DOPAMINE D_1 AND D_2 RECEPTOR GENE EXPRESSION AND cGMP, IP3 AND Ca^{2+} REGULATION IN THE BRAIN REGIONS OF HYPOXIC NEONATAL RATS: ROLE OF GLUCOSE, OXYGEN AND EPINEPHRINE SUPPLEMENTATION

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Dedicated To My Beloved Parents and Sisters . . .

DECLARATION

I hereby declare that the thesis entitled "**Dopamine D_1 and D_2 Receptor Gene Expression and cGMP, IP3 and Ca^{2+} Regulation in the Brain Regions of Hypoxic Neonatal Rats: Role of Glucose, Oxygen and Epinephrine Supplementation"** is based on the original research carried out by me at the Department of Biotechnology, Cochin University of Science and Technology, under the guidance of Dr. C. S. Paulose, Director, Centre for Neuroscience, Professor & Head, Department of Biotechnology and no part thereof has been presented for the award of any other degree, diploma, associateship or other similar titles or recognition.

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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). Although systemic and cerebrovascular physiologic factors play an important role in the initial phases of hypoxic-ischemic injuries, the intrinsic vulnerability of specific cell types and systems in the developing brain is more important in determining the effect of damage and functional disability. 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. Drowning, strangling, choking, suffocation, cardiac arrest, head trauma, carbon monoxide poisoning and complications of general anesthesia create conditions that lead to cerebral hypoxia. 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).

Hypoxia has been implicated in CNS pathology in a number of disorders including stroke, head trauma, neoplasia, vascular malformations and neurodegenerative diseases. Hypoxia in newborn infants results in severe lifelong consequences. The brain, lungs, heart and kidneys are particularly sensitive to low oxygenation (Li & Jackson, 2002). Brain cells are extremely sensitive to oxygen deprivation and begin to die within five minutes after oxygen supply has been blocked. Cerebral cortex is comprised of layers of neurons exhibiting distinct morphologies and synaptic connections (McConnell, 1991). 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).

Dopamine (DA), a major neurotransmitter in central nervous system is involved in the control of motor and cognitive programmes. Dopaminergic neurons

appear early during development (6-8 weeks) in humans. The dopamine turnover is relatively high during perinatal period compared to adults (Herlenius & Lagercrantz, 2001). DA is synthesised from tyrosine, stored in vesicles in axon terminals and released when the neuron is depolarised. DA interacts with specific membrane receptors to produce its effects. These effects are terminated by reuptake of dopamine into the presynaptic neuron by a dopamine transporter or by metabolic inactivation by monoamine oxidase B (MAO-B) or catechol-*O*-methyltransferase (COMT). DA plays an important role both centrally and peripherally. The recent identification of five dopamine receptor subtypes provides a basis for understanding dopamine's central and peripheral actions. DA receptors are classified into two major groups: DA D_1 like and DA D_2 like. DA D_1 like receptors consists of DA D_1 and DA D_5 receptors. DA D_2 like receptors consists of DA D_2, DA D_3 and DA D_4 receptors. Stimulation of the DA D_1 receptor gives rise to increased production of cAMP. DA D_2 receptors inhibit cAMP production, but activate the inositol phosphate second messenger system (Seeman, 1980). Disturbances of the development of the dopmaninergic system lead to dyskinesia, dystonia, tics and abnormal eye movements. An imbalance between dopaminergic neurotransmission and DA receptors is known to be associated with the symptomatology of numerous neuropsychiatric disorders, like schizophrenia, psychosis, mania and depression as well as neuropathological disorders, like Parkinson's disease and Huntington's disease (Carlsson, 1988, 1993; Bermanzohn *et al.*, 1992; Brown & Gershon, 1993; Jakel & Maragos, 2000; Kostrzewa & Segura-Aguilar, 2003). The dopaminergic cells in particular are highly sensitive to excitotoxicity and oxidative stress when the energy metabolism is impaired (Callahan *et al.*, 1998). During postnatal development, extensive changes takes place in neurotransmitter systems including glutamate, GABA, serotonin, dopamine and acetylcholine in the cortex of primates (Weickert *et al.*, 2007). Of these neurotransmitter systems, dopamine is of particular interest in relation to the development of cognitive abilities subserved by the prefrontal cortex. The most

