5 [a]	13.90 ± 0.99 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared with control
[d] $p<0.001$, [e] $p<0.05$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 88
Real Time PCR amplification of $GABA_{A\acute{u}1}$ receptor subunit mRNA from the brain stem of control and experimental neonatal rats

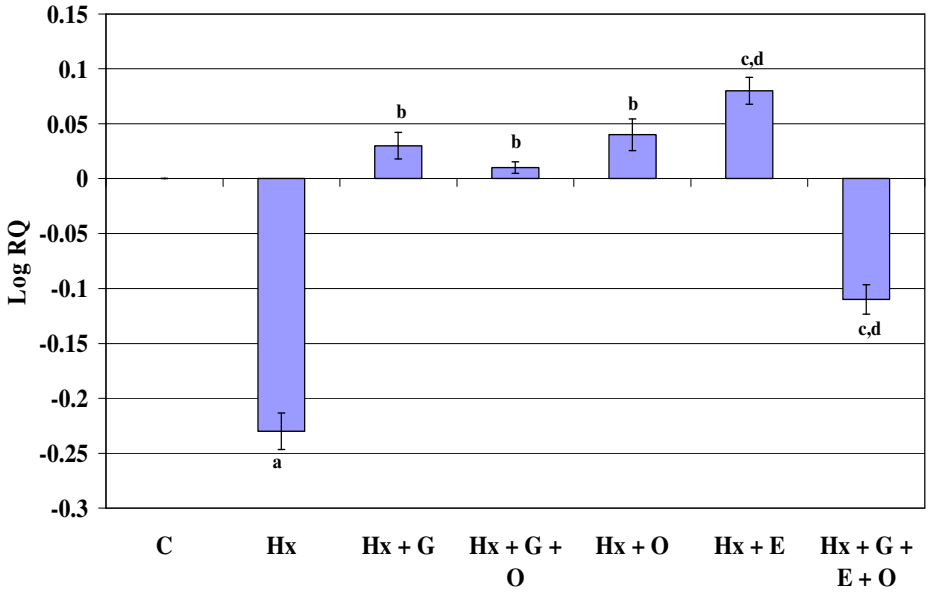


Table- 92
Real Time PCR amplification of $GABA_{A\acute{u}1}$ receptor subunit mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.23 ± 0.02 [a]
Hx + G	0.03 ± 0.01 [b]
Hx + G + O	0.01 ± 0.005 [b]
Hx + O	0.04 ± 0.01 [b]
Hx + E	0.08 ± 0.01 [c, d]
Hx + G + E + O	-0.11 ± 0.01 [c, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ [c] $p<0.05$ when compared to Control
[b] $p<0.001$ [d] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 89
Real Time PCR amplification of $GABA_{A\delta 5}$ receptor subunit mRNA from the brain stem of control and experimental neonatal rats

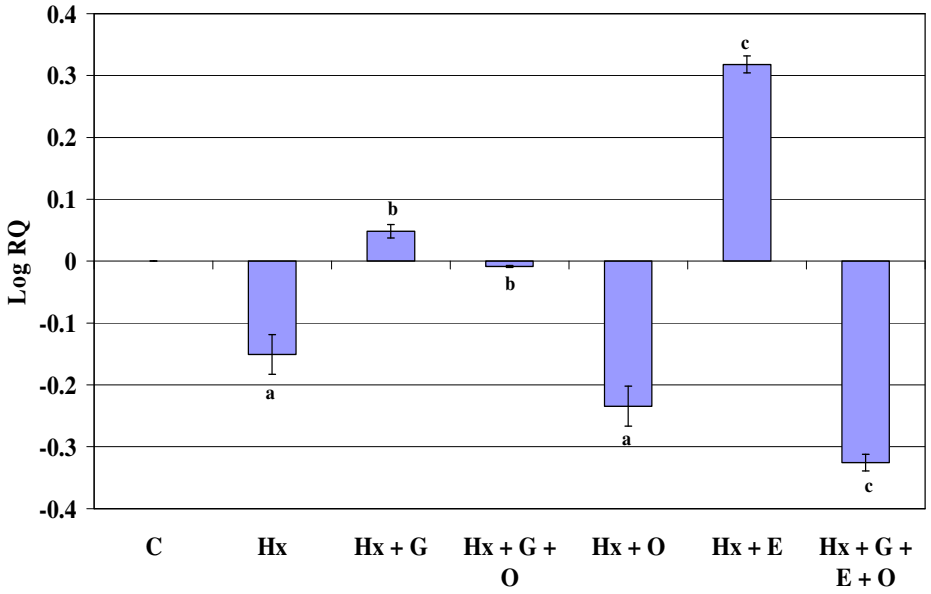


Table- 93
Real Time PCR amplification of $GABA_{A\delta 5}$ receptor subunit mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.15 ± 0.03 [a]
Hx + G	0.05 ± 0.01 [b]
Hx + G + O	-0.009 ± 0.002 [b]
Hx + O	-0.23 ± 0.03 [a]
Hx + E	0.32 ± 0.01 [c]
Hx + G + E + O	-0.33 ± 0.01 [c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.01$, [c] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 90
Real Time PCR amplification of $GABA_{A\gamma3}$ receptor subunit mRNA from the brain stem of control and experimental neonatal rats

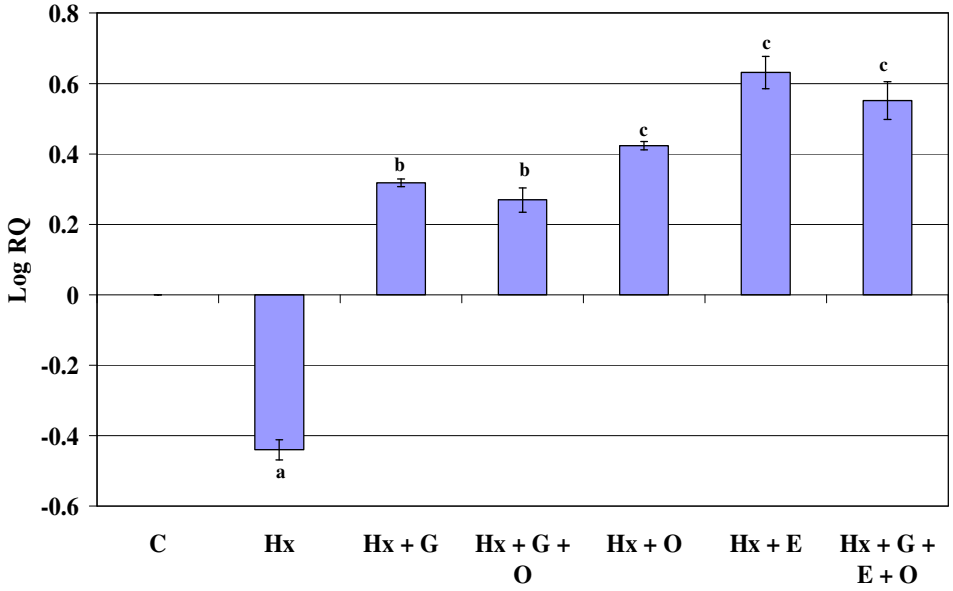


Table- 94
Real Time PCR amplification of $GABA_{A\ \gamma3}$ receptor subunit mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.44 ± 0.02 [a]
Hx + G	0.32 ± 0.01 [b]
Hx + G + O	0.23 ± 0.03 [b]
Hx + O	0.42 ± 0.01 [c]
Hx + E	0.63 ± 0.05 [c]
Hx + G + E + O	0.55 ± 0.05 [c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.01$, [c] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 91
Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the brain stem of control and experimental neonatal rats.

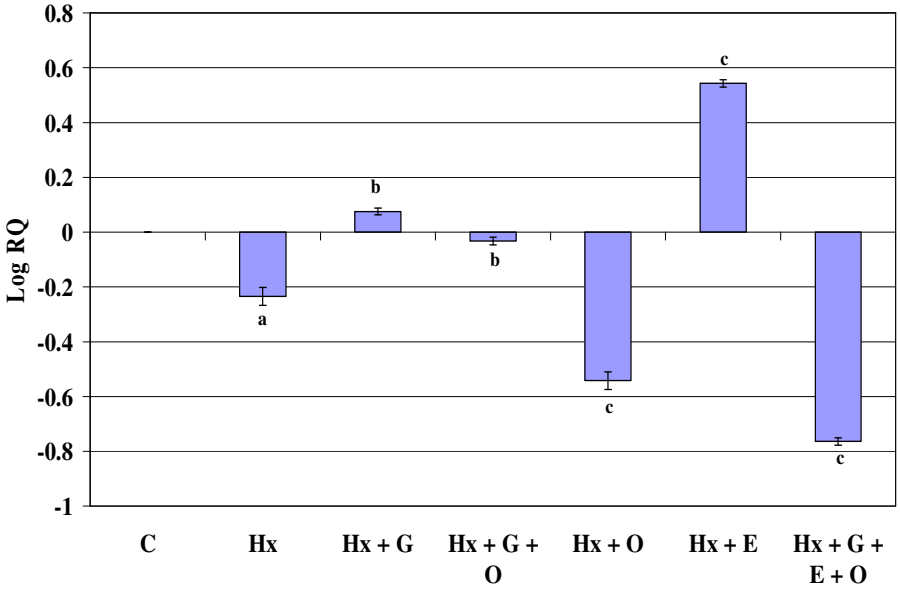


Table- 95
Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.23 ± 0.03 [a]
Hx + G	0.08 ± 0.01 [b]
Hx + G + O	-0.03 ± 0.01 [b]
Hx + O	-0.54 ± 0.03 [c]
Hx + E	0.54 ± 0.01 [c]
Hx + G + E + O	-0.76 ± 0.01 [c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.05$, [c] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 92
Real Time PCR amplification of $GABA_B$ receptor subunit mRNA from the brain stem of control and experimental neonatal rats

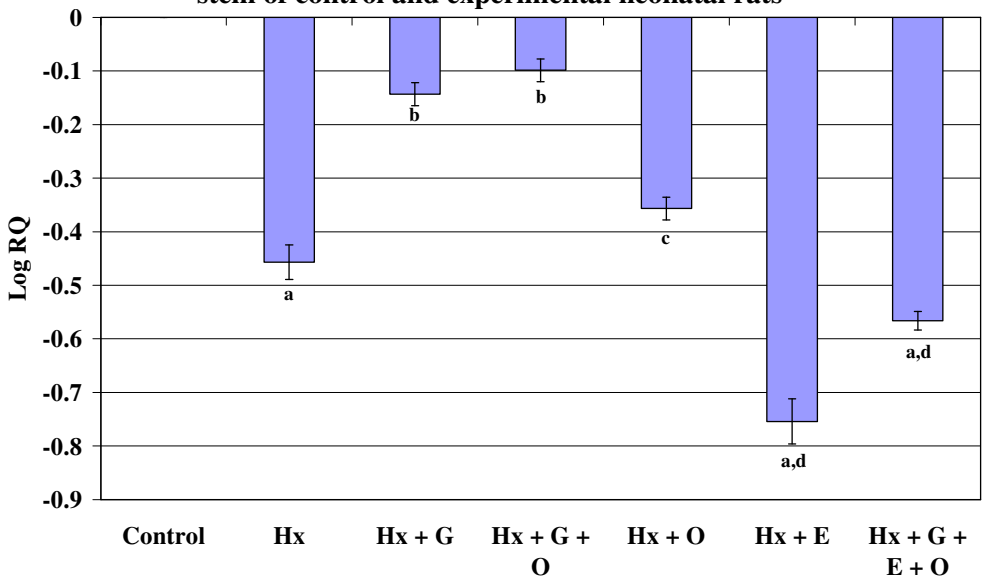


Table- 96
Real Time PCR amplification of $GABA_B$ receptor subunit mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.46 ± 0.03 [a]
Hx + G	-0.14 ± 0.02 [b]
Hx + G + O	-0.10 ± 0.02 [b]
Hx + O	-0.36 ± 0.02 [c]
Hx + E	-0.75 ± 0.04 [a, d]
Hx + G + E + O	-0.57 ± 0.02 [a, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.01$ when compared to Control
[b] $p<0.001$, [d] $p<0.05$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 93
Real Time PCR amplification of GAD mRNA from the brain stem of control and experimental neonatal rats

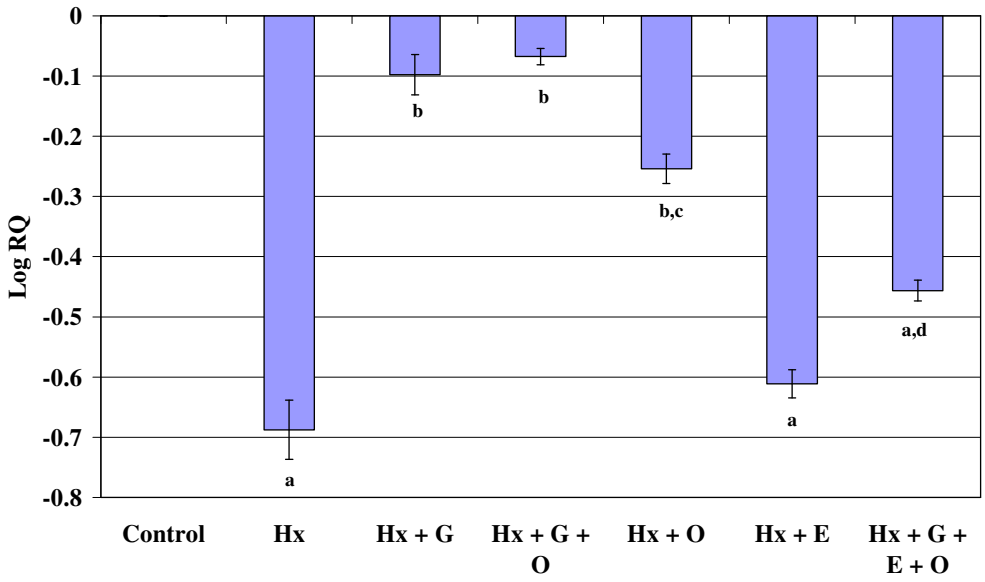


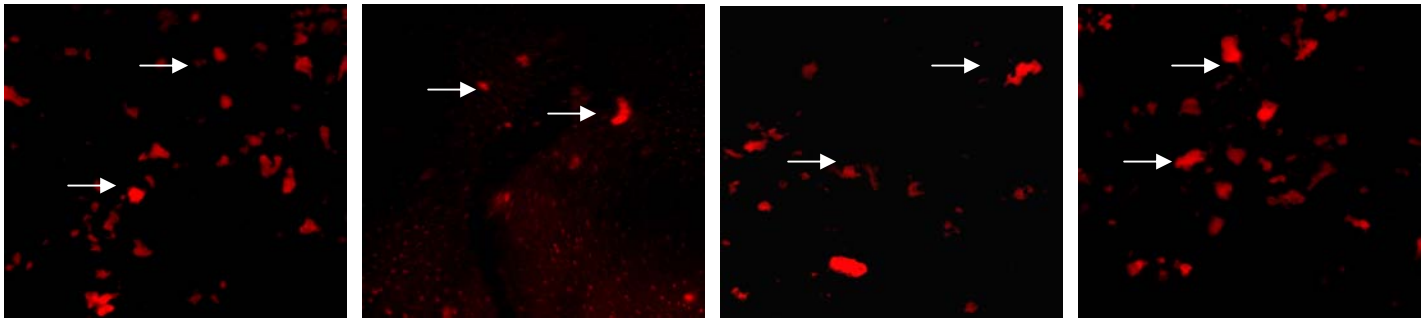
Table- 97
Real Time PCR amplification of GAD mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.69 ± 0.05 [a]
Hx + G	-0.10 ± 0.03 [b]
Hx + G + O	-0.07 ± 0.01 [b]
Hx + O	-0.25 ± 0.02 [b, c]
Hx + E	-0.61 ± 0.02 [a, d]
Hx + G + E + O	-0.46 ± 0.02 [a]

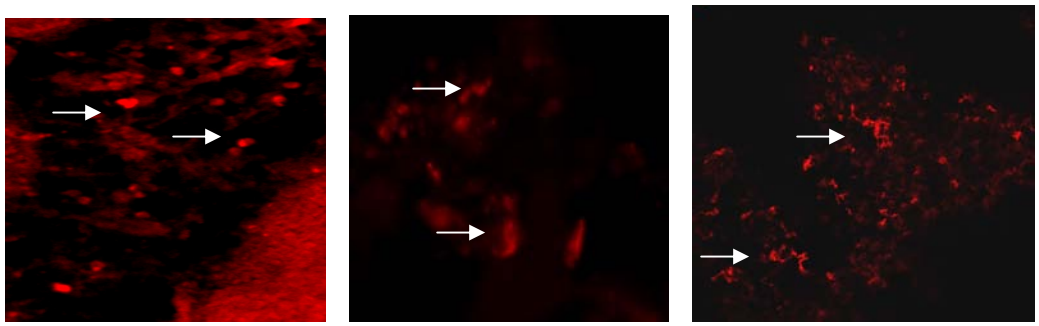
Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.01$ when compared to Control
[b] $p<0.001$, [d] $p<0.05$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure-94

$GABA_{A\alpha1}$ receptor expression in the brain stem of control and experimental neonatal rats



Hx **Hx + G**



Hx + O **Hx + E** **Hx + G + E + O**

Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table- 98

$GABA_{A\alpha1}$ receptor expression in the brain stem of control and experimental neonatal rats

Experimental groups	Mean Pixel intensity
Control	78.32 ± 4.32
Hx	41.50 ± 5.20 [a]
Hx + G	74.65 ± 5.05 [b]
Hx + G + O	76.45 ± 4.55 [b]
Hx + O	53.22 ± 5.75 [a]
Hx + E	43.55 ± 4.55 [a]
Hx + G + E + O	46.46 ± 5.32 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.

[a] $p<0.001$ when compared to Control

[b] $p<0.001$ when compared to hypoxic group

Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 95
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the brain stem of control and experimental neonatal rats.

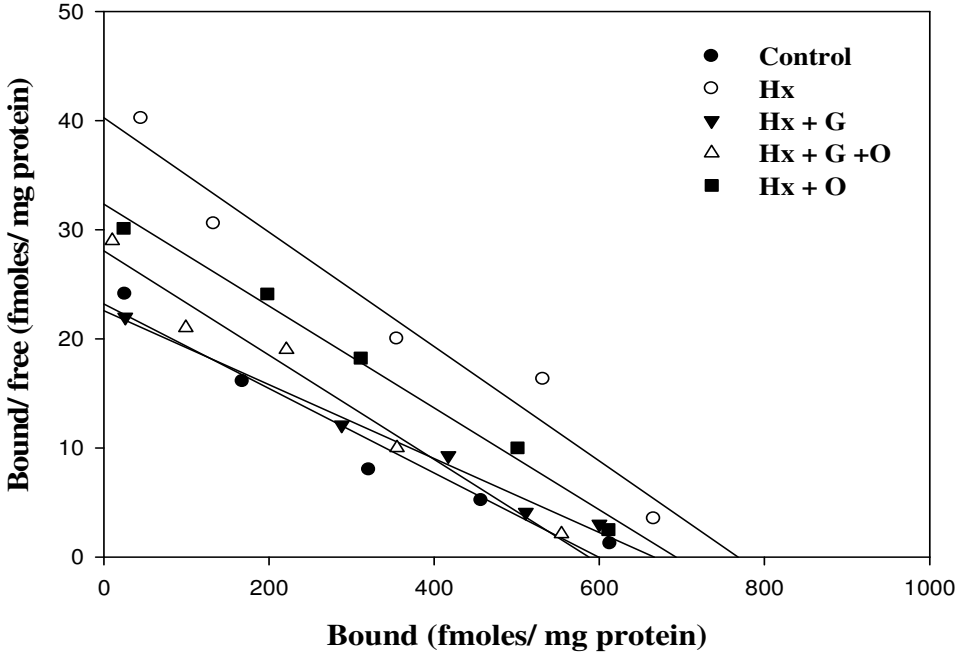


Table- 99
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the brain stem of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	598.86 ± 5.0	26.08 ± 2.50
Hx	766.10 ± 6.5 [a]	18.92 ± 1.40 [a]
Hx + G	655.96 ± 4.2 [b]	28.77 ± 2.55 [b]
Hx + G + O	584.03 ± 5.5 [b]	20.49 ± 2.05 [b]
Hx + O	689.90 ± 4.0 [c]	21.29 ± 2.00 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$, [c] $p<0.01$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 96
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the brain stem of control and experimental neonatal rats

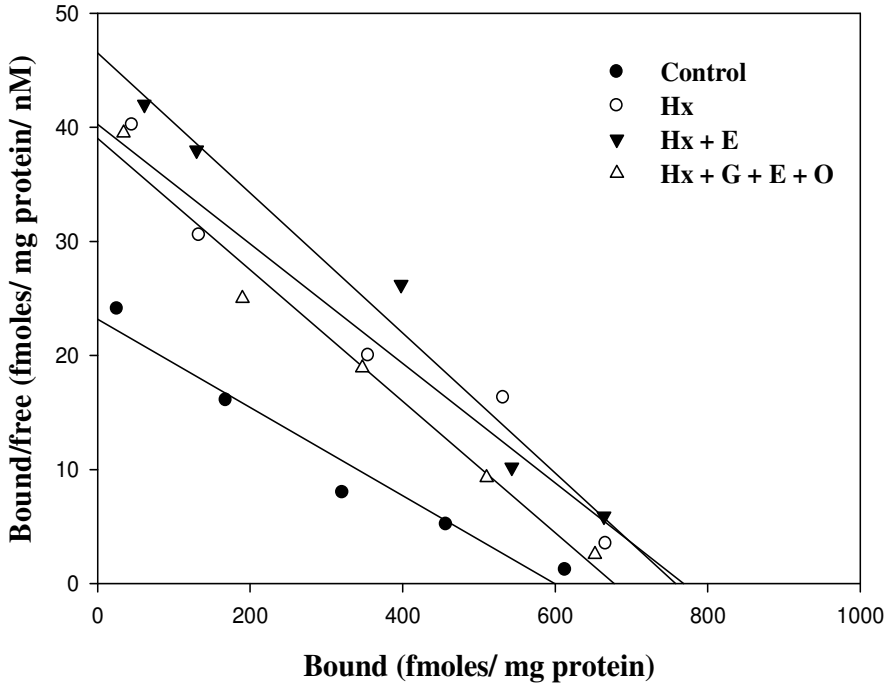


Table- 100
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the brain stem of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	598.86 ± 5.0	26.08 ± 2.50
Hx	766.10 ± 6.5 [a]	18.92 ± 1.40 [a]
Hx + E	755.54 ± 5.5 [a]	16.30 ± 1.10 [a]
Hx + G + E + O	677.10 ± 5.0 [a, c]	17.19 ±1.50 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$, [c] $p<0.05$when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 97
Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT$_{2A}$ receptor in the brain stem of control and experimental neonatal rats.

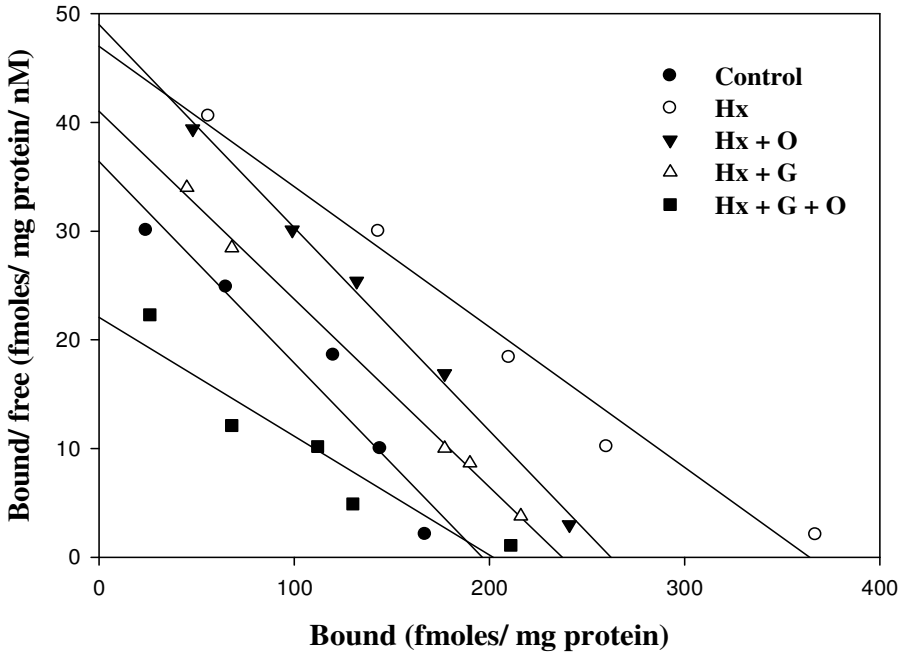


Table- 101
Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT$_{2A}$ receptor in the brain stem and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	197.19 ± 4.1	5.40 ± 1.14
Hx	363.95 ± 5.5 [a]	7.69 ± 1.04 [a]
Hx + G	236.13 ± 3.8 [b]	5.73 ± 1.10 [b]
Hx + G + O	203.13 ± 4.2 [b]	9.02 ± 1.15 [a]
Hx + O	262.35 ± 4.3 [a, b]	5.35 ± 1.24 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 98
Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT$_{2A}$ receptor in the brain stem of control and experimental neonatal rats.

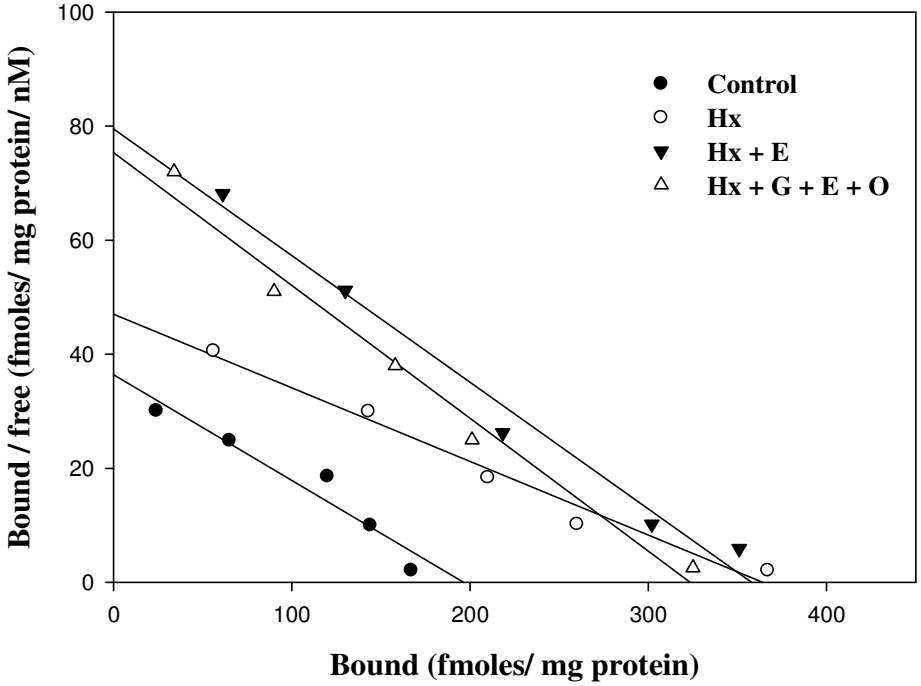


Table- 102
Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT$_{2A}$ receptor in the brain stem and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	197.19 ± 4.1	5.40 ± 1.14
Hx	363.95 ± 5.5 [a]	7.69 ± 1.04 [a]
Hx + E	358.10 ± 6.5 [a]	4.48 ± 1.10 [b]
Hx + G + E + O	323.81 ± 5.8 [a]	4.33 ±1.12 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] p<0.001 when compared with control
[b] p<0.001 when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 99
Real Time PCR amplification of 5-HT_{2A} receptor subunit mRNA from the brain stem of control and experimental neonatal rats

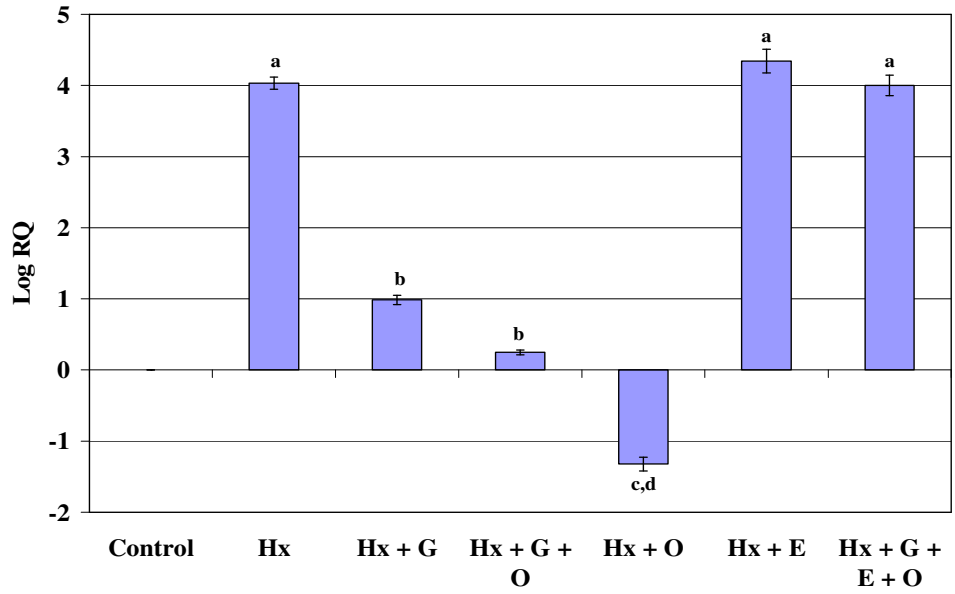


Table- 103
Real Time PCR amplification of 5-HT_{2A} receptor subunit mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	4.03 ± 0.09 [a]
Hx + G	0.99 ± 0.06 [b]
Hx + G + O	0.25 ± 0.03 [b]
Hx + O	-1.32 ± 0.10 [c, d]
Hx + E	4.34 ± 0.17 [a]
Hx + G + E + O	4.00 ± 0.14 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.05$ when compared to Control
[b] $p<0.001$, [d] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 100
Real Time PCR amplification of 5-HT transporter mRNA from the brain stem of control and experimental neonatal rats

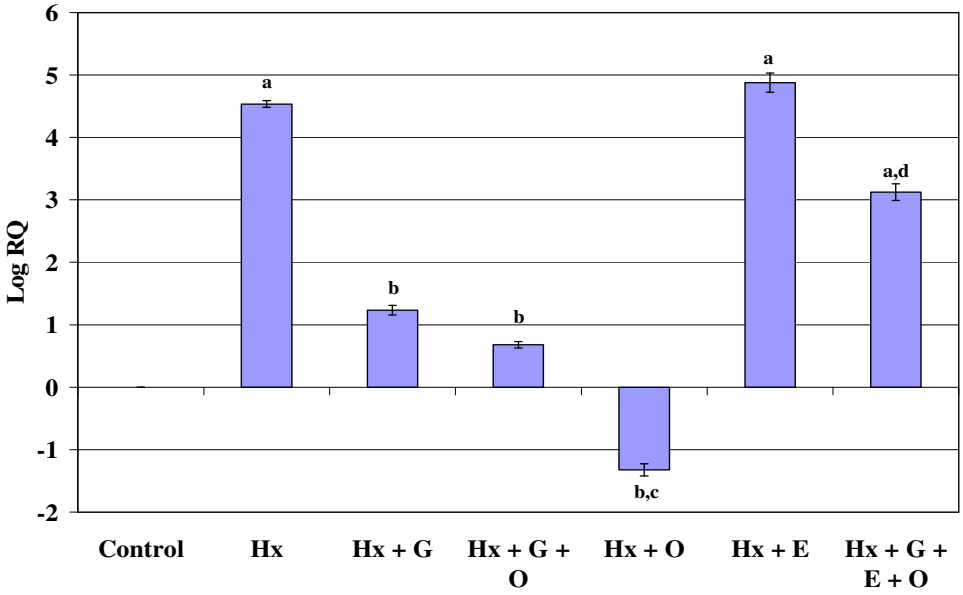
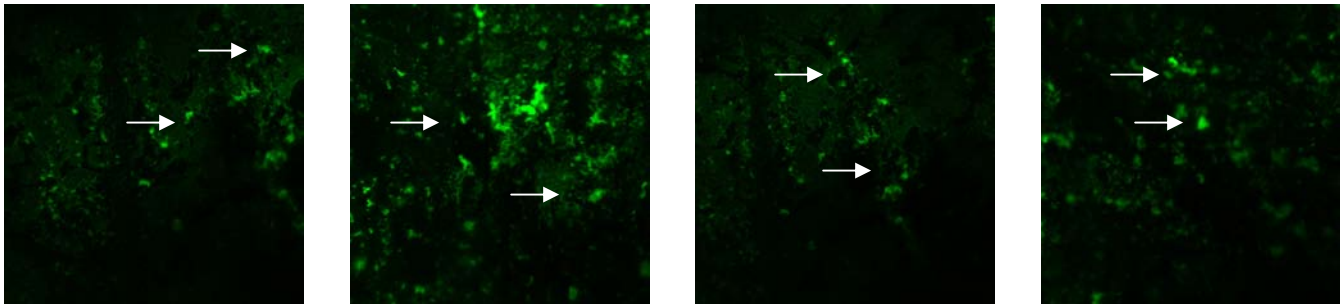


Table- 104
Real Time PCR amplification of 5-HT transporter mRNA from the brain stem of control and experimental neonatal rats

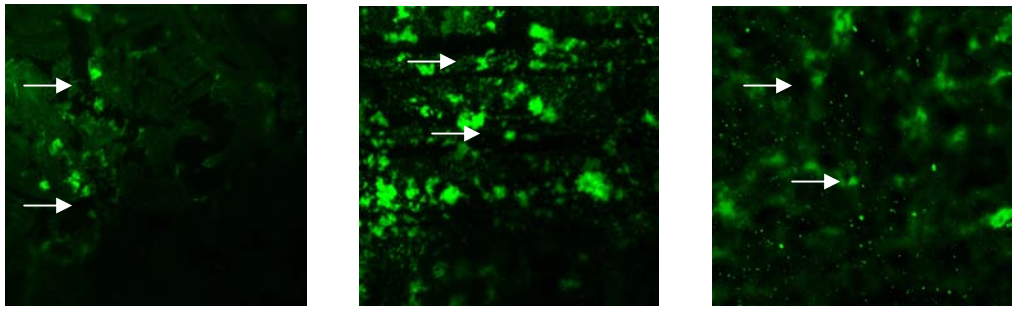
Experimental groups	Log RQ
Control	0
Hx	4.53 ± 0.05 [a]
Hx + G	1.23 ± 0.07 [b]
Hx + G + O	0.68 ± 0.05 [b]
Hx + O	-1.32 ± 0.10 [b, c]
Hx + E	4.88 ± 0.15 [a]
Hx + G + E + O	3.12 ± 0.13 [a, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.05$ when compared to Control
[b] $p<0.001$, [d] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure - 101
5-HT_{2A} receptor expression in the brain stem of control and experimental neonatal rats



Control **Hx** **Hx + G** **Hx + G + O**



Hx + O **Hx + E** **Hx + G + E + O**

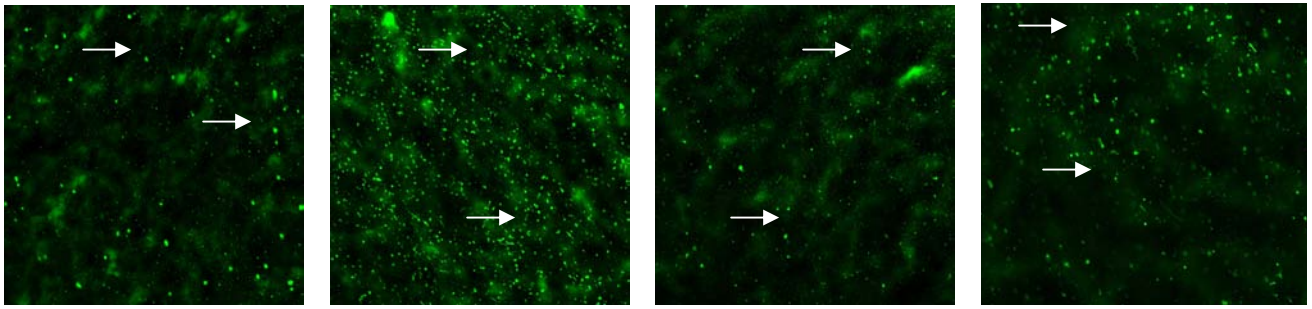
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table-105
5-HT_{2A} receptor expression in the brain stem of control and experimental neonatal rats

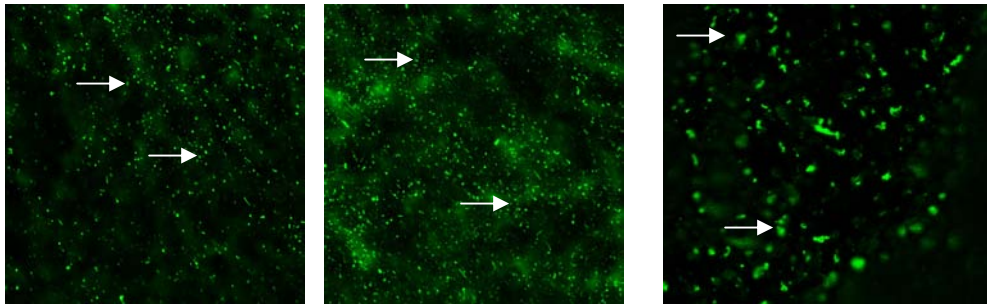
Experimental groups	Mean Pixel intensity
Control	19.78 ± 2.15
Hx	35.20 ± 3.50 [a]
Hx + G	17.55 ± 2.06 [b]
Hx + G + O	18.99 ± 1.56 [b]
Hx + O	29.89 ± 2.55 [a]
Hx + E	45.20± 3.25 [a]
Hx + G + E + O	32.12 ± 4.07 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure - 102
5-HT transporter expression in the brain stem of control and experimental neonatal rats



Control **Hx** **Hx + G** **Hx + O**



Hx + O **Hx + E** **Hx + G + E + O**

Table-106
5-HT transporter expression in the brain stem of control and experimental neonatal rats

Experimental groups	Mean Pixel intensity
Control	12.66 ± 1.22
Hx	34.25 ± 1.45 [a]
Hx + G	14.20 ± 2.05 [b]
Hx + G + O	15.25 ± 3.05 [b]
Hx + O	22.45 ± 2.50
Hx + E	32.12 ± 2.65 [a]
Hx + G + E + O	29.87 ± 2.00 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure-103
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the brain stem of control and experimental neonatal rats.

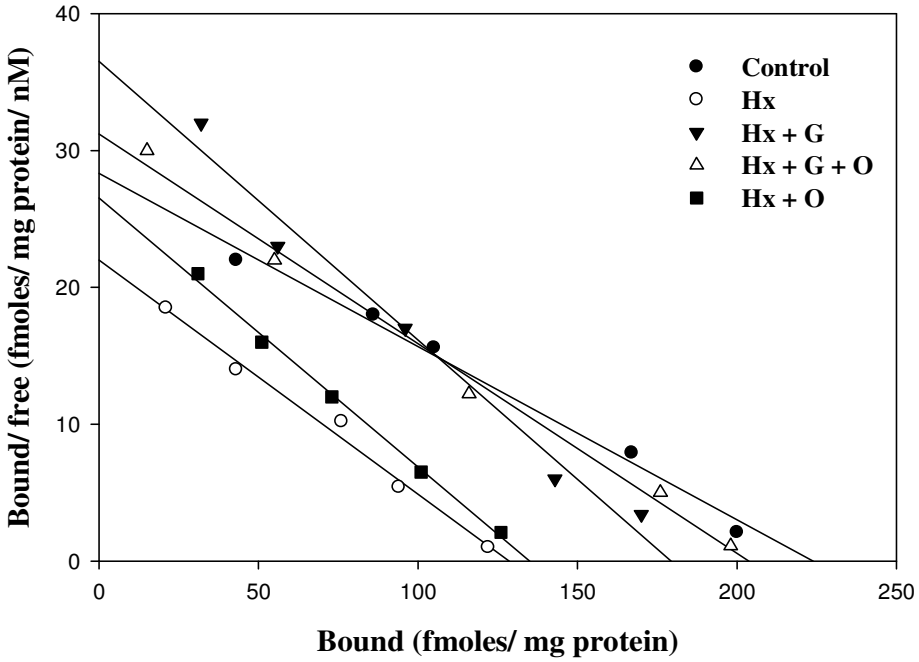


Table- 107
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the brain stem of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	223.95 ± 3.6	7.80 ±0.14
Hx	127.10 ± 3.8 [a]	5.74 ± 0.12 [a]
Hx + G	178.15 ± 2.9 [c]	4.88 ± 0.10 [a]
Hx + G + O	202.60 ± 3.8 [b]	6.56 ± 0.15 [b]
Hx + O	134.40 ± 2.5 [a]	5.07 ± 0.14 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$, [c] $p<0.01$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O.

Figure- 104
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the brain stem of control and experimental neonatal rats.

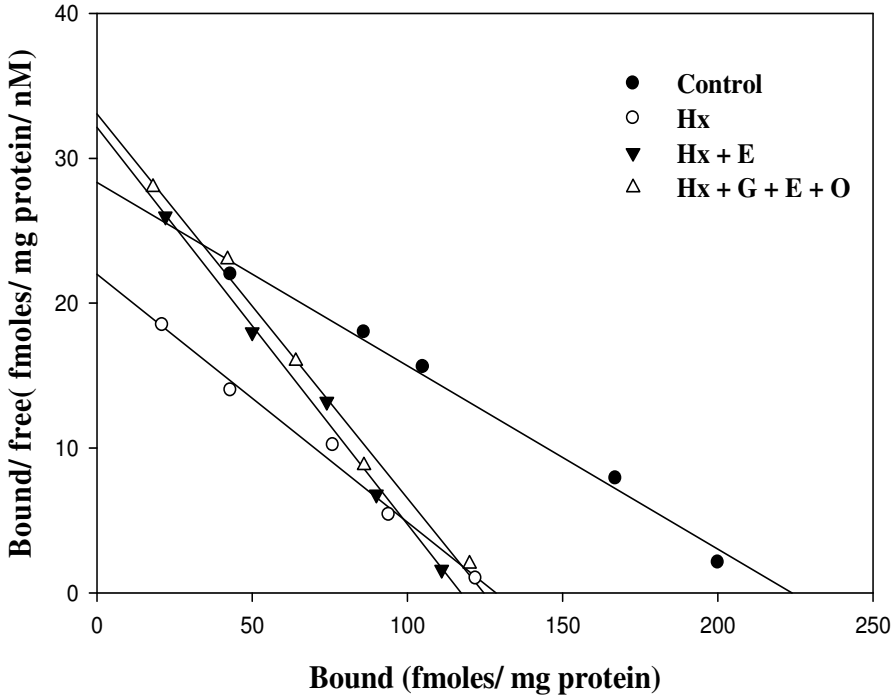


Table- 108
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the brain stem of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	223.95 ± 3.6	7.80 ±0.14
Hx	127.10 ± 3.8 [a]	5.74 ± 0.12 [a]
Hx + E	116.15 ±2.6 [a]	3.62 ±0.10 [a]
Hx + G + E + O	123.95 ±4.5 [a]	3.70 ±0.12 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure-105
Real Time PCR amplification of Muscarinic M_1 receptor subunit mRNA from the brain stem of control and experimental neonatal rats

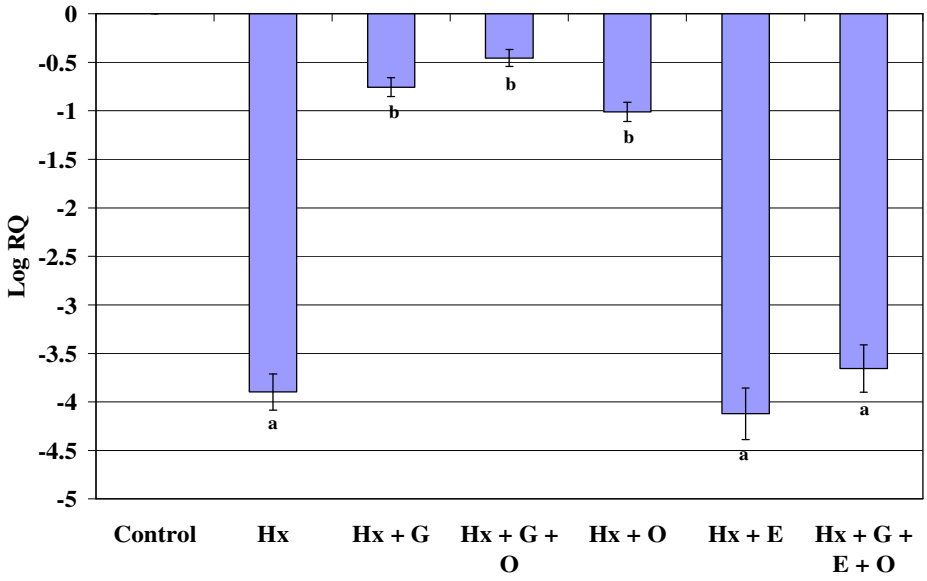


Table- 109
Real Time PCR amplification of Muscarinic M_1 receptor subunit mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-3.89 ± 0.18 [a]
Hx + G	-0.76 ± 0.10 [b]
Hx + G + O	-0.46 ± 0.09 [b]
Hx + O	-1.01 ± 0.10 [b]
Hx + E	-4.12 ± 0.26 [a]
Hx + G + E + O	-3.66 ± 0.24 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 106
Real Time PCR amplification of Muscarinic M_2 receptor subunit mRNA from the brain stem of control and experimental neonatal rats