postsynaptic markers of the DA system are its receptors. Studies done by Gurevich *et al*., (2000) showed that DA D_2 mRNA was found to be expressed in foetal human temporal cortex in differentiated neurons of the cortical plate and cortical sub-plate. Studies on piglets have also shown that the use of dopaminergic receptor antagonists in neonates protects the striatum without the adverse effects of completely blocking the NMDA receptors in the developing brain. The effects of DA D_2 receptor actions in the cortex have to be studied in detail.

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. Brain injury in newborns can cause deficits in motor and sensory function (Frances *et al*., 2001). A large amount of investigation has focused on cytokine and hypoxia-ischemia-mediated injury to the developing cortex and periventricular white matter as the cause of the neurodevelopmental handicaps suffered by infants who have experienced perinatal brain injury. Energy failure, free radical, cytokine and excitatory amino acid release and caspase-dependant cell death are known to contribute to injury in the neo-cortex, striatum and periventricular white matter (Back *et al*., 1998; Cheng *et al*., 1998). However, the degeneration of thalamus and other non-forebrain structures after hypoxia-ischemia is studied less frequently. Injuries to somatosensory thalamus have been described in human newborns after hypoxic-ischemia (Barkovich, 1995; Roland *et al*., 1998) and contribute to sensory motor deficits in infants with perinatal brain injury and cerebral palsy. Damage to the brain during development affects typical patterns of neuronal connectivity (Finlay *et al*., 1979). The foetal brain can protect itself from hypoxia by increasing cerebral blood flow for a period between one and three hours, but as the brain becomes increasingly acidotic, the blood pressure falls, inducing ischemic injury. Apoptosis, which involves activation of genetically determined cell-suicide programme, has been observed in postmortem brain tissue

from infants after hypoxic-ischemic insults (Pulera *et al*., 1998; Yue *et al*., 1997). Comparison of adult and immature animal models of hypoxic-ischemia suggests that apoptosis is more prevalent in the immature brain (McDonald *et al*., 1997; Li *et al*., 1998). Nakajima *et al*., (2000) reported that the relative numbers of apoptotic versus necrotic cells in a rodent model of hypoxic ischemia indicate that many regions such as the cerebral cortex and basal ganglia contain high numbers of apoptotic cells for over 7 days after hypoxia-ischemia.

Cerebral palsy (CP) meaning "brain paralysis" refers to motor or postural abnormalities that are noted during early development. These anomalies are thought to be associated with prenatal, perinatal or postnatal events of varying etiologies. CP generally is considered to be a static encephalopathy that is nonprogressive in nature. The immature brain has only a limited number of ways of responding to acute or chronic injury and these essentially consist of neuronal and white matter loss and glial proliferation. These changes occur over many days and weeks. By the time a child presents with cerebral palsy during the first years of life, the neuropathological effects of any hypoxic-ischemic injury or other injury will have become modified by these changes and by further postnatal brain development (Blair & Stanley, 1988). During the perinatal period and infancy (first 2 years post natal), several incidences can cause brain damage. Complications with the endocrine system due to hypoxia include respiratory distress syndrome, hypoglycemia or hypothyroidism (Nelson & Ellenberg, 1986). Nevertheless CP is secondary to prenatal, perinatal or neonatal insult; or is secondary to neuronal damage at the cellular level in the neurotransmitter or receptor systems. The global effects are the result of impaired communication between the brain and the muscles which decreased the control of movements that cause poor motor coordination, balance and abnormal movements. As a result, these motor difficulties are secondary to brain damage or abnormal brain development. In individuals with CP and epilepsy, this disruption is spread throughout the brain and cause varied symptoms all over the body as in tonic-clonic seizures or is confined to

just one part of the brain and cause more specific symptoms, as in partial seizures. Neonatal and infantile seizures suggest underlying structural brain disease with the possibility of adverse motor consequences (Singhi *et al*., 2003). Multiple neuropsychological tests have revealed neuropsychological dysfunction, which is largely due to brain hypoxia. 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).