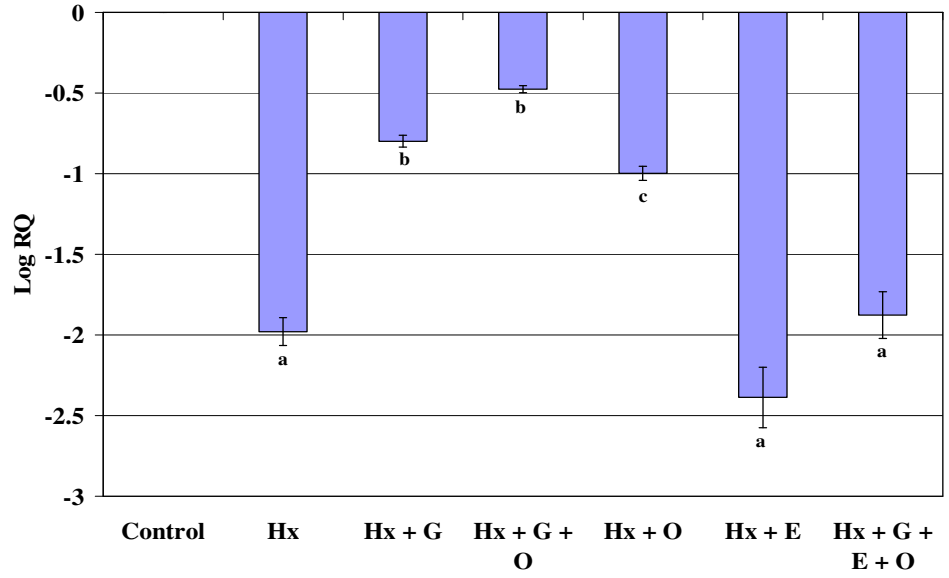


Table- 110
Real Time PCR amplification of Muscarinic M_2 receptor subunit mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.98 ± 0.09 [a]
Hx + G	-0.80 ± 0.03 [b]
Hx + G + O	-0.48 ± 0.02 [b]
Hx + O	-0.99 ± 0.04 [c]
Hx + E	-2.39 ± 0.19 [a]
Hx + G + E + O	-1.88 ± 0.14 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$, [c] $p<0.01$when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure-107
Real Time PCR amplification of Muscarinic M_3 receptor subunit mRNA from the brain stem of control and experimental neonatal rats

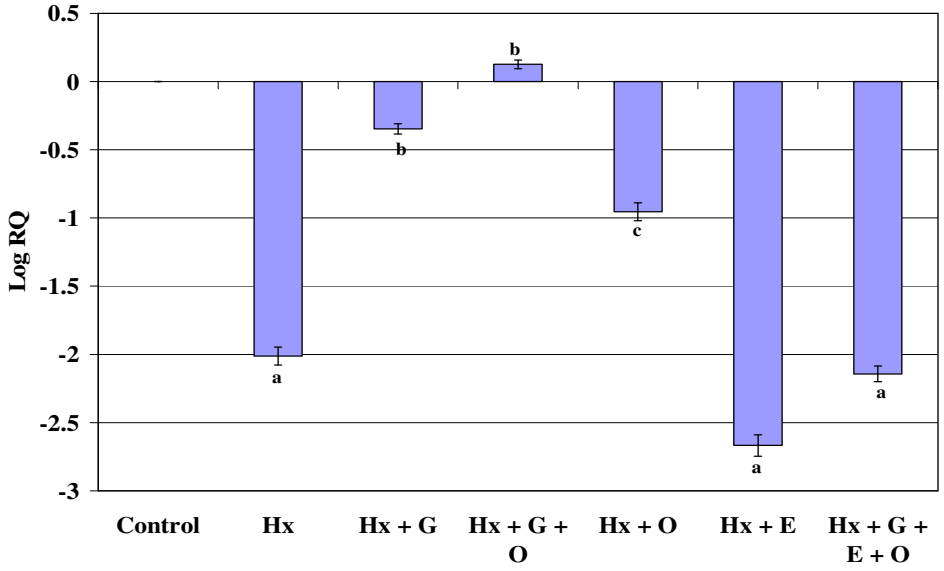


Table- 111
Real Time PCR amplification of Muscarinic M_3 receptor subunit mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-2.01 ± 0.09 [a]
Hx + G	-0.35 ± 0.03 [b]
Hx + G + O	0.13 ± 0.02 [b]
Hx + O	-0.95 ± 0.04 [c]
Hx + E	-2.67 ± 0.19 [a]
Hx + G + E + O	-2.14 ± 0.14 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$, [c] $p<0.01$when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure-108
Real Time PCR amplification of acetylcholine esterase mRNA from the brain stem of control and experimental neonatal rats.

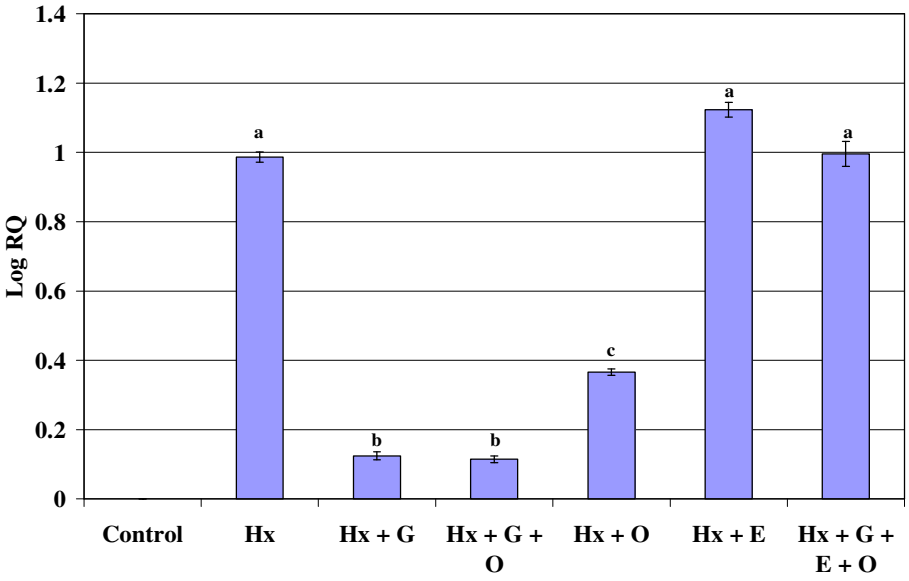


Table- 112
Real Time PCR amplification of acetylcholine esterase mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	0.99 ± 0.09 [a]
Hx + G	0.12 ± 0.03 [b]
Hx + G + O	0.11 ± 0.02 [b]
Hx + O	0.37 ± 0.04 [c]
Hx + E	1.12 ± 0.19 [a]
Hx + G + E + O	1.00 ± 0.14 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] p<0.001when compared to Control
[b] p<0.001, [c] p <0.01when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 109
Real Time PCR amplification of choline acetyl transferase mRNA from the brain stem of control and experimental neonatal rats.

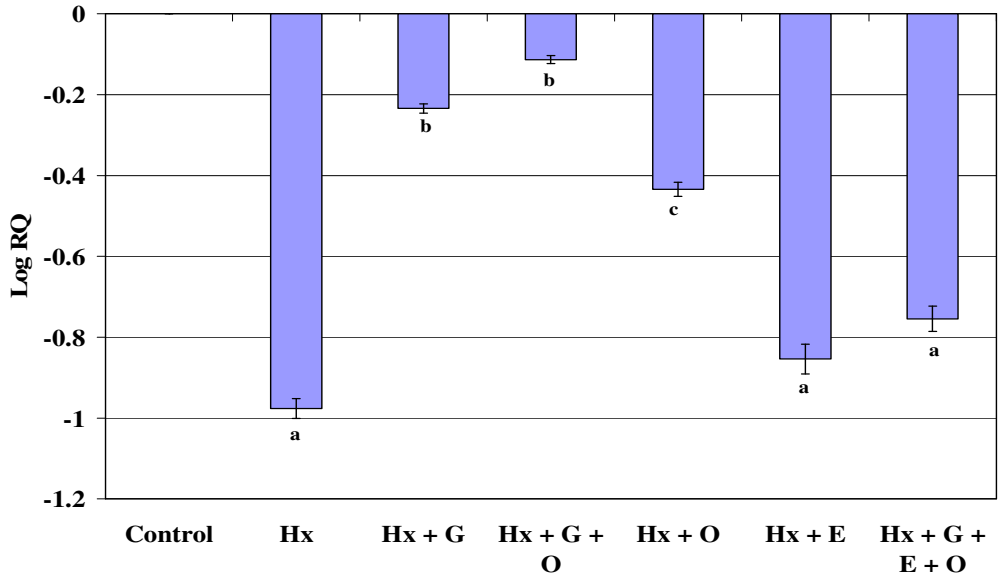


Table- 113
Real Time PCR amplification of choline acetyl transferase mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.98 ± 0.02 [a]
Hx + G	-0.24 ± 0.01 [b]
Hx + G + O	-0.11 ± 0.01 [b]
Hx + O	-0.43 ± 0.02 [c]
Hx + E	-0.85 ± 0.04 [a]
Hx + G + E + O	-0.75 ± 0.03 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$, [c] $p<0.01$when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 110
Real Time PCR amplification of Hif 1 mRNA from the brain stem of control and experimental neonatal rats.

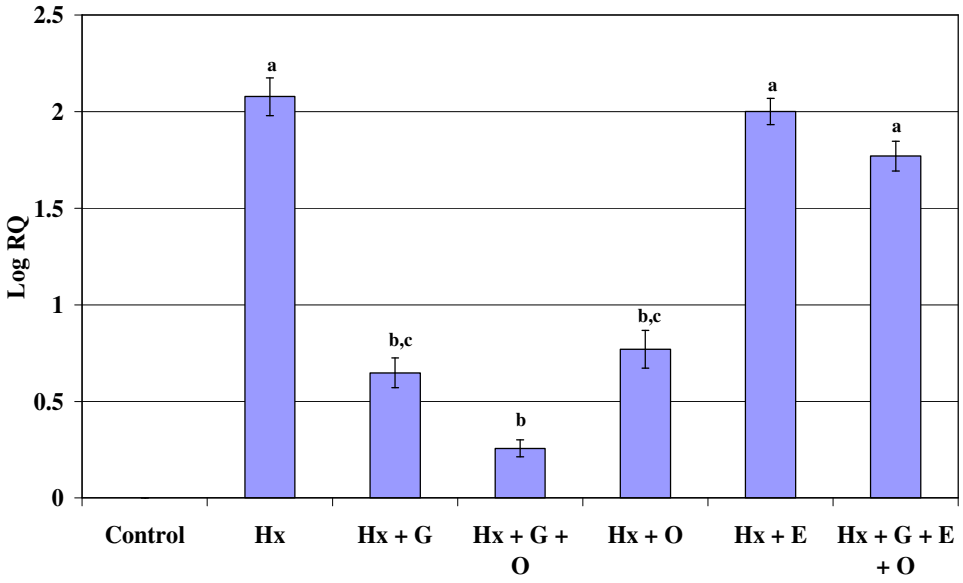


Table- 114
Real Time PCR amplification of Hif 1 mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	2.08 ± 0.10 [a]
Hx + G	0.65 ± 0.08 [b,c]
Hx + G + O	0.26 ± 0.04 [b]
Hx + O	0.77 ± 0.10 [b,c]
Hx + E	1.99 ± 0.08 [a]
Hx + G + E + O	1.77 ± 0.07 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.05$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 111
Real Time PCR amplification of SOD mRNA from the brain stem of control and experimental neonatal rats.

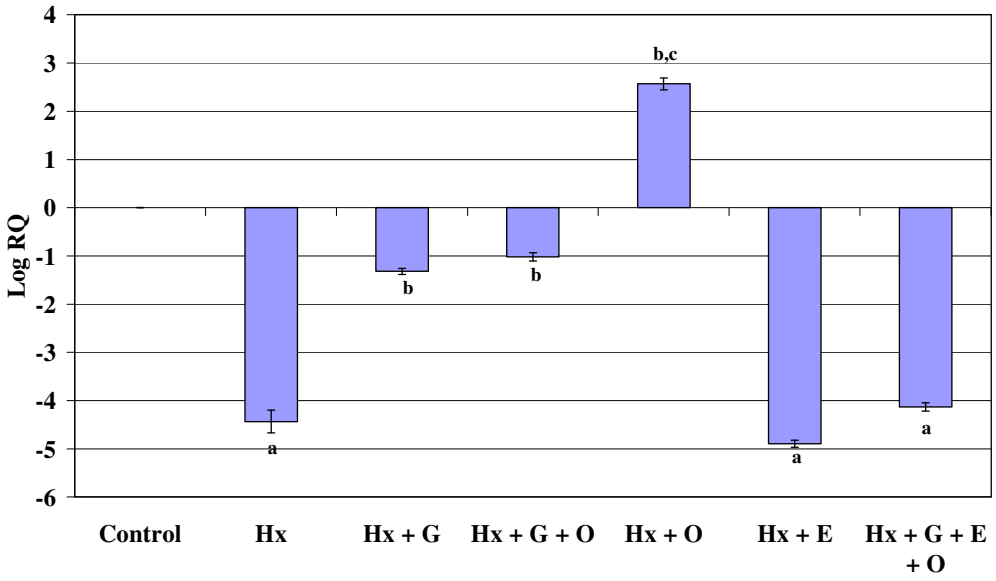


Table- 115
Real Time PCR amplification of SOD mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-4.44 ± 0.23 [a]
Hx + G	-1.32 ± 0.06 [b]
Hx + G + O	-1.02 ± 0.09 [b]
Hx + O	2.57 ± 0.12 [b, c]
Hx + E	-4.89 ± 0.08 [a]
Hx + G + E + O	-4.13 ± 0.09 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.05$ when compared to Control
[b] $p<0.001$when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 112
Real Time PCR amplification of GPx mRNA from the brain stem of control and experimental neonatal rats.

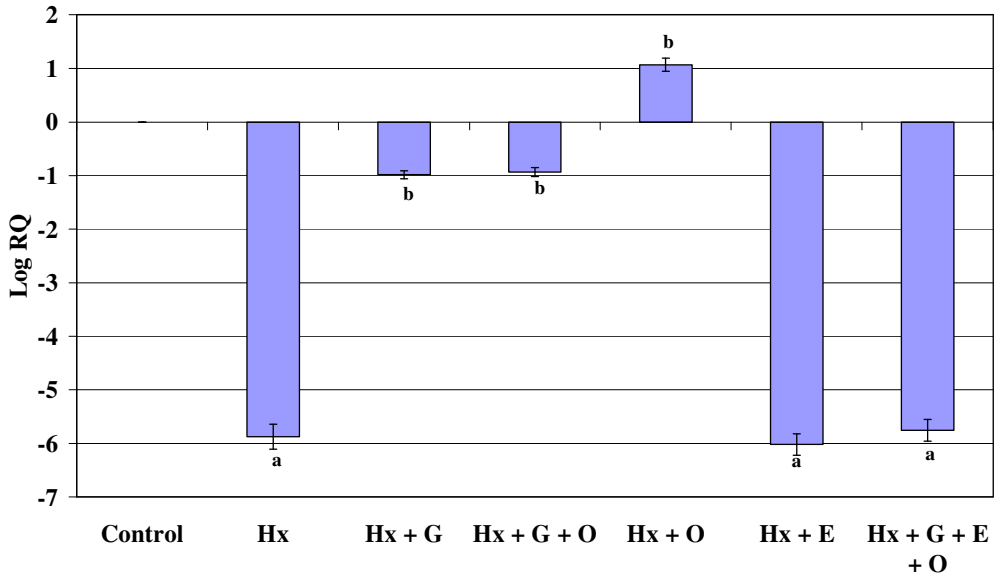


Table- 116
Real Time PCR amplification of GPx mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-5.88 ± 0.23 [a]
Hx + G	-0.99 ± 0.08 [b]
Hx + G + O	-0.94 ± 0.08 [b]
Hx + O	1.06 ± 0.12 [b]
Hx + E	-6.02 ± 0.20 [a]
Hx + G + E + O	-5.76 ± 0.20 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 113
Real Time PCR amplification of BAX mRNA from the brain stem of control and experimental neonatal rats

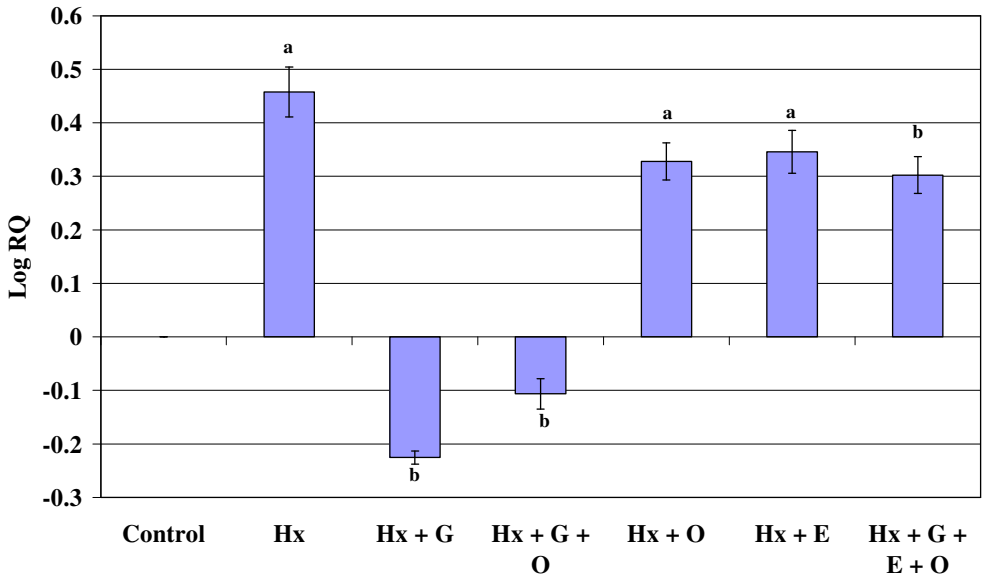


Table- 117
Real Time PCR amplification of BAX mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	0.46 ± 0.05 [a]
Hx + G	-0.23 ± 0.01 [b]
Hx + G + O	-0.11 ± 0.03 [b]
Hx + O	0.33 ± 0.03 [a]
Hx + E	0.35 ± 0.04 [a]
Hx + G + E + O	0.30 ± 0.03 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 114
Real Time PCR amplification of CREB mRNA from the brain stem of control and experimental neonatal rats

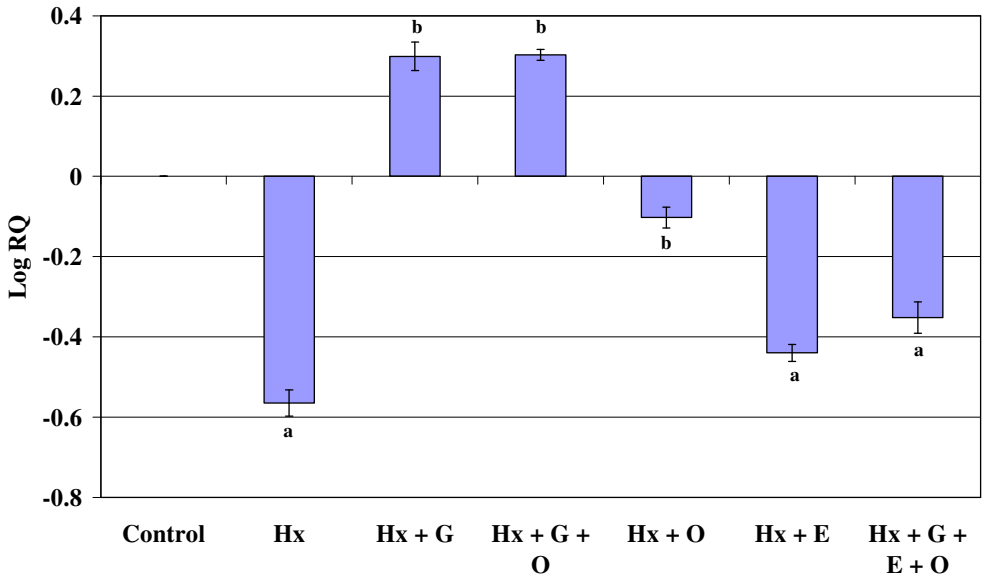


Table- 118
Real Time PCR amplification of CREB mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.56 ± 0.05 [a]
Hx + G	0.29 ± 0.01 [b]
Hx + G + O	0.30 ± 0.03 [b]
Hx + O	-0.10 ± 0.03 [b]
Hx + E	-0.44 ± 0.04 [a]
Hx + G + E + O	-0.35 ± 0.03 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 115
Real Time PCR amplification of phospholipase C mRNA from the brain stem of control and experimental neonatal rats.

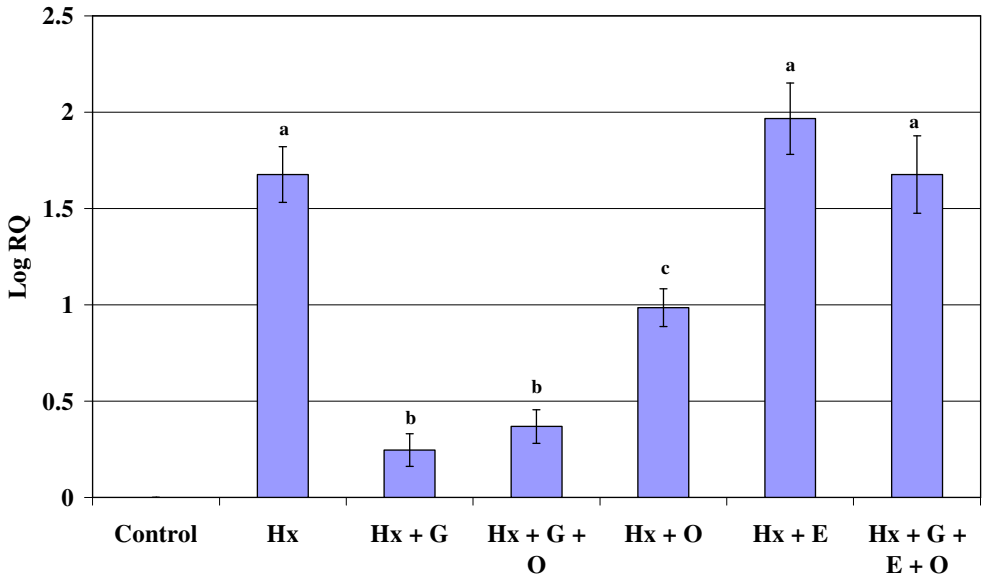


Table- 119
Real Time PCR amplification of phospholipase C mRNA from the brain stem of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	1.68 ± 0.14 [a]
Hx + G	0.25 ± 0.09 [b]
Hx + G + O	0.37 ± 0.09 [b]
Hx + O	0.98 ± 0.10 [c]
Hx + E	1.97 ± 0.19 [a]
Hx + G + E + O	1.68 ± 0.20 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$, [c] $p<0.01$when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 116
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the corpus striatum of control and experimental neonatal rats.

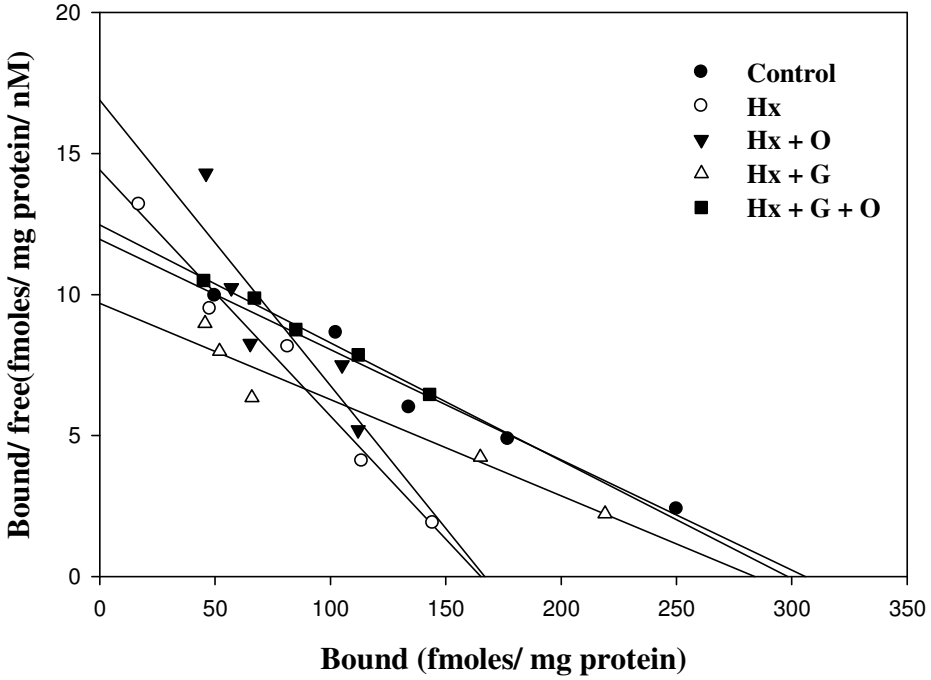


Table- 120
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the corpus striatum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	304.08 ± 3.7	26.77 ± 0.44
Hx	164.78 ± 2.8 [a]	11.35 ± 0.22 [a]
Hx + G	281.47 ± 2.5 [b]	28.72 ± 0.35 [b]
Hx + G + O	297.50 ± 3.4 [b]	24.15 ± 0.26 [b]
Hx + O	165.55 ± 2.3 [a]	9.74 ± 0.29 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 117
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the corpus striatum of control and experimental neonatal rats.

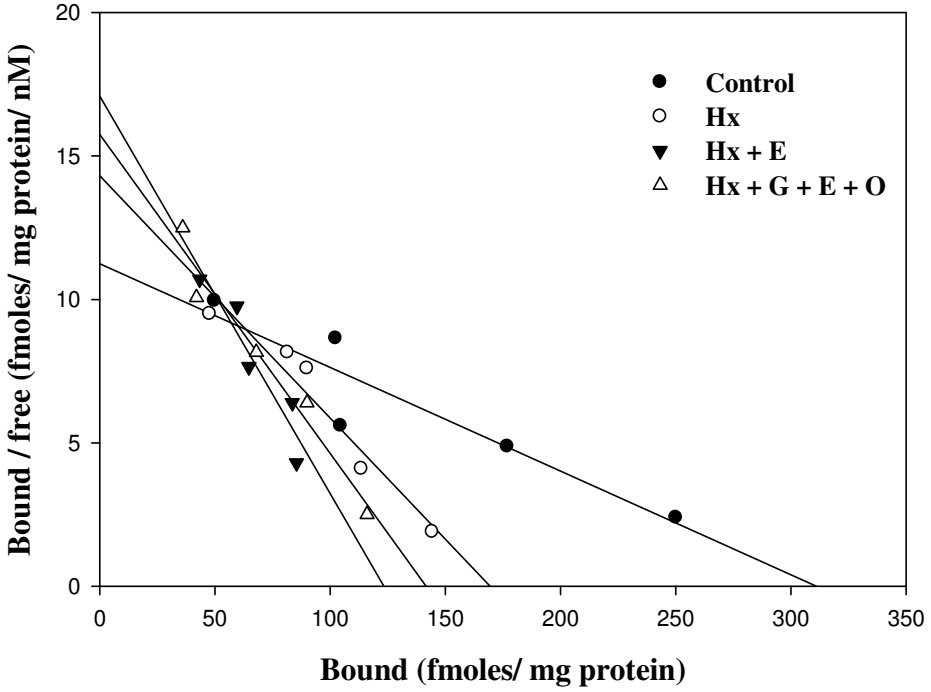


Table- 121
Scatchard analysis of [^{3}H] GABA binding against GABA to total GABA receptor in the corpus striatum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	304.08 ± 3.7	26.77 ± 0.44
Hx	164.78 ± 2.8 [a]	11.35 ± 0.22 [a]
Hx + E	121.80 ± 2.6 [a]	7.16 ± 0.14 [a]
Hx + G + E + O	141.47 ± 4.5 [a]	8.89 ± 0.22 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure - 118

Binding parameters of [^{3}H] GABA against GABA in the corpus striatum of control and experimental neonatal rats.

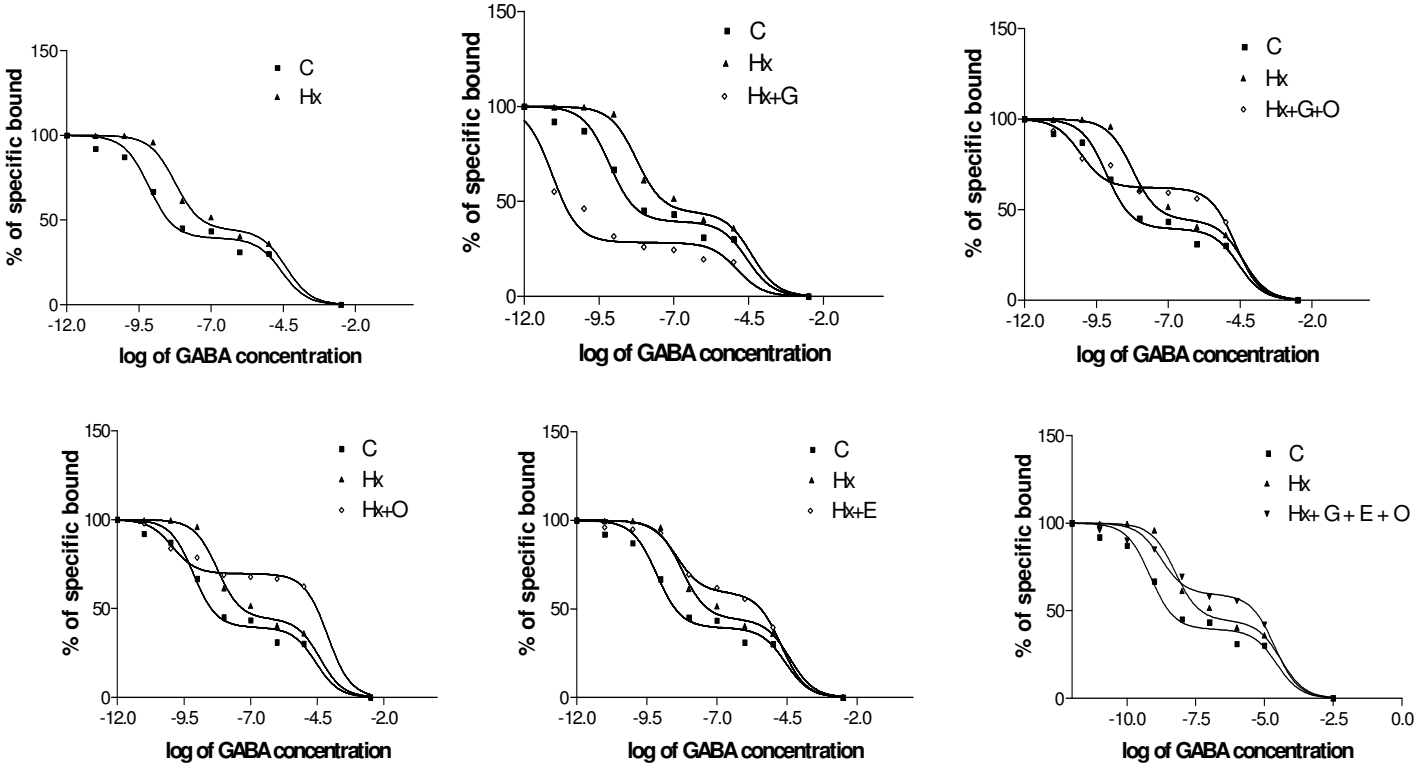


Table - 122

Binding parameters of [^{3}H] GABA against GABA in the corpus striatum of control and experimental neonatal rats.

Group	Best-fit model	Log (EC_{50})-1	Log (EC_{50})-2	Ki $_{(H)}$	Ki $_{(L)}$	Hill slope
C	Two- site	-6.147	-3.550	2.3090x 10^{-9}	1.3123x 10^{-5}	- 0.6832
Hx	Two- site	-7.243	-4.398	2.0543 x 10^{-10}	1.4350 x 10^{-6}	- 0.6944
Hx + G	Two- site	-6.06	-3.880	3.9210 x 10^{-9}	4.1230 x 10^{-5}	- 0.5522
Hx + G + O	Two- site	-6.05	-2.667	3.9740 x 10^{-9}	5.5530 x 10^{-5}	- 0.6831
Hx + O	Two- site	-7.927	-4.106	4.4550 x 10^{-9}	3.3450 x 10^{-5}	- 0.7926
Hx + E	Two- site	-9.432	-4.708	2.7180 x 10^{-10}	8.0340 x 10^{-6}	- 0.7957
Hx + G + E + O	Two- site	-8.564	-4.623	2.4586 x 10^{-10}	8.0455 x 10^{-6}	- 0.6964

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 119
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the corpus striatum of control and experimental neonatal rats.

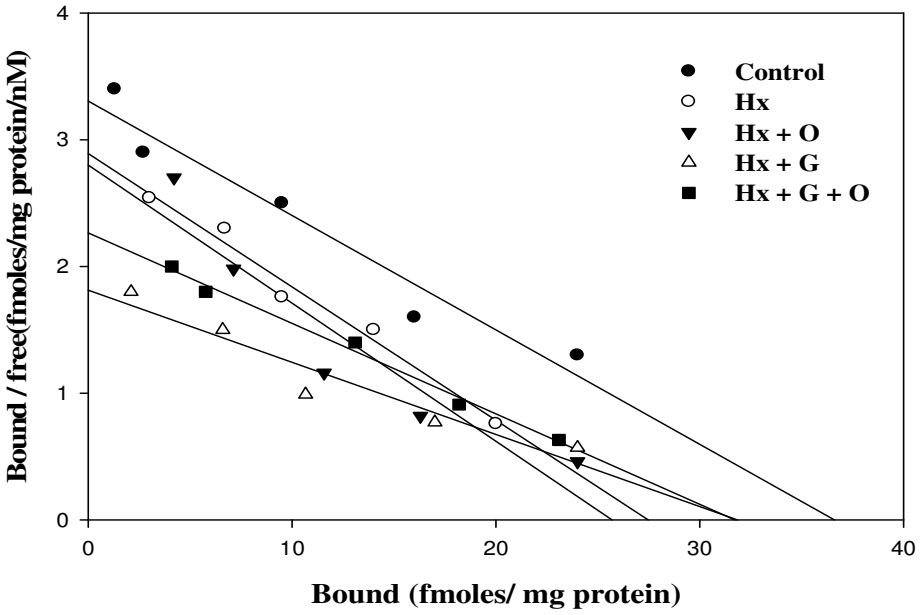


Table- 123
Scatchard analysis of [^{3}H] bicuculline binding against $GABA_A$ receptor antagonist bicuculline in the corpus striatum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	36.58 ± 2.6	10.98 ±1.20
Hx	27.42 ± 1.5 [a]	9.46 ± 1.02 [a]
Hx + G	31.75 ± 2.6 [b]	17.26 ± 2.12 [b]
Hx + G + O	31.76 ± 3.0 [b]	13.99 ± 2.00 [b]
Hx + O	25.67 ± 2.0 [a]	9.17 ± 1.34 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 120
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the corpus striatum of control and experimental neonatal rats.

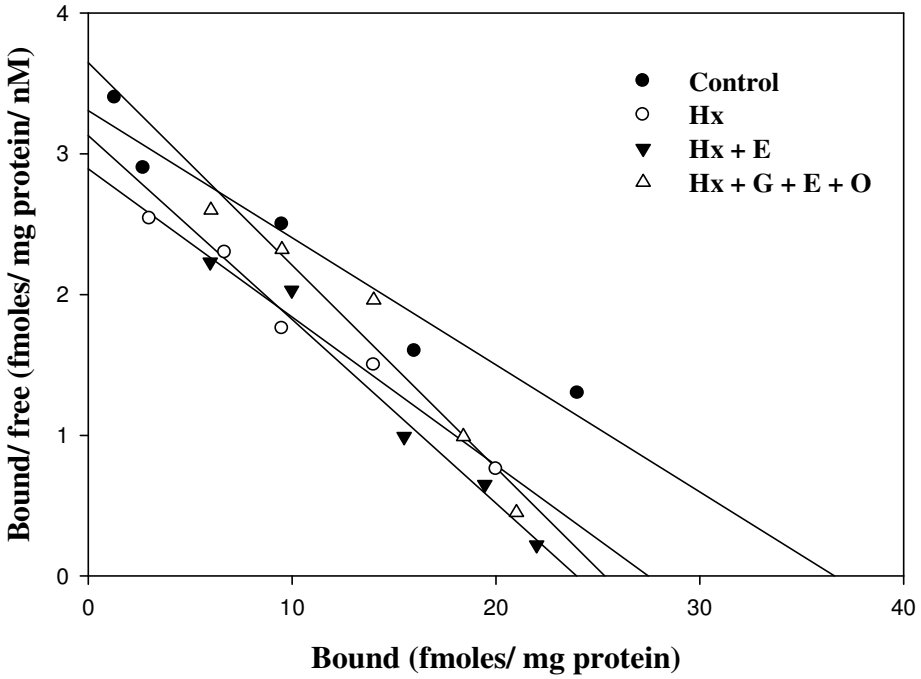


Table- 124
Scatchard analysis of [^{3}H] bicuculline binding against bicuculline to $GABA_A$ receptor in the corpus striatum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	36.58 ± 2.6	10.98 ±1.20
Hx	27.42 ± 1.5 [a]	9.46 ± 1.02 [a]
Hx + E	23.75 ± 2.6 [a]	7.66 ±1.85 [a]
Hx + G + E + O	25.08 ± 4.5 [a]	6.97 ±1.20 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 121
Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the corpus striatum of control and experimental neonatal rats.

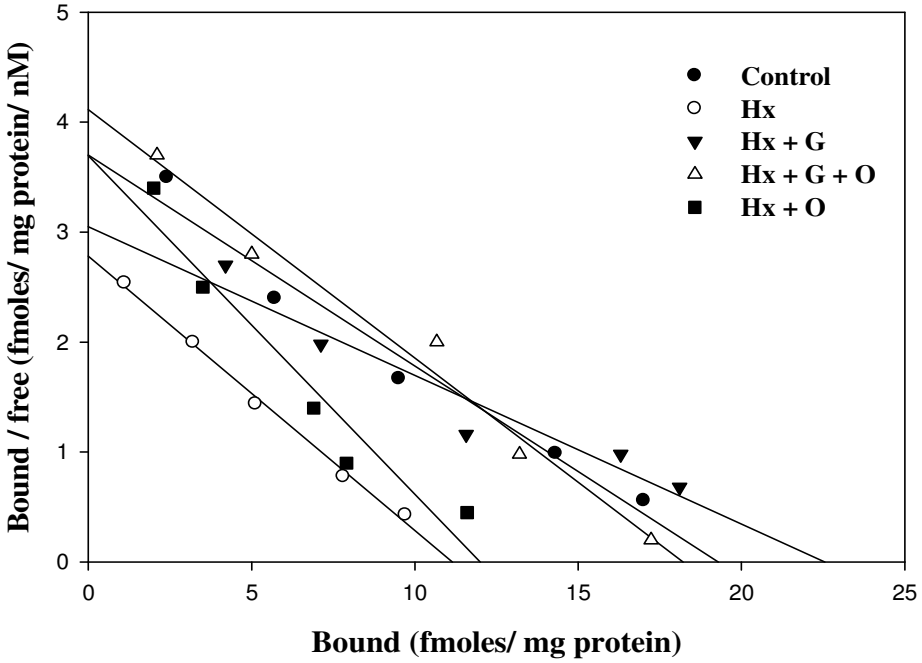


Table- 125
Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the corpus striatum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	19.12 ±1.20	5.18 ± 1.00
Hx	11.05 ± 1.50 [a]	3.96 ± 0.99 [a]
Hx + G	22.35 ±1.40 [b]	7.21 ± 0.99 [b]
Hx + G + O	18.18 ±1.10 [b]	4.43 ± 1.20 [d]
Hx + O	11.93 ± 1.10 [a]	3.23 ± 1.50 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 122
Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the corpus striatum of control and experimental neonatal rats.

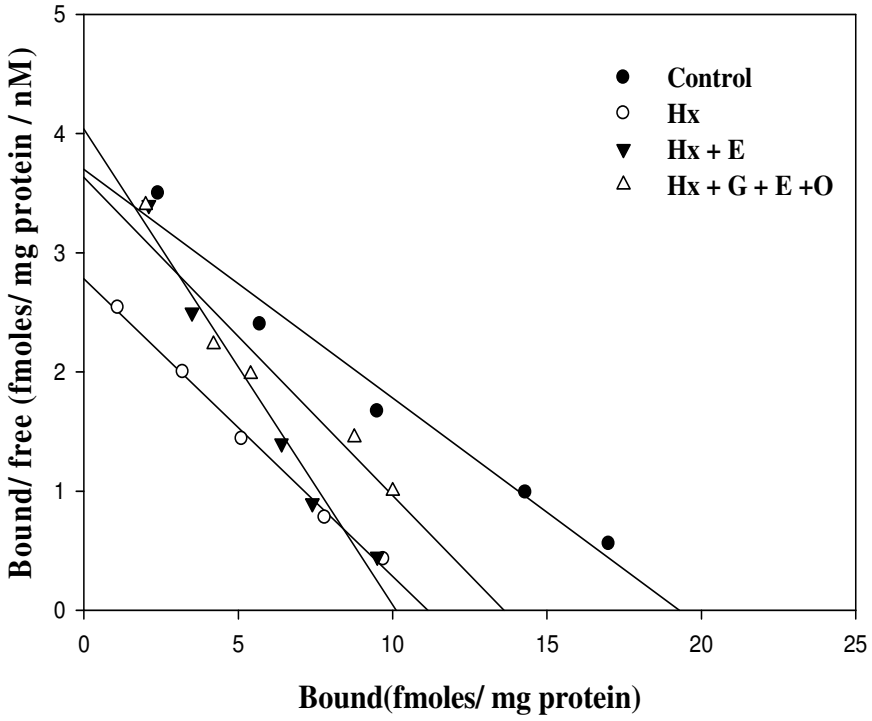


Table- 126
Scatchard analysis of [^{3}H] baclofen binding against baclofen to $GABA_B$ receptor in the corpus striatum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	19.12 ±1.20	5.18 ± 1.00
Hx	11.05 ± 1.50 [a]	3.96 ± 0.99 [a]
Hx + E	10.00 ±1.50 [a]	2.50 ± 0.99 [a]
Hx + G + E + O	13.55 ±1.50 [a]	3.76 ± 0.99 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 123
Real Time PCR amplification of $GABA_{A\acute{u}1}$ receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

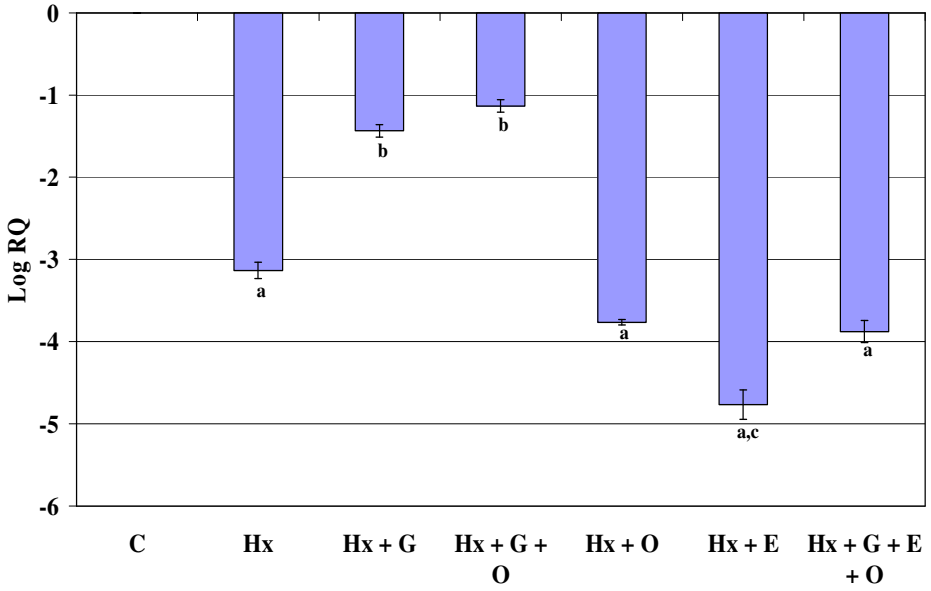


Table- 127
Real Time PCR amplification of $GABA_{A\acute{u}1}$ receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-3.13 ± 0.10 [a]
Hx + G	-1.44 ± 0.08 [b]
Hx + G + O	-1.13 ± 0.08 [b]
Hx + O	-3.76 ± 0.03 [a]
Hx + E	-4.77 ± 0.18 [a, c]
Hx + G + E + O	-3.87 ± 0.13 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$, [c] $p<0.05$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 124
Real Time PCR amplification of $GABA_{A\delta 5}$ receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