In the present work, the role of glucose, oxygen and epinephrine supplementation in regulating neurotransmitter contents, dopaminergic binding parameters in the brain regions of experimental groups of neonatal rats were investigated. The study of neurotransmitters and their receptors in the cerebral cortex and the Ca^{2+} release patterns in brain regions of neonatal rats were taken as index for brain damage due to hypoxia, oxygen and epinephrine. Real-Time PCR work was done to confirm the binding parameters. Second messengers - cyclic guanosine monophosphate (cGMP), cyclic adenosine monophospahte (cAMP) and inositol 1, 4, 5-trisphosphate (IP3) were assayed to find the functional correlation of the receptors. Behavioural studies were carried out to confirm the biochemical and molecular studies. 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 and supplement glucose, epinephrine and oxygen in the Wistar neonatal rats.

2. To measure the blood glucose level in the serum of experimental groups of Wistar neonatal rats.

3. To measure the dopamine (DA) and homovanillic acid (HVA) in the brain regions - BS and CB and serum of the experimental groups of neonatal rats using HPLC.

4. To study DA, DA D_1, DA D_2 receptors binding parameters in the brain regions - CC, BS and CB of experimental groups of Wistar neonatal rats.

5. To study DA D_1 and DA D_2 receptor gene expression in the brain regions - CC, BS and CB of experimental groups of Wistar neonatal rats using Real-Time PCR.

6. To study NMDA receptor binding parameters in the brain regions - CC, BS and CB of experimental groups of Wistar neonatal rats.

7. To study mGLU5 and NMDA 2b receptor gene expression in the brain regions - CC, BS and CB of experimental groups of Wistar neonatal rats using Real-Time PCR.

8. To study the second messengers - cAMP, cGMP and IP3 content in the brain regions of experimental groups of Wistar neonatal rats.

9. To study the behavioural activities of the experimental groups of Wistar neonatal rats using Rotarod test.

10. To study the Ca^{2+} patterns in the cortical cells of neonatal rats *in vitro* in confocal microscope.

11. To study the apoptotic pattern in the cortical cells using TO-PRO-3 staining using confocal microscope.

Literature Review

Impact of Hypoxia

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).

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. The number of deaths coded as being due to the latter would have been even higher if infants weighing 500 to 999 g at birth were included. Death rates in pre-term infants were particularly high in cities, towns and rural areas where neonatal high care facilities are very limited. The large number of deaths associated with perinatal hypoxia in all three groups suggested problems and inadequacies in care of women in labour and the resuscitation of newborn infants. Many of these deficiencies were identified as modifiable factors in all regions. Specifically, only a few infants were recorded as having died as a result of poor neonatal resuscitation and care. Given the large number

of neonatal deaths due to "hypoxia", it is inconceivable that this is a true reflection of the actual circumstances. 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).

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 lead 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.

Dopamine

Dopamine is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake and endocrine regulation. This catecholamine also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function and gastrointestinal motility (Missale *et al.*, 1998).

DA containing neurons arise mainly from DA cell bodies in the substantia nigra and ventral tegmental area in mid-brain region (Royh *et. al*., 1991; Carlsson, 1993; Lookingland *et al*., 1995; Tepper *et. al*., 1997; Tarazi *et al*., 1997 a, b, 1998 a, b, 2001;). Dopaminergic system is organized into four major subsystems (i) the

nigrostriatal system involving neurons projecting from the substantia nigra *pars compacta* to