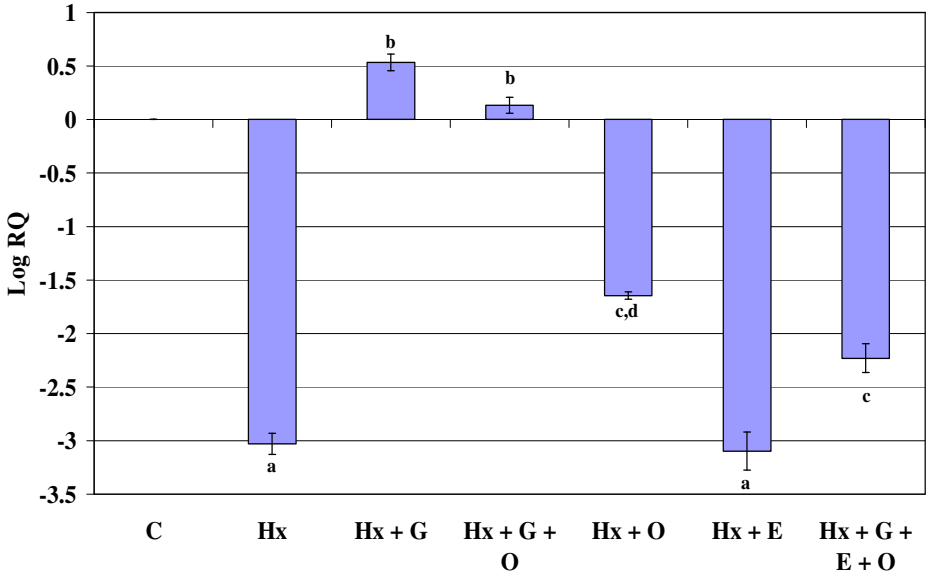


Table- 128
Real Time PCR amplification of $GABA_{A\delta 5}$ receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-3.03 ± 0.10 [a]
Hx + G	0.53 ± 0.08 [b]
Hx + G + O	0.13 ± 0.08 [b]
Hx + O	-1.65 ± 0.03 [c, d]
Hx + E	-3.09 ± 0.18 [a]
Hx + G + E + O	-2.23 ± 0.13 [c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$, [c] $p<0.05$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 125
Real Time PCR amplification of $GABA_{A\gamma3}$ receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

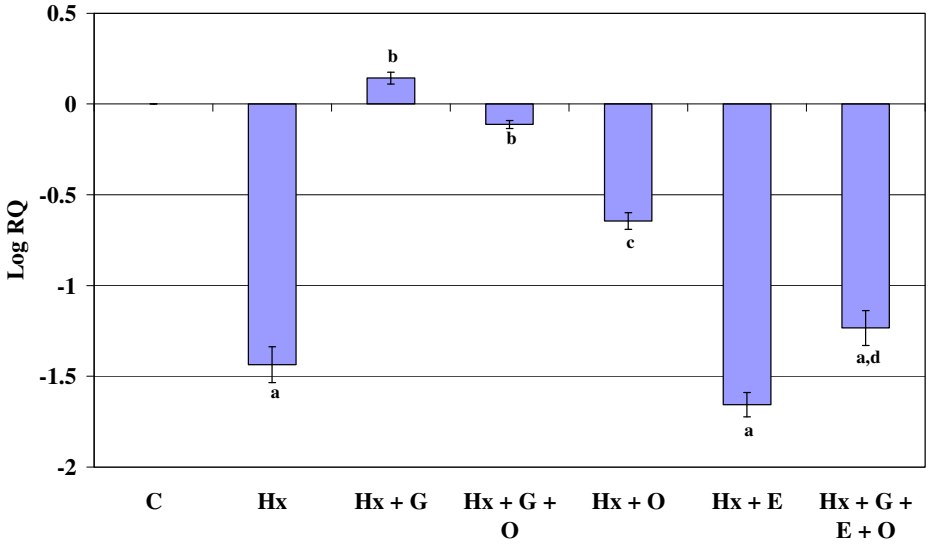


Table- 129
Real Time PCR amplification of $GABA_{A\ \gamma3}$ receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.44 ± 0.10 [a]
Hx + G	0.14 ± 0.03 [b]
Hx + G + O	-0.11 ± 0.02 [b]
Hx + O	-0.64 ± 0.05 [c]
Hx + E	-1.66 ± 0.07 [a]
Hx + G + E + O	-1.23 ± 0.10 [a, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$, [c] $p<0.01$, [d] $p<0.05$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 126
Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats.

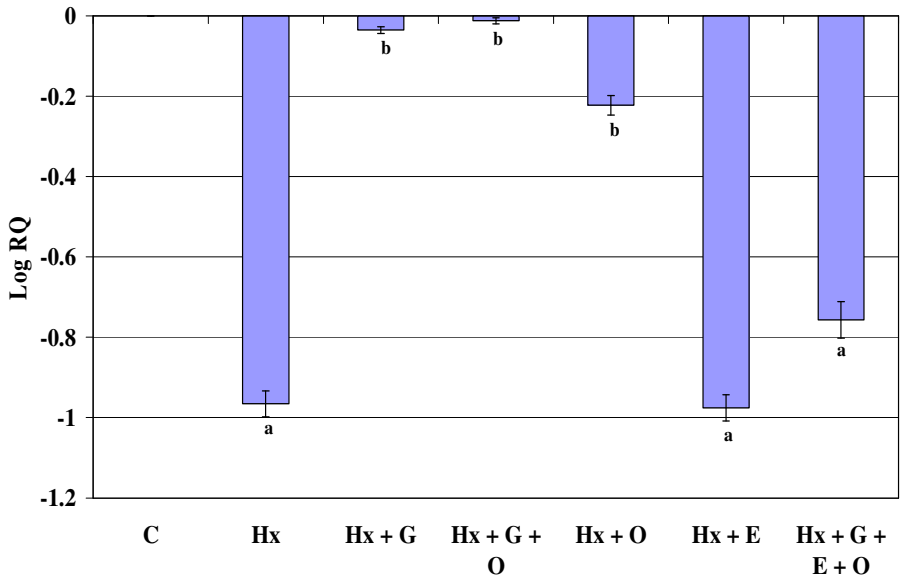


Table- 130
Real Time PCR amplification of $GABA_{A\delta}$ receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.97 ± 0.03 [a]
Hx + G	-0.04 ± 0.008 [b]
Hx + G + O	-0.01 ± 0.007 [b]
Hx + O	-0.22 ± 0.02 [b]
Hx + E	-0.98 ± 0.03 [a]
Hx + G + E + O	-0.76 ± 0.05 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$, [c] $p<0.05$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 127
Real Time PCR amplification of $GABA_B$ receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

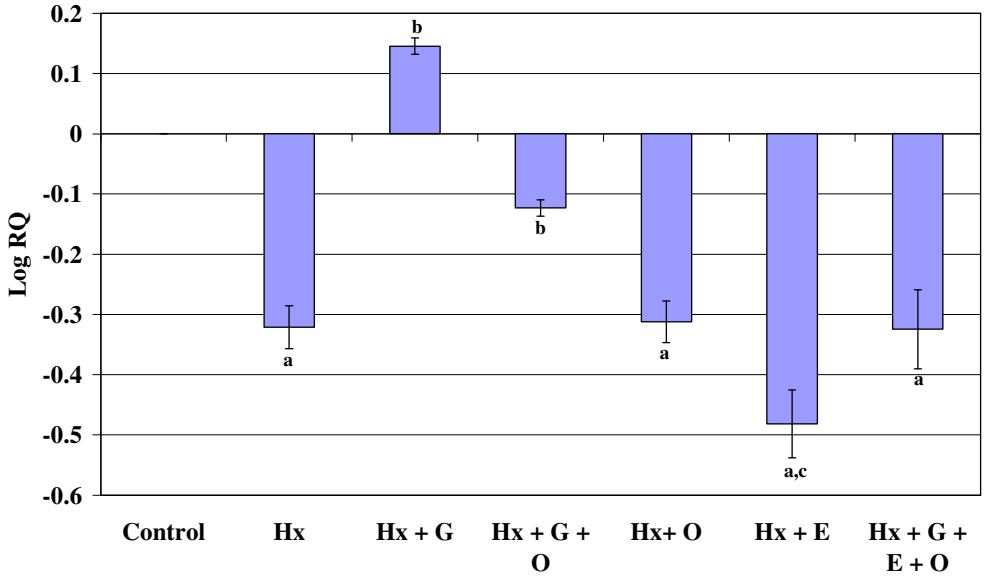


Table- 131
Real Time PCR amplification of $GABA_B$ receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.32 ± 0.04 [a]
Hx + G	0.15 ± 0.01 [b]
Hx + G + O	-0.12 ± 0.01 [b]
Hx + O	-0.31 ± 0.03 [a]
Hx + E	-0.48 ± 0.06 [a]
Hx + G + E + O	-0.32 ± 0.07 [a, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$, [c] $p<0.05$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 128
Real Time PCR amplification of GAD mRNA from the corpus striatum of control and experimental neonatal rats

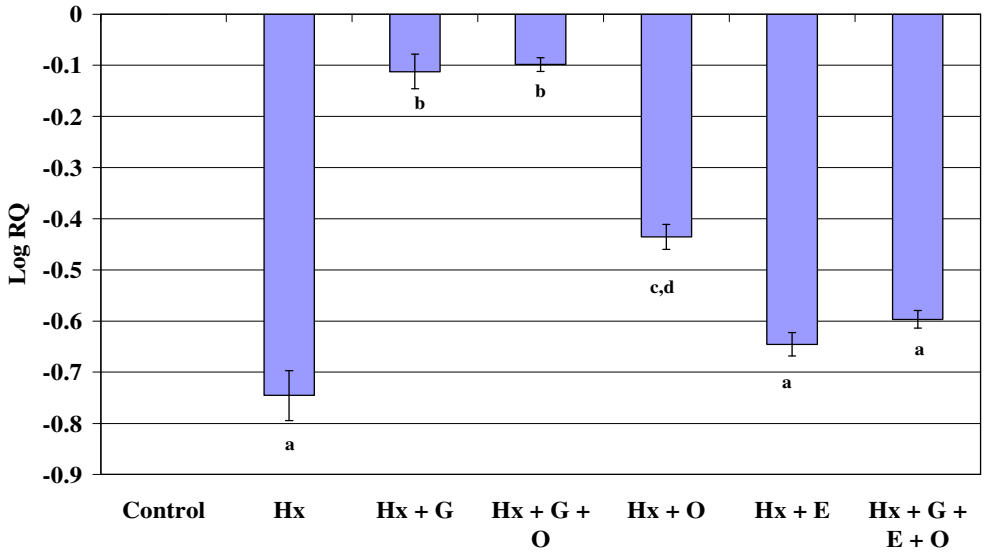


Table- 132
Real Time PCR amplification of GAD mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.75 ± 0.04 [a]
Hx + G	-0.11 ± 0.03 [b]
Hx + G + O	-0.10 ± 0.01 [b]
Hx + O	-0.44 ± 0.02 [c,d]
Hx + E	-0.65 ± 0.02 [a]
Hx + G + E + O	-0.60 ± 0.02 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$when compared to Control
[b] $p<0.001$, [c] $p<0.05$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 129
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the corpus striatum of control and experimental neonatal rats.

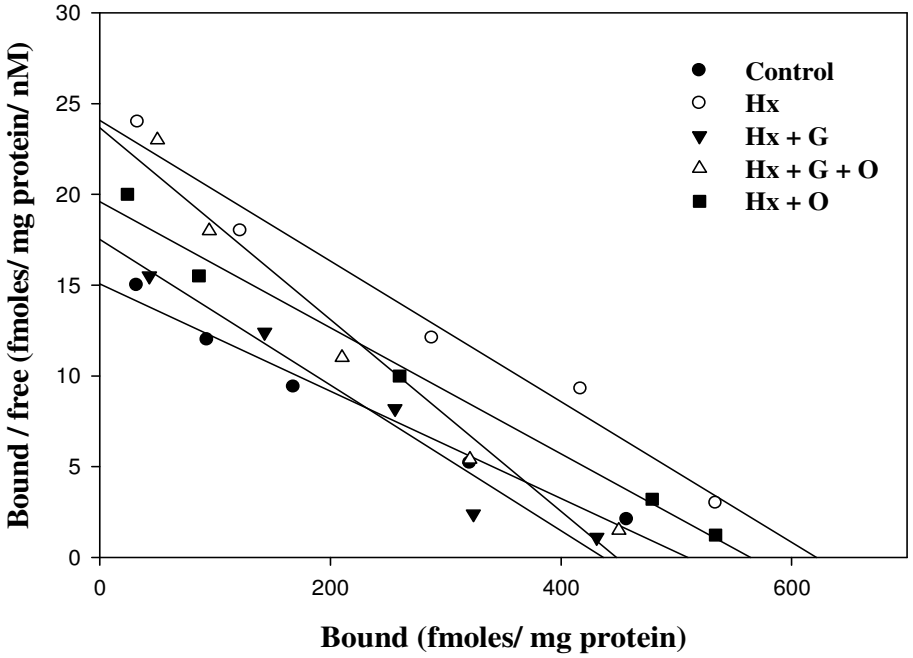


Table- 133
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the corpus striatum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	522.06 ± 5.0	36.51 ± 2.50
Hx	618.38 ± 6.5 [a]	25.59 ± 1.40 [a]
Hx + G	433.16 ± 4.2 [b]	24.75 ± 2.55 [c]
Hx + G + O	443.38 ± 5.5 [b]	18.71 ± 2.05 [a]
Hx + O	560.00 ± 4.0 [c]	28.43 ± 2.00 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$, [c] $p<0.05$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O.

Figure- 130
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the corpus striatum of control and experimental neonatal rats

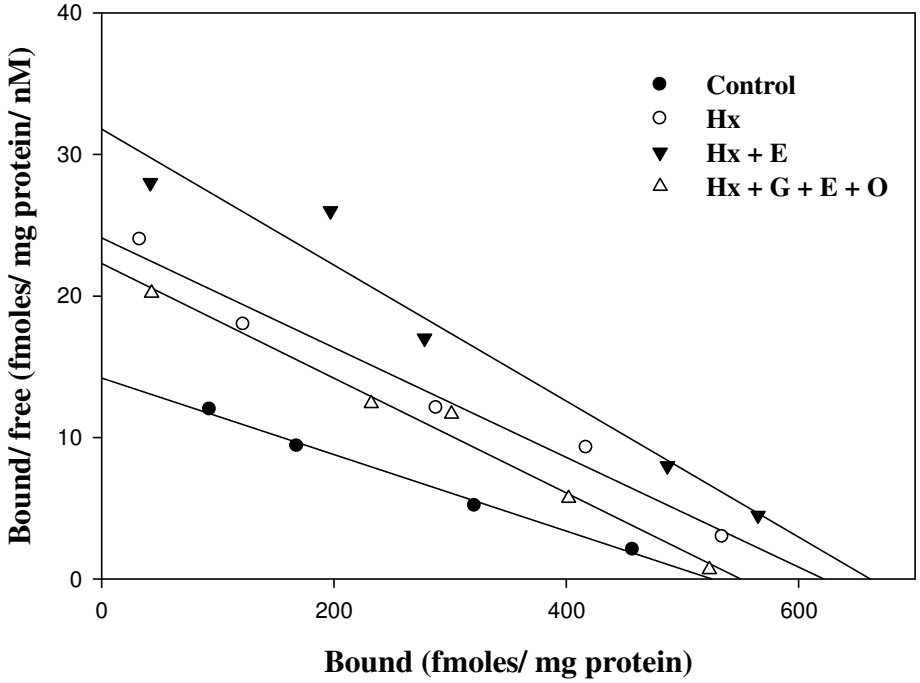


Table- 134
Scatchard analysis of [^{3}H] 5- HT binding against 5-HT to total 5-HT receptor in the corpus striatum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	522.06 ± 5.00	36.51 ± 2.50
Hx	618.38 ± 6.50 [a]	25.59 ± 1.40 [a]
Hx + E	660.66 ± 5.50 [a]	20.65 ± 1.10 [a]
Hx + G + E + O	546.98 ± 5.00 [b]	24.42 ±1.50 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 131
Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the corpus straitum of control and experimental neonatal rats.

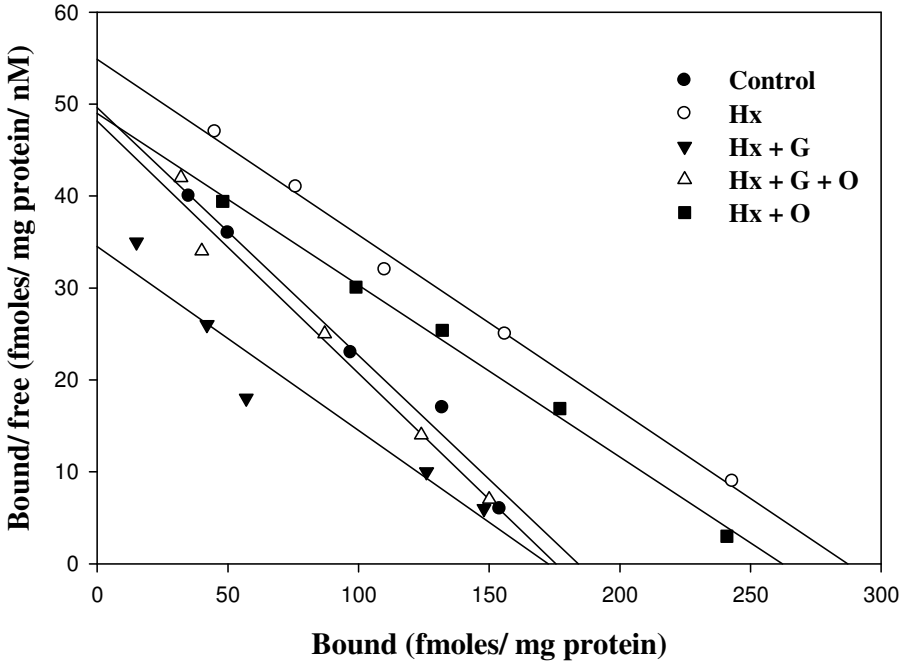


Table- 135
Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the corpus striatum and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	184.38 ± 4.10	3.70 ± 1.14
Hx	287.50 ± 5.50 [a]	5.21 ± 1.04 [a]
Hx + G	172.50 ± 3.80 [b]	4.96 ± 1.10 [c]
Hx + G + O	175.02 ± 4.20 [b]	3.63 ± 1.15 [b]
Hx + O	261.90 ± 4.30 [a]	5.39 ± 1.24 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$, [c] $p<0.05$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O.

Figure- 132
Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the corpus striatum of control and experimental neonatal rats.

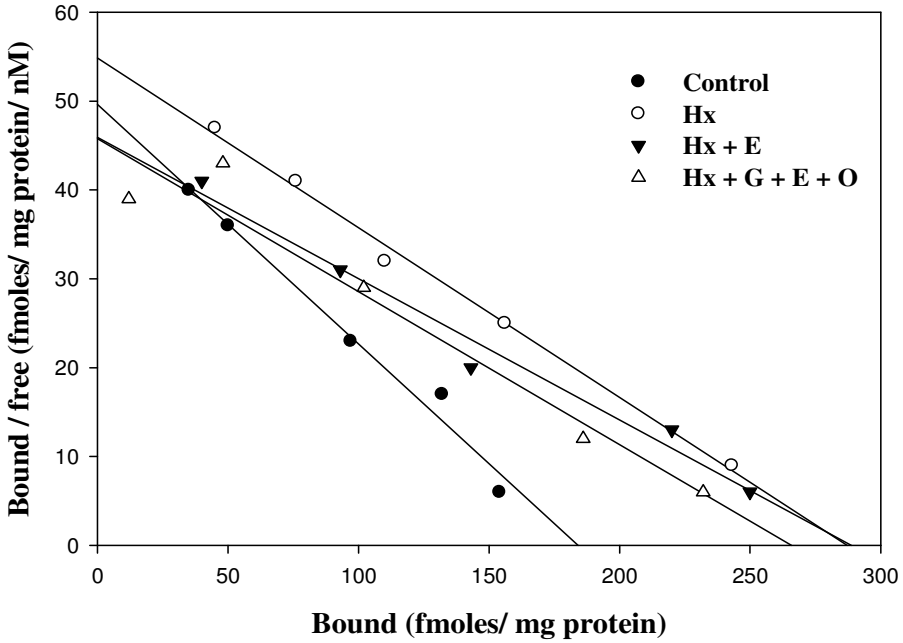


Table- 136
Scatchard analysis of [^{3}H] ketanserin binding against ketanserin to 5-HT_{2A} receptor in the corpus striatum and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	184.38 ± 4.1	3.70 ± 1.14
Hx	287.50 ± 5.5 [a]	5.21 ± 1.04 [a]
Hx + E	287.52 ± 6.5 [a]	6.29 ± 1.10 [a]
Hx + G + E + O	265.62 ± 5.8 [a]	5.79 ±1.12 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$, [c] $p<0.05$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O.

Figure- 133
Real Time PCR amplification of 5-HT_{2A} receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

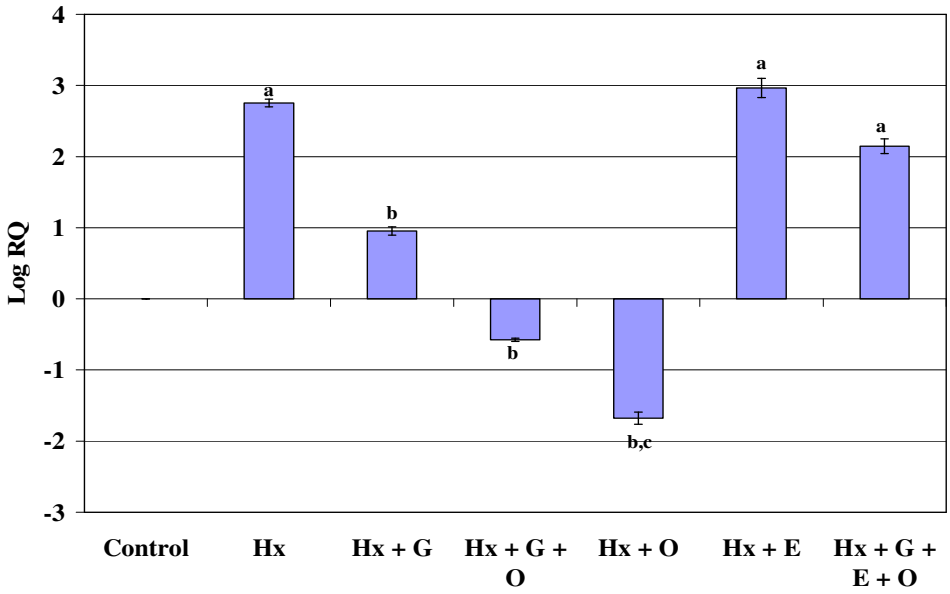


Table- 137
Real Time PCR amplification of 5-HT_{2A} receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	2.75 ± 0.05 [a]
Hx + G	0.95 ± 0.06 [b]
Hx + G + O	-0.58 ± 0.02 [b]
Hx + O	-1.68 ± 0.09 [b, c]
Hx + E	2.97 ± 0.13 [a]
Hx + G + E + O	2.15 ± 0.10 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$, [c] $p<0.05$ when compared to Control
[b] $p<0.001$when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 134
Real Time PCR amplification of 5-HT transporter mRNA from the corpus striatum of control and experimental neonatal rats

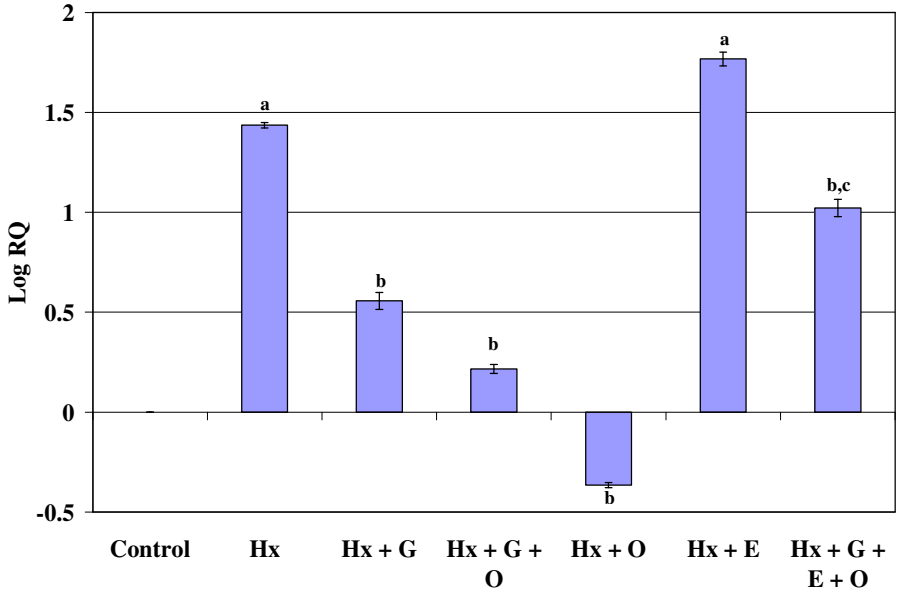


Table- 138
Real Time PCR amplification of 5-HT transporter mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	1.44 ± 0.01[a]
Hx + G	0.56 ± 0.04 [b]
Hx + G + O	0.22 ± 0.02 [b]
Hx + O	-0.37 ± 0.01 [b]
Hx + E	1.77 ± 0.03 [a]
Hx + G + E + O	1.02 ± 0.04 [b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] p<0.001, [c] p<0.05 when compared to Control
[b] p<0.001when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 135
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the corpus striatum of control and experimental neonatal rats.

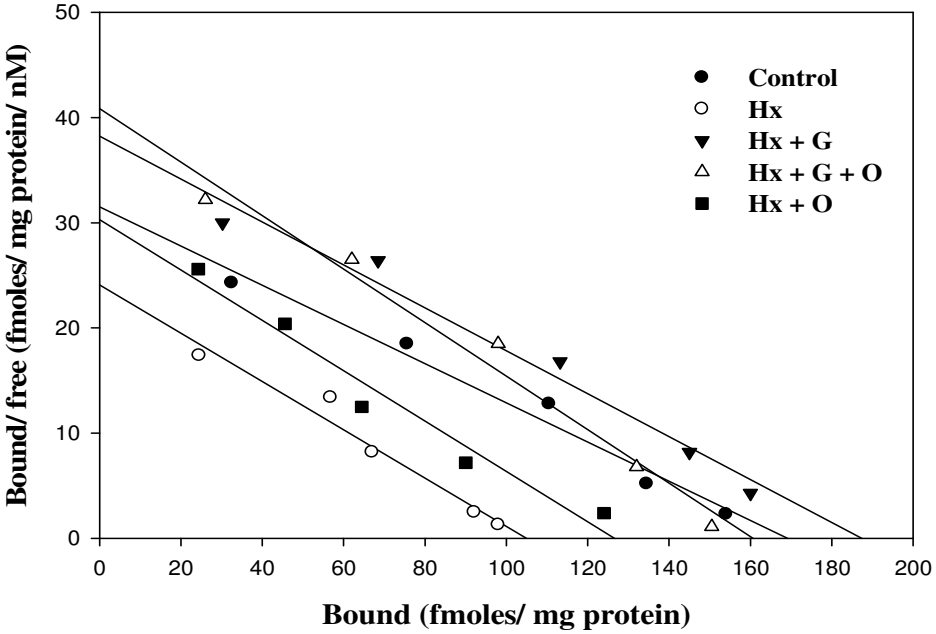


Table- 139
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the corpus striatum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	169.16 ± 3.60	5.40 ± 0.14
Hx	104.60 ± 3.80 [a]	4.29 ± 0.12 [a]
Hx + G	187.52 ± 2.90 [b]	4.88 ± 0.10 [a]
Hx + G + O	160.44 ± 3.80 [b]	3.93 ± 0.15 [a]
Hx + O	125.84 ± 2.50 [a]	4.19 ± 0.14 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure- 136
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the corpus striatum of control and experimental neonatal rats.

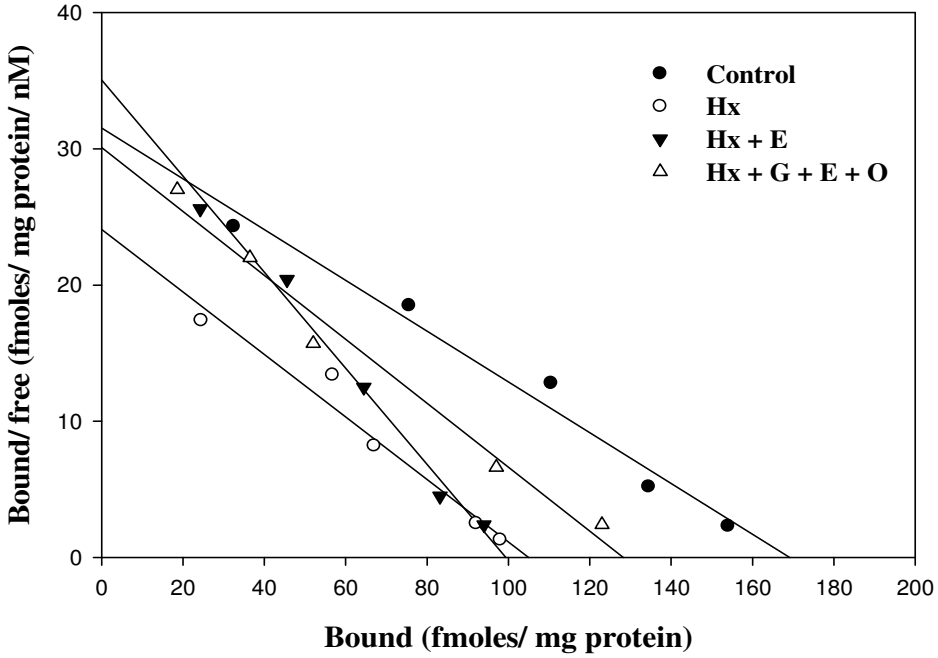


Table- 140
Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the corpus striatum of control and experimental neonatal rats.

Experimental groups	Bmax (fmoles/mg protein)	Kd (nM)
Control	169.16 ± 3.60	5.40 ± 0.14
Hx	104.60 ± 3.80 [a]	4.29 ± 0.12 [a]
Hx + E	100.00 ± 2.60 [a]	2.86 ± 0.10 [a]
Hx + G + E + O	127.92 ± 4.50 [a]	4.26 ± 0.12 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared with control
[b] $p<0.001$ when compared with hypoxic group.
Hypoxic rats- Hx, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 137

Real Time PCR amplification of Muscarinic M_1 receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

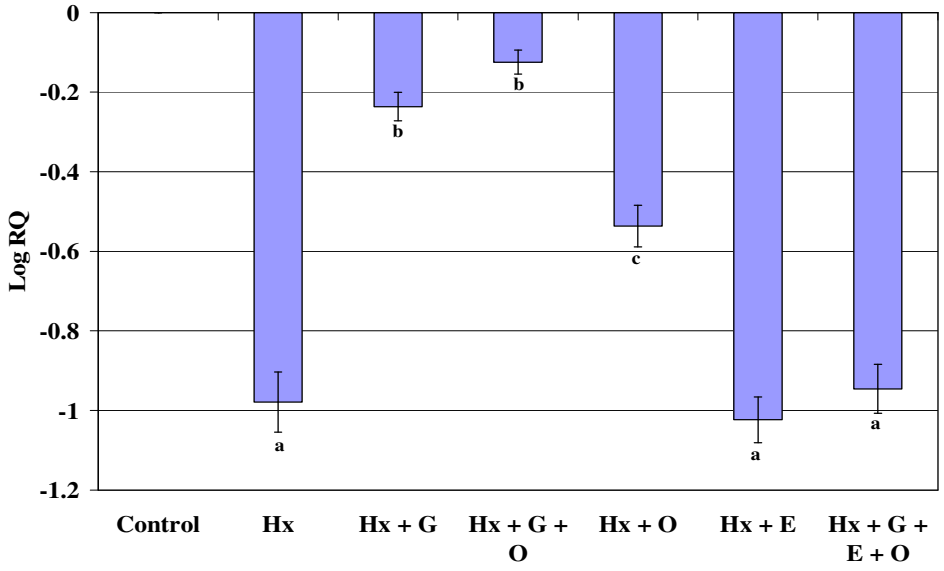


Table- 141

Real Time PCR amplification of Muscarinic M_1 receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.98 ± 0.08 [a]
Hx + G	-0.24 ± 0.04 [b]
Hx + G + O	-0.12 ± 0.03 [b]
Hx + O	-0.54 ± 0.05 [c]
Hx + E	-1.02 ± 0.06 [a]
Hx + G + E + O	-0.95 ± 0.06 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 138
Real Time PCR amplification of Muscarinic M_2 receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

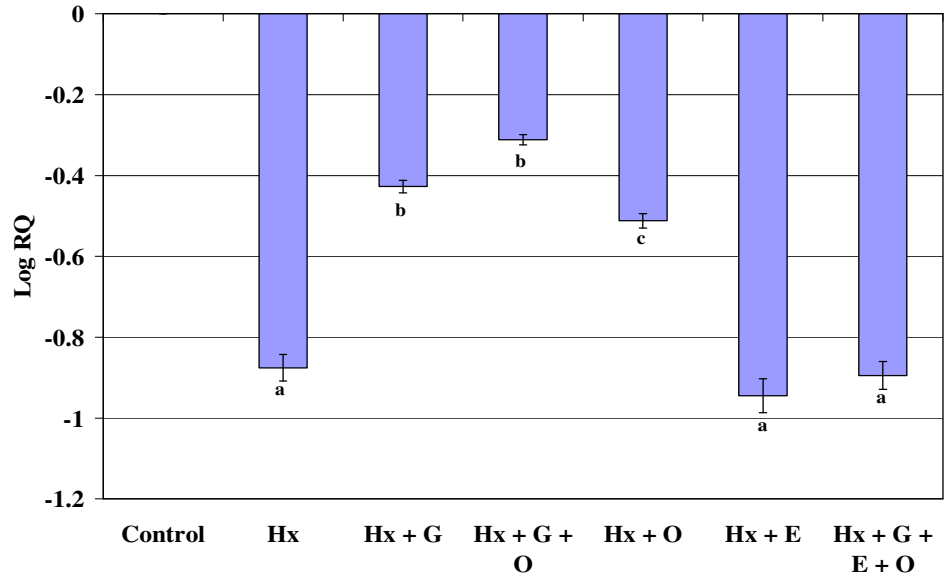


Table- 142
Real Time PCR amplification of Muscarinic M_2 receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.88 ± 0.03 [a]
Hx + G	-0.43 ± 0.02 [b]
Hx + G + O	-0.31 ± 0.01 [b]
Hx + O	-0.51 ± 0.02 [c]
Hx + E	-0.94 ± 0.04 [a]
Hx + G + E + O	-0.89 ± 0.03 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 139
Real Time PCR amplification of Muscarinic M_3 receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

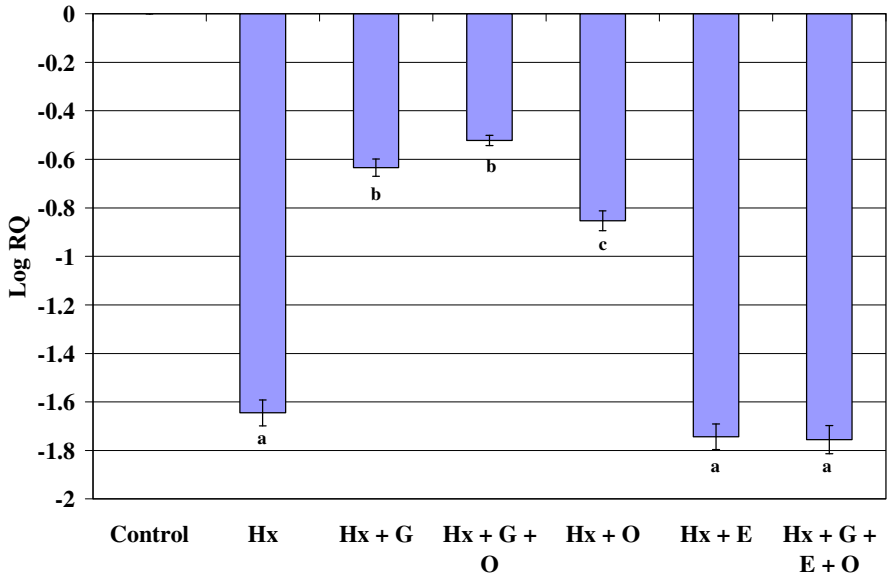


Table- 143
Real Time PCR amplification of Muscarinic M_3 receptor subunit mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-1.65 ± 0.05 [a]
Hx + G	-0.63 ± 0.04 [b]
Hx + G + O	-0.52 ± 0.02 [b]
Hx + O	-0.85 ± 0.04 [c]
Hx + E	-1.74 ± 0.05 [a]
Hx + G + E + O	-1.76 ± 0.06 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 140

Real Time PCR amplification of acetylcholine esterase mRNA from the corpus striatum of control and experimental neonatal rats.

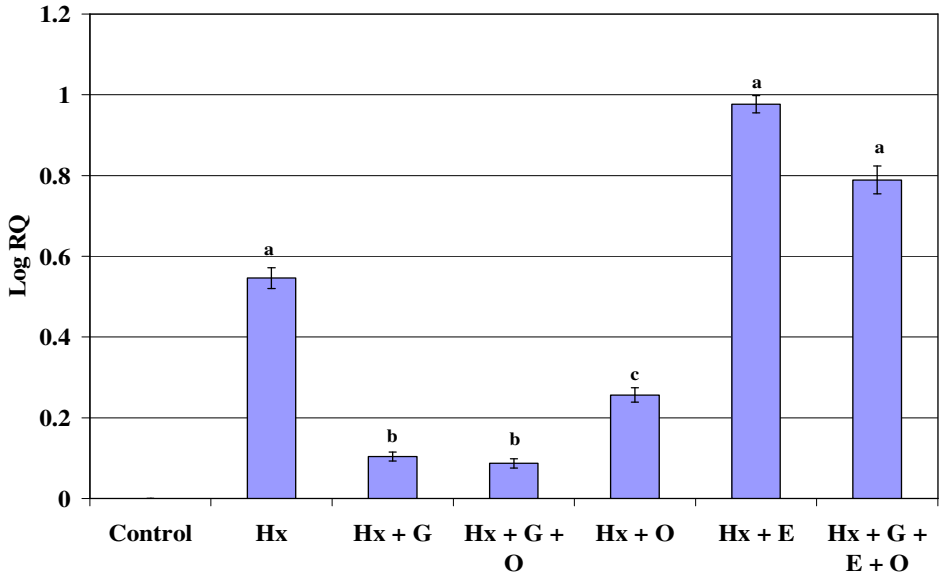


Table- 144

Real Time PCR amplification of acetylcholine esterase mRNA from the corpus striatum of control and experimental neonatal rats.

Experimental groups	Log RQ
Control	0
Hx	0.55 ± 0.05 [a]
Hx + G	0.10 ± 0.04 [b]
Hx + G + O	0.09 ± 0.02 [b]
Hx + O	0.26 ± 0.04 [c]
Hx + E	0.98 ± 0.05 [a]
Hx + G + E + O	0.79 ± 0.06 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 141

Real Time PCR amplification of choline acetyl transferase mRNA from the corpus striatum of control and experimental neonatal rats.

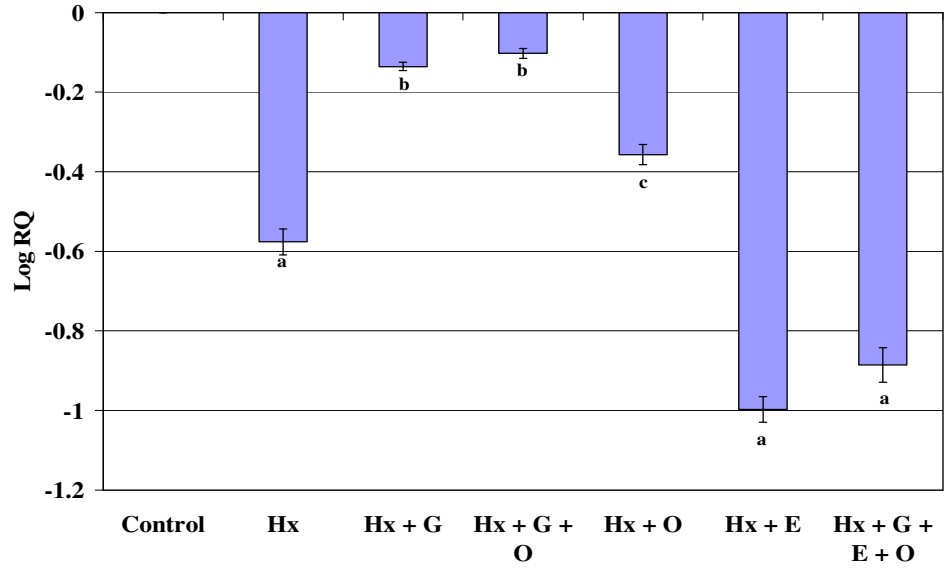


Table- 145

Real Time PCR amplification of acetylcholine esterase mRNA from the corpus striatum of control and experimental neonatal rats.

Experimental groups	Log RQ
Control	0
Hx	-0.58 ± 0.03 [a]
Hx + G	-0.14 ± 0.01 [b]
Hx + G + O	-0.10 ± 0.01 [b]
Hx + O	-0.36 ± 0.03 [c]
Hx + E	-0.10 ± 0.03 [a]
Hx + G + E + O	-0.89 ± 0.04 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 142
Real Time PCR amplification of Hif 1 mRNA from the corpus striatum of control and experimental neonatal rats.

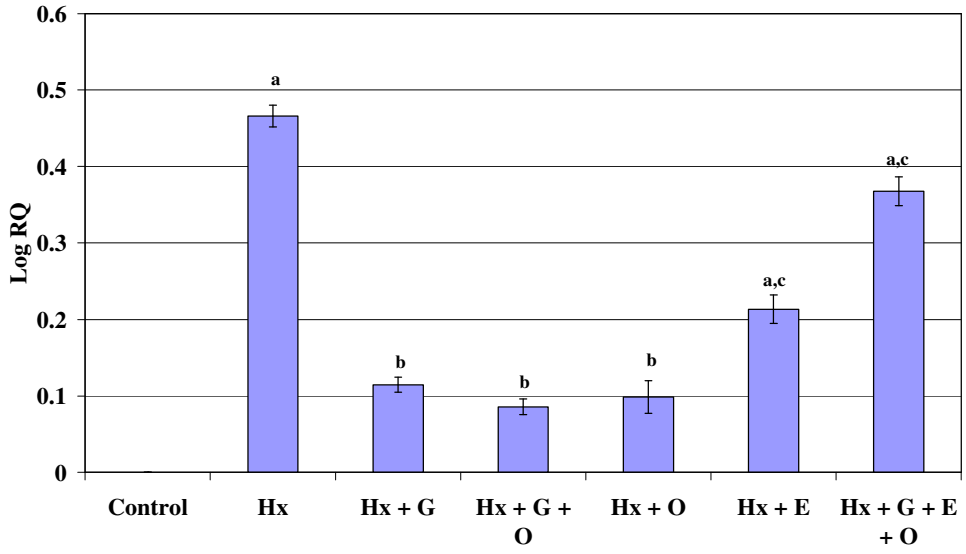


Table- 146
Real Time PCR amplification of Hif 1 mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	0.47 ± 0.01 [a]
Hx + G	0.11 ± 0.01 [b]
Hx + G + O	0.09 ± 0.01 [b]
Hx + O	0.10 ± 0.02 [b]
Hx + E	0.21 ± 0.02 [a, c]
Hx + G + E + O	0.37 ± 0.02 [a, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.05$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 143
Real Time PCR amplification of SOD mRNA from the corpus striatum of control and experimental neonatal rats.

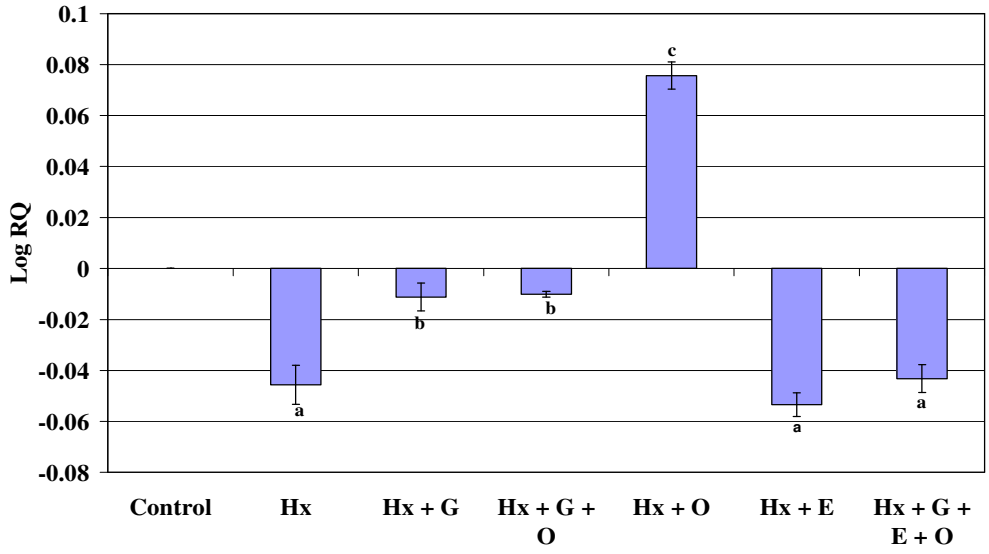


Table- 147
Real Time PCR amplification of SOD mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.046 ± 0.008 [a]
Hx + G	-0.011 ± 0.005 [b]
Hx + G + O	-0.010 ± 0.001 [b]
Hx + O	0.076 ± 0.005 [c]
Hx + E	-0.053 ± 0.004 [a]
Hx + G + E + O	-0.043 ± 0.005 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.01$, [c] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 144
Real Time PCR amplification of GPx mRNA from the corpus striatum of control and experimental neonatal rats.

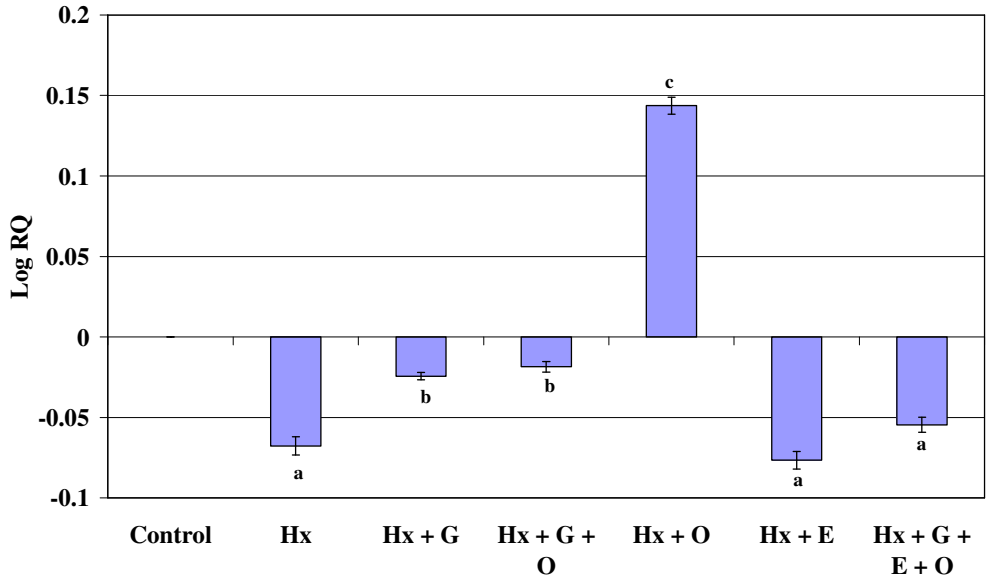


Table- 148
Real Time PCR amplification of GPx mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-0.067 ± 0.006 [a]
Hx + G	-0.024 ± 0.002 [b]
Hx + G + O	-0.018 ± 0.003 [b]
Hx + O	0.144 ± 0.005 [c]
Hx + E	-0.077 ± 0.005 [a]
Hx + G + E + O	-0.055 ± 0.005 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.01$, [c] $p<0.001$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 145
Real Time PCR amplification of BAX mRNA from the corpus striatum of control and experimental neonatal rats

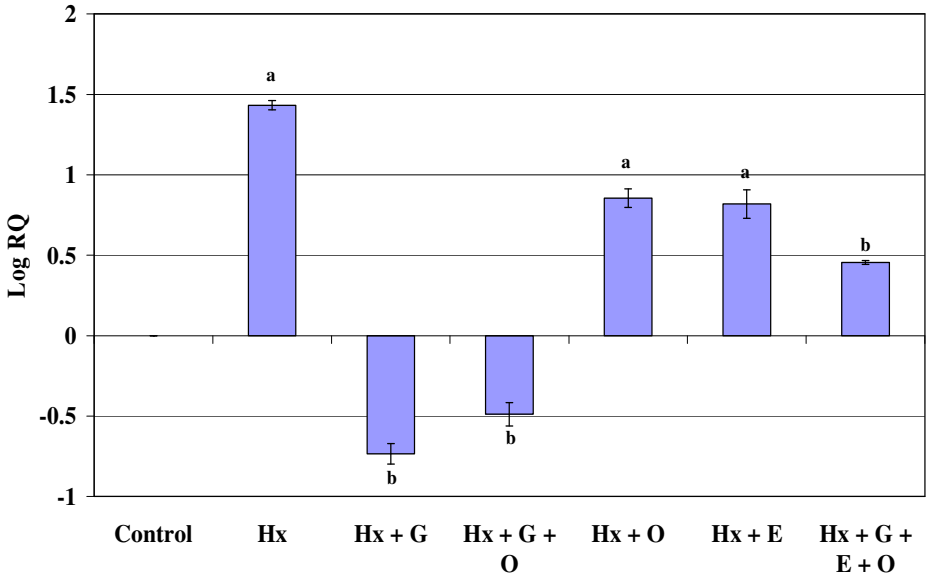


Table- 149
Real Time PCR amplification of BAX mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	1.43 ± 0.03 [a]
Hx + G	-0.73 ± 0.06 [b]
Hx + G + O	-0.49 ± 0.07 [b]
Hx + O	0.85 ± 0.06 [a]
Hx + E	0.82 ± 0.09 [a]
Hx + G + E + O	0.45 ± 0.01 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.01$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 146
Real Time PCR amplification of CREB mRNA from the corpus striatum of control and experimental neonatal rats

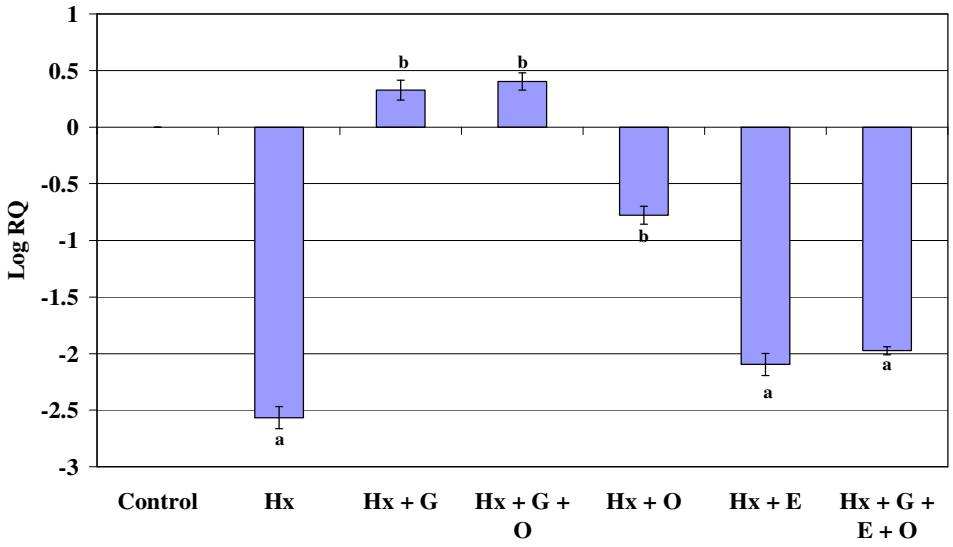


Table- 150
Real Time PCR amplification of CREB mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	-2.57 ± 0.10 [a]
Hx + G	0.33 ± 0.09 [b]
Hx + G + O	0.40 ± 0.08 [b]
Hx + O	-0.78 ± 0.08 [b]
Hx + E	-2.09 ± 0.10 [a]
Hx + G + E + O	-1.97 ± 0.03 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.01$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 147
Real Time PCR amplification of phospholipase C mRNA from the corpus striatum of control and experimental neonatal rats.

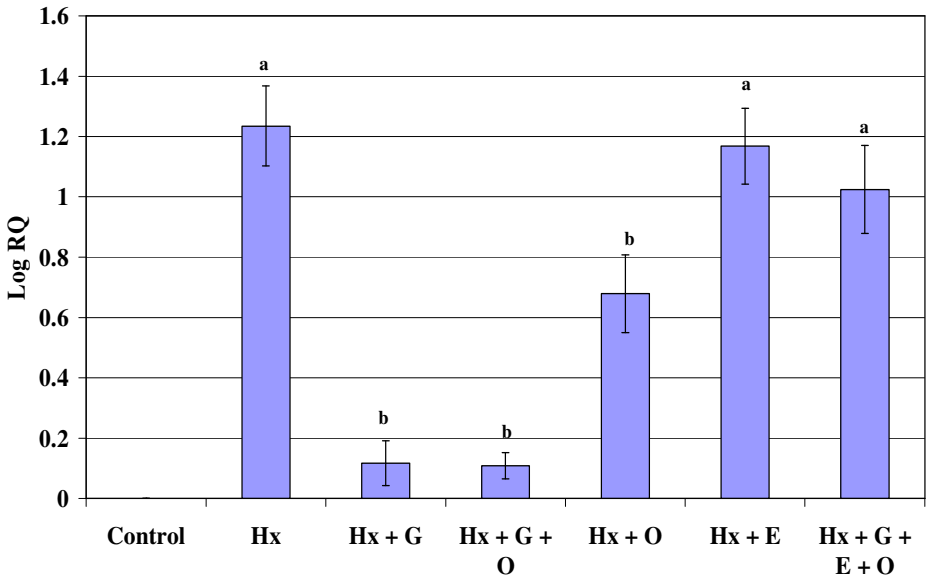


Table- 151
Real Time PCR amplification of phospholipase C mRNA from the corpus striatum of control and experimental neonatal rats

Experimental groups	Log RQ
Control	0
Hx	1.23 ± 0.13 [a]
Hx + G	0.12 ± 0.07 [b]
Hx + G + O	0.11 ± 0.04 [b]
Hx + O	0.68 ± 0.13 [b]
Hx + E	1.17 ± 0.12 [a]
Hx + G + E + O	1.02 ± 0.15 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.01$ when compared to Control
[b] $p<0.001$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure-148
cGMP Content in the Corpus striatum of Control and Experimental Groups of Neonatal Rats

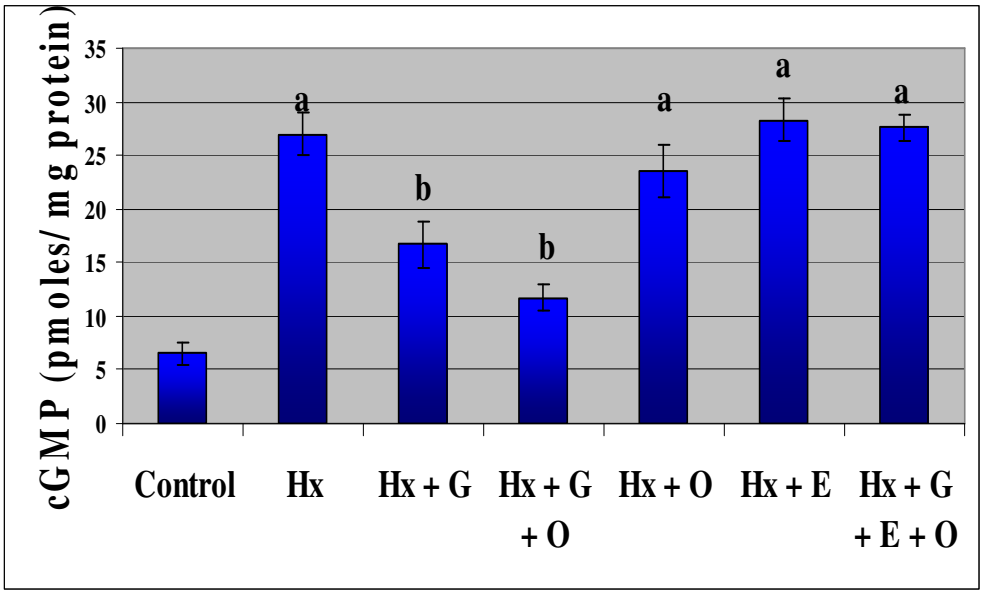


Table-152
cGMP Content in the Corpus striatum of Control and Experimental Groups of Neonatal Rats

Experimental groups	cGMP (pmoles/mg protein)
C	6.57 ± 1.02
Hx	26.97 ± 2.01 [a]
Hx+O	23.46 ± 2.45 [a]
Hx+G	16.67± 2.14 [b]
Hx+G+O	11.69 ± 1.23 [b]
Hx + E	28.30 ± 1.98 [a]
Hx+G+E+O	27.65 ± 1.22 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
[a] $p<0.001$when compared to Control
[b] $p<0.001$ when compared to Hx
Control rats – C, Hypoxic rats- Hx , Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O , Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure-149
cAMP Content in the Corpus striatum of Control and Experimental Groups of Neonatal Rats

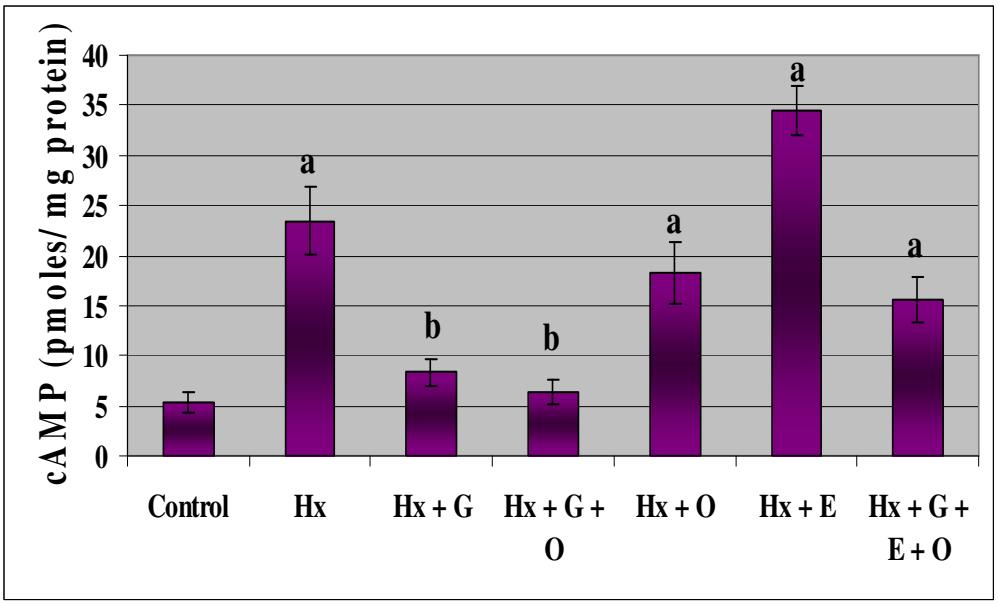


Table-153
cAMP Content in the Corpus striatum of Control and Experimental Groups of Neonatal Rats

Experimental groups	cGMP (pmoles/mg protein)
C	5.31 ± 1.02
Hx	23.48 ± 2.01[a]
Hx+O	18.25 ± 2.45 [a]
Hx+G	8.31 ± 2.14 [b]
Hx+G+O	6.45 ± 1.23 [b]
Hx + E	34.42 ± 1.98 [a]
Hx+G+E+O	15.65 ± 1.22 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
[a] p<0.001when compared to Control
[b] p<0.001 when compared to Hx
Control rats – C, Hypoxic rats- Hx , Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O , Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure-150
IP3 Content in the Corpus striatum of Control and Experimental Groups of Neonatal Rats

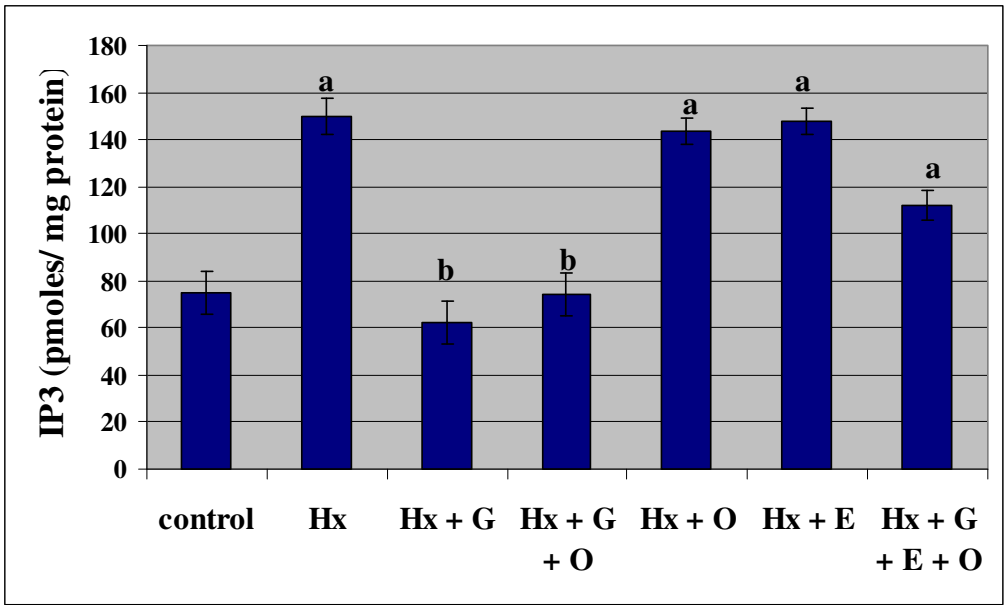


Table-154
IP3 Content in the Corpus striatum of Control and Experimental Groups of Neonatal Rats

Experimental groups	IP3 (pmoles/mg protein)
C	75.00 ± 9.01
Hx	150.00 ± 7.50 [a]
Hx+O	143.50 ± 5.55 [a]
Hx+G	62.40 ± 9.25 [b]
Hx+G+O	74.50 ± 9.05 [b]
Hx + E	148.00 ± 5.65 [a]
Hx+G+E+O	112.02 ± 6.50 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
[a] $p<0.001$when compared to Control
[b] $p<0.001$ when compared to Hx
Control rats – C, Hypoxic rats- Hx , Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O , Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table-155
Body Weight of Experimental Animals (1 month old) used for Behavioural Study

Experimental groups	Body weight (g)
C	75.32 ± 0.35
Hx	65.53 ± 0.74 [a]
Hx+G	72.35 ± 0.57 [d]
Hx+G+O	73.61 ± 0.94 [d]
Hx+O	68.22 ± 0.25 [a]
Hx+G+E+O	63.44 ± 0.54 [b, c]
Hx + E	60.24 ± 0.82 [b, c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
[a] $p<0.01$, [b] $p<0.001$ when compared to Control
[c] $p<0.05$, [d] $p<0.01$when compared to Hx

Control rats - C
Hypoxic rats- Hx
Hypoxic rats glucose treated - Hx+G
Hypoxic rats oxygen treated - Hx+O
Hypoxic rats glucose and oxygen treated - Hx+G+O
Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 151
Behavioural response of one month old control and experimental rats on Y- maze

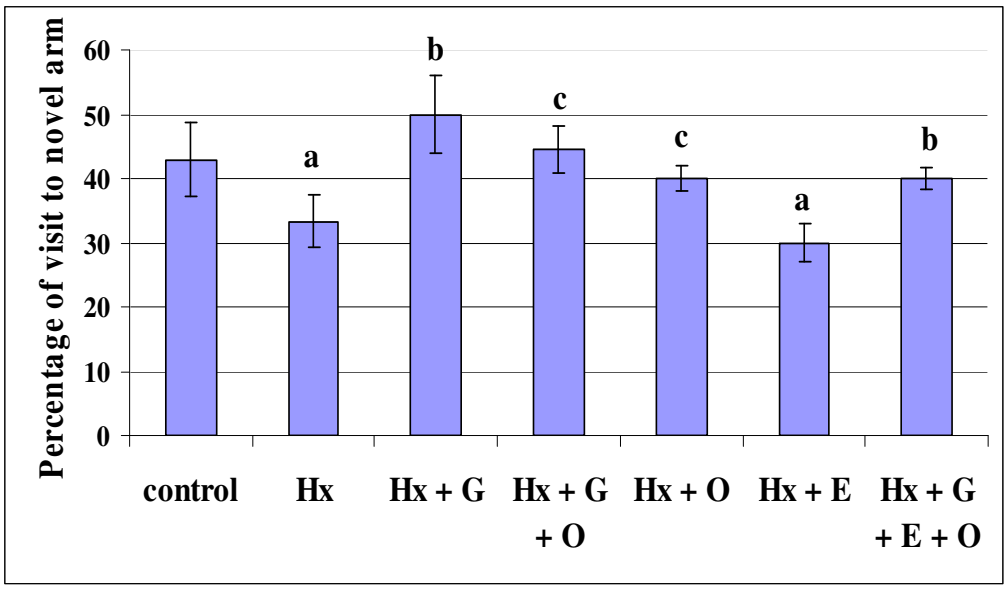


Table- 156
Behavioural response of one month old control and experimental rats on Y- maze

Experimental groups	% of visit to novel arm
Control	42.86 ± 5.80
Hx	33.33 ± 4.12 [a]
Hx + G	50.00 ± 6.11 [c]
Hx + G + O	44.55 ± 3.66 [c]
Hx + O	40.00 ± 2.11 [b]
Hx + E	30.00 ± 2.98 [a]
Hx + G + E + O	40.00 ± 1.65 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ [b] $p<0.01$ when compared to Control
[c] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 152
Behavioural response of one month old control and experimental rats on Y- maze

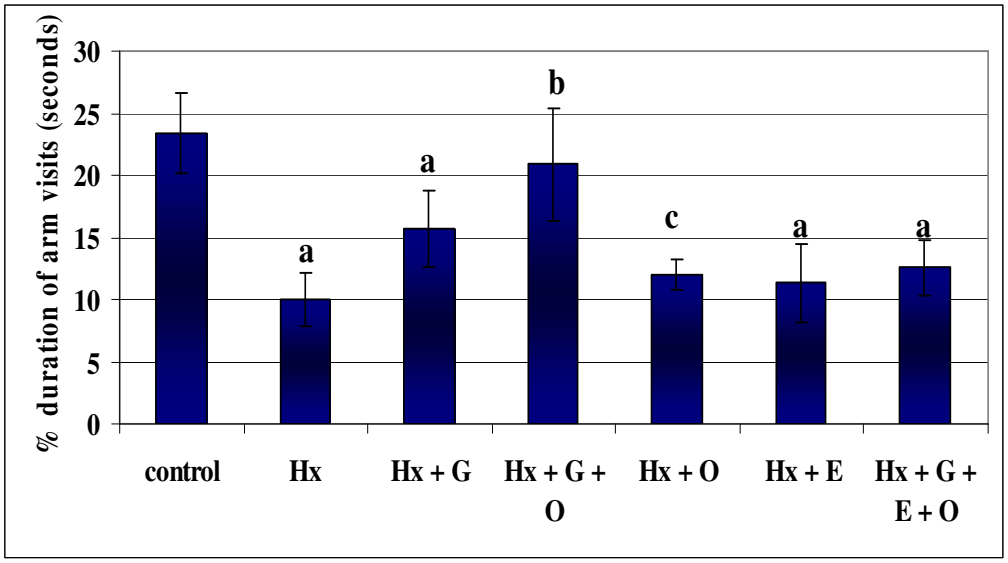


Table- 157
Behavioural response of one month old control and experimental rats on Y- maze

Experimental groups	% duration of arm visits (seconds)
Control	23.33 ± 3.25
Hx	10.05 ± 2.15 [a]
Hx + G	15.65 ± 3.05 [c]
Hx + G + O	20.87 ± 4.51 [b]
Hx + O	12.05 ± 1.25 [a]
Hx + E	11.33 ± 3.11 [a]
Hx + G + E + O	12.56 ± 2.18 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 153
Number of trials to criteria of one month old control and experimental rats on Radial arm maze

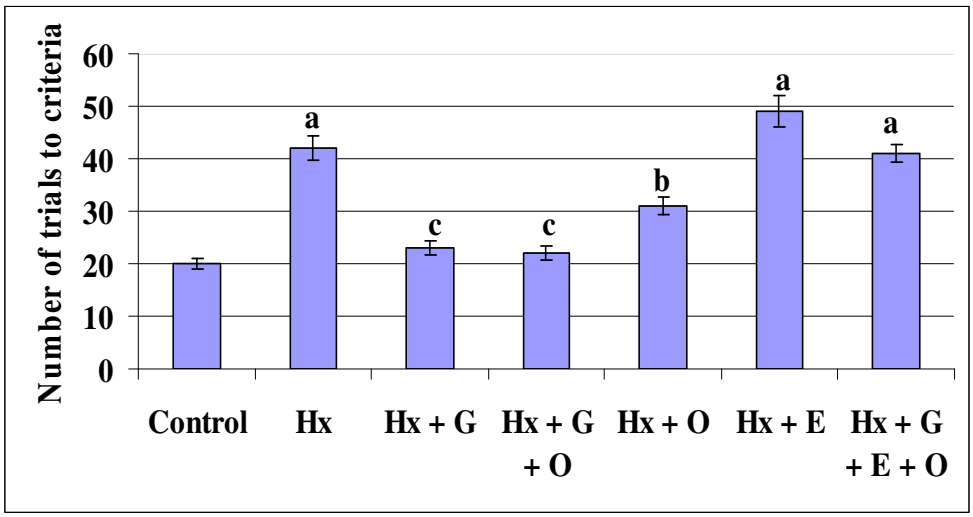
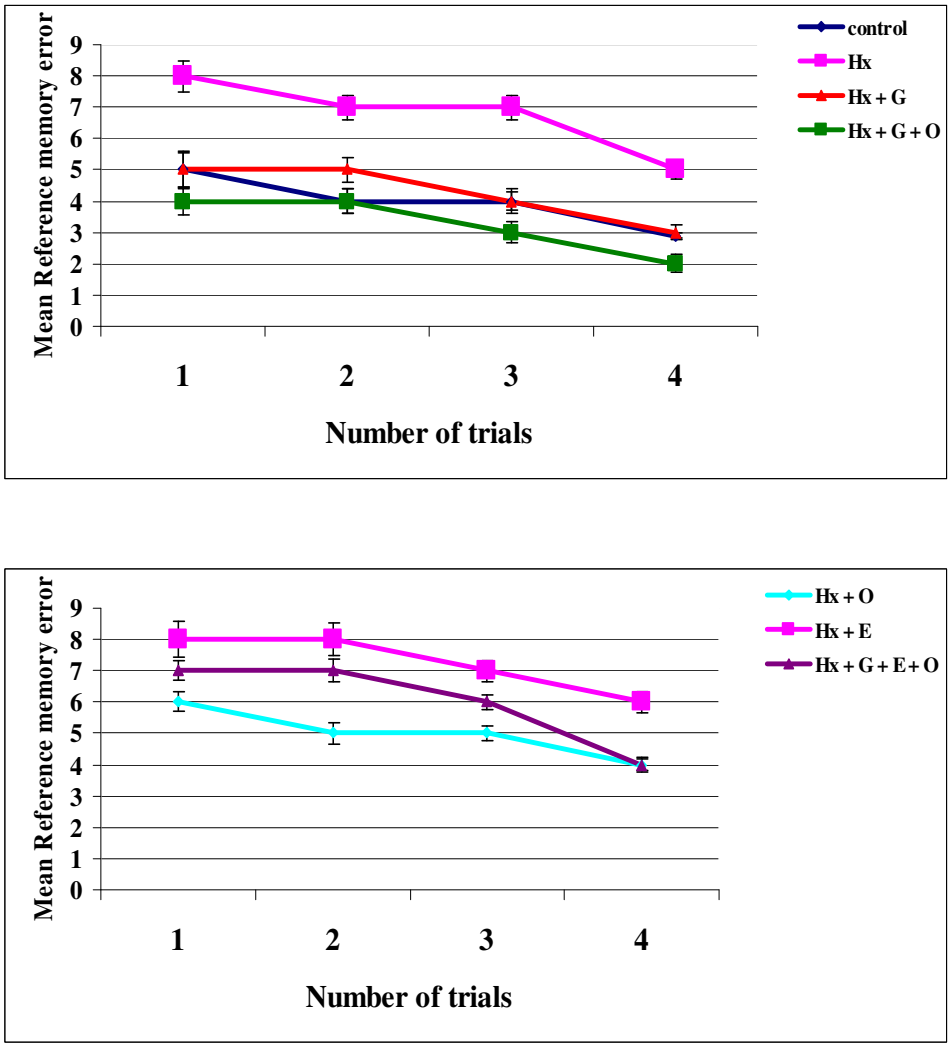


Table- 158
Behavioural response of one month old control and experimental rats on Radial arm maze

Experimental groups	Number of trials to criteria
Control	20.00 ± 1.10
Hx	42.00 ± 2.20 [a]
Hx + G	23.00 ± 1.30 [b]
Hx + G + O	22.00 ± 1.45 [b]
Hx + O	31.00 ± 1.80 [c]
Hx + E	49.00 ± 3.10 [a]
Hx + G + E + O	41.00 ± 1.65 [b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.01$, [b] $p<0.001$ when compared to Control
[c] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 154
Mean Reference memory error of one month old control and experimental rats on Radial arm maze



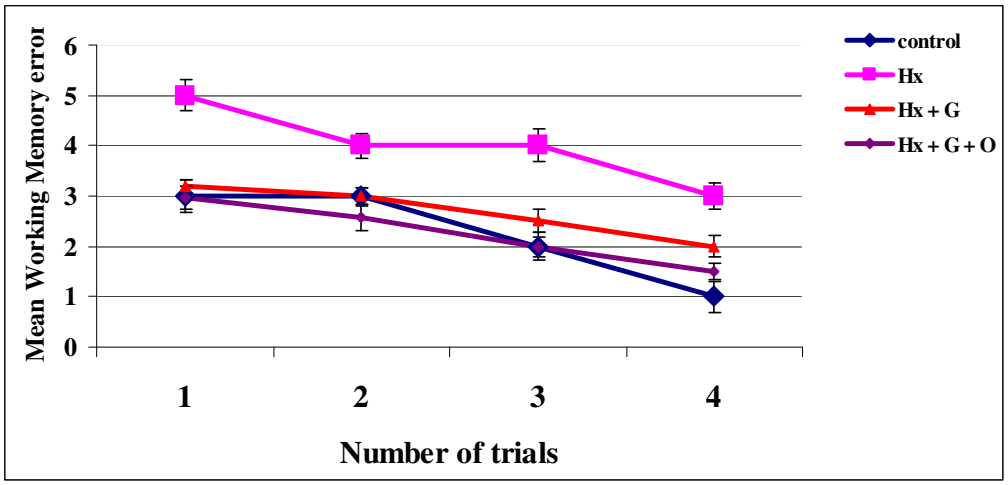
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

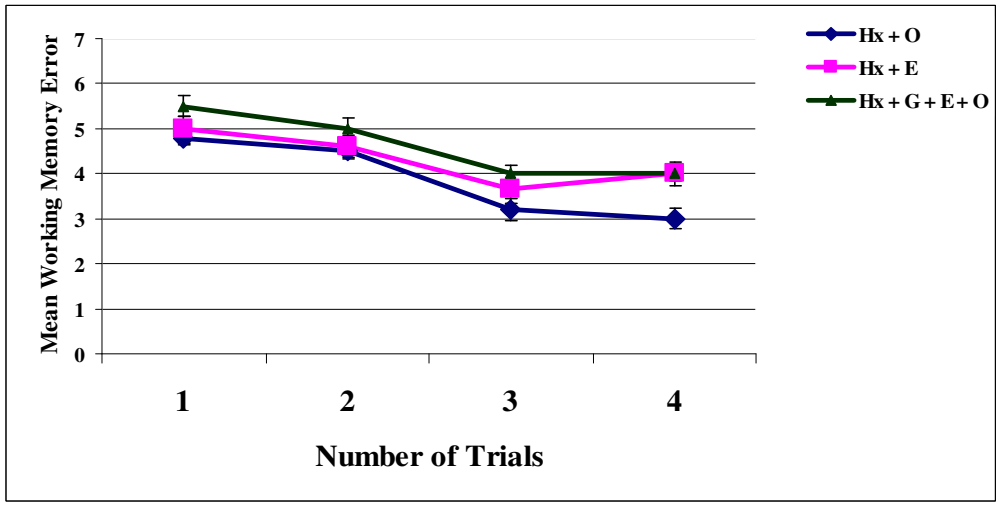
Table- 159
Mean Reference memory error of one month old control and experimental rats on Radial arm maze

Experimental group	1st Trial	2nd Trial	3rd Trial	4th Trial
Control	5.0±0.60	4.0±0.40	4.0±0.30	3.0±0.10
Hx	8.0±0.50 [a]	7.0±0.40 [a]	7.0±0.40 [a]	5.0±0.30 [a]
Hx + G	5.0±0.54 [b]	5.0±0.41 [b]	4.0±0.39 [b]	3.0±0.22 [b]
Hx + G + O	4.0±0.43 [b]	4.0±0.41 [b]	3.0±0.33 [b]	2.0±0.29 [b]
Hx + O	6.0±0.31 [a,b]	5.0±0.33 [a,b]	5.0±0.23 [a,b]	4.0±0.19 [a,b]
Hx + E	8.0±0.56 [a]	8.0±0.54 [a]	7.0±0.33 [a]	6.0±0.34 [a]
Hx + G + E + O	7.0±0.30 [a]	7.0±0.37 [a]	6.0±0.22 [a]	4.0±0.24 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.01$ when compared to Control
[b] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 155
Mean working memory error of one month old control and experimental rats on Radial arm maze





Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Table- 160
Mean working memory error of one month old control and experimental rats on Radial arm maze

Experimental group	1st Trial	2nd Trial	3rd Trial	4th Trial
Control	3.0 ±0.32	3.0 ±0.16	2.0 ±0.28	1.0 ±0.31
Hx	5.0 ±0.30 [a]	4.0±0.24 [a]	4.0±0.33 [a]	3.0±0.26 [a]
Hx + G	3.2±0.14 [b]	3.0±0.16 [b]	2.5±0.23 [b]	2.0±0.22 [b]
Hx + G + O	3.0±0.23 [b]	2.6±0.25 [b]	2.0±0.20 [b]	1.5±0.16 [b]
Hx + O	4.8±0.30 [a]	4.5±0.24 [a]	3.2±0.33 [a]	3.0±0.26 [a]
Hx + E	5.0±0.14 [a]	4.6±0.16 [a]	3.7±0.23 [a]	4.0±0.22 [a]
Hx + G + E + O	5.5±0.23 [a]	5.0±0.25 [a]	4.0±0.20 [a]	4.0±0.16 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.01$ when compared to Control
[b] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 156
Escape latency of one month old control and experimental rats in Morris Water maze experiment

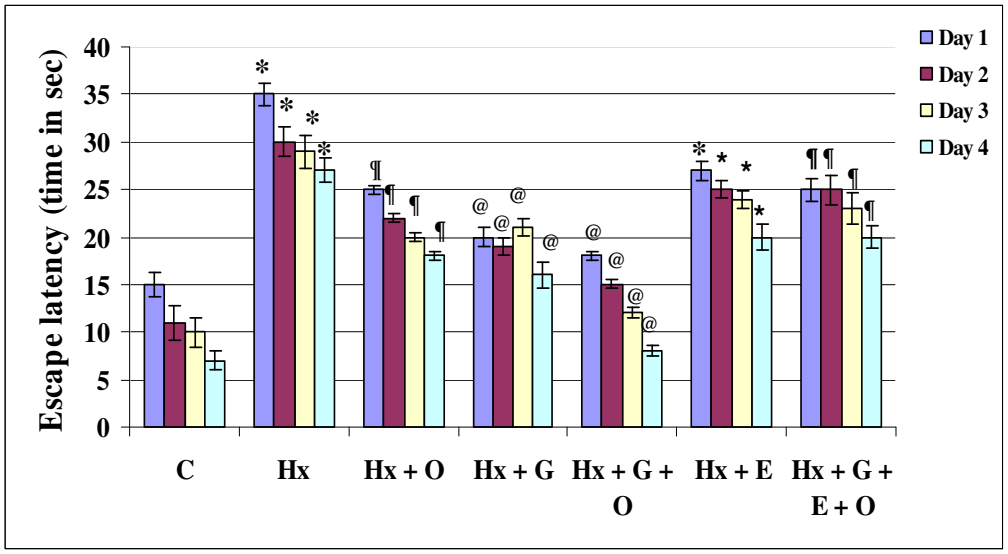


Table- 161
Escape latency of one month old control and experimental rats in Morris Water maze experiment

Experimental groups	1st Trial	2nd Trial	3rd Trial	4th Trial
Control	15.0±1.34	11±1.78	10±1.55	7±0.98
Hx	35.6±1.21 *	30±1.56 *	29±1.71 *	27±1.22 *
Hx + O	25.4±0.45 ¶	22±0.44 ¶	20±0.52 ¶	18±0.51 ¶
Hx + G	20.4±0.98 @	19±0.87 @	21±0.90 @	16±1.33 @
Hx + G + O	27.2±1.25 @	23±1.42 @	17±1.20 @	15±1.32 @
Hx + E	27.0±0.46 *	25±0.48 *	24±0.55 *	20±0.65 *
Hx + G + E + O	25.4±1.10 ¶	25±0.99 ¶	23±1.12 ¶	20±1.50 ¶

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
* p<0.001 ¶ p< 0.01 when compared to Control
@ p<0.001 when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure- 157
Time spent in platform quadrant by one month old control and experimental rats in Morris Water maze experiment

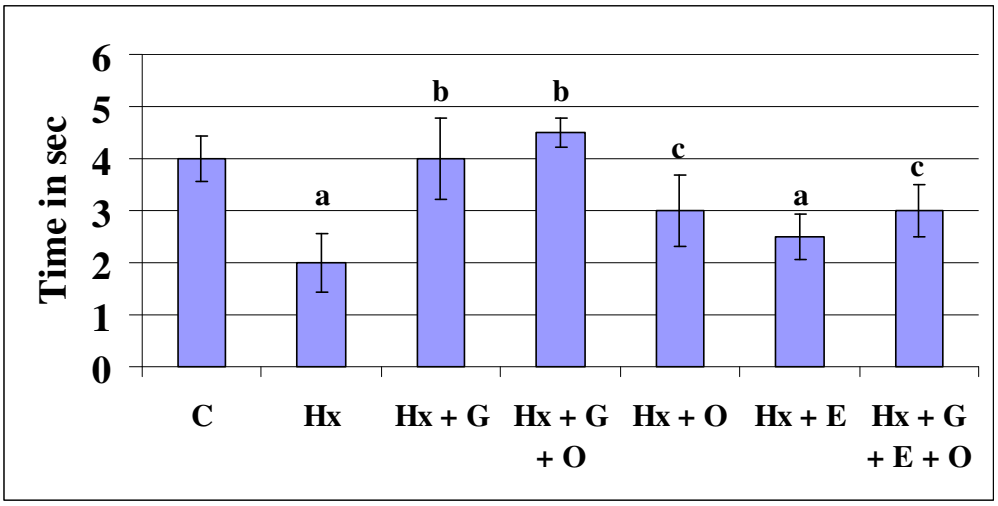


Table- 162
Time spent in platform quadrant by one month old control and experimental rats in Morris Water maze experiment

Experimental groups	Number of trials to criteria
Control	4.00 ± 0.43
Hx	2.00 ± 0.56 [a]
Hx + O	3.00 ± 0.69 [c]
Hx + G	4.00 ± 0.77 [b]
Hx + G + O	4.50 ± 0.28 [b]
Hx + E	2.50 ± 0.44 [a]
Hx + G + E + O	3.00 ± 0.51 [c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 neonatal rats.
[a] $p<0.001$ when compared to Control
[b] $p<0.001$, [c] $p<0.01$ when compared to hypoxic group
Hypoxic rats- Hx, Hypoxic rats glucose treated - Hx+G, Hypoxic rats oxygen treated - Hx+O, Hypoxic rats glucose and oxygen treated - Hx+G+O, Hypoxic rats epinephrine treated – Hx + E, Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure Legends

Figure 24

Confocal image of $GABA_{A\alpha1}$ receptor subunit expression in the cerebral cortex of control and experimental neonatal rats using immunofluorescent $GABA_{A\alpha1}$ receptor specific primary antibody and rhodamine coated secondary antibody. ⟶ in white shows $GABA_{A\alpha1}$ receptors. There was a significant decrease of $GABA_{A\alpha1}$ receptor subunit in hypoxic neonatal rats which was reversed to near control in glucose resuscitated groups.

Figure 31

Confocal image of $5\text{-}HT_{2A}$ receptor expression in the cerebral cortex of control and experimental neonatal rats using immunofluorescent $5\text{-}HT_{2A}$ receptor specific primary antibody and FITC tagged secondary antibody. ⟶ in white shows $5\text{-}HT_{2A}$ receptors. There was a significant increase of $5\text{-}HT_{2A}$ receptor subunit in hypoxic neonatal rats which was reversed to near control in glucose resuscitated groups.

Figure 32

Confocal image of 5-HT transporter expression in the cerebral cortex of control and experimental neonatal rats using immunofluorescent 5-HT transporter specific primary antibody and FITC tagged secondary antibody. ⟶ in white shows 5-HT transporters. There was a significant increase of 5-HT transporter in hypoxic neonatal rats which was reversed to near control in glucose resuscitated groups.

Figure 59

Confocal image of $GABA_{A\alpha1}$ receptor subunit expression in the cerebellum of control and experimental neonatal rats using immunofluorescent $GABA_{A\alpha1}$ receptor specific primary antibody and rhodamine coated secondary

antibody. ⟶ in white shows $GABA_{A\alpha1}$ receptors. There was a significant decrease of $GABA_{A\alpha1}$ receptor subunit in hypoxic neonatal rats which was reversed to near control in glucose resuscitated groups.

Figure 66

Confocal image of 5-HT_{2A} receptor expression in the cerebellum of control and experimental neonatal rats using immunofluorescent 5-HT_{2A} receptor specific primary antibody and FITC tagged secondary antibody. ⟶ in white shows 5-HT_{2A} receptors. There was a significant increase of 5-HT_{2A} receptor subunit in hypoxic neonatal rats which was reversed to near control in glucose resuscitated groups.

Figure 67

Confocal image of 5-HT transporter expression in the cerebellum of control and experimental neonatal rats using immunofluorescent 5-HT transporter specific primary antibody and FITC tagged secondary antibody. ⟶ in white shows 5-HT transporters. There was a significant increase of 5-HT transporter in hypoxic neonatal rats which was reversed to near control in glucose resuscitated groups.

Figure 94

Confocal image of $GABA_{A\alpha1}$ receptor subunit expression in the brain stem of control and experimental neonatal rats using immunofluorescent $GABA_{A\alpha1}$ receptor specific primary antibody and rhodamine coated secondary antibody. ⟶ in white shows $GABA_{A\alpha1}$ receptors. There was a significant decrease of $GABA_{A\alpha1}$ receptor subunit in hypoxic neonatal rats which was reversed to near control in glucose resuscitated groups.

Figure 101

Confocal image of 5-HT_{2A} receptor expression in the brain stem of control and experimental neonatal rats using immunofluorescent 5-HT_{2A} receptor specific primary antibody and FITC tagged secondary antibody. ⟶ in white shows 5-HT_{2A} receptors. There was a significant increase of 5-HT_{2A} receptor subunit in hypoxic neonatal rats which was reversed to near control in glucose resuscitated groups.

Figure 102

Confocal image of 5-HT transporter expression in the brain stem of control and experimental neonatal rats using immunofluorescent 5-HT transporter specific primary antibody and FITC tagged secondary antibody. ⟶ in white shows 5-HT transporters. There was a significant increase of 5-HT transporter in hypoxic neonatal rats which was reversed to near control in glucose resuscitated groups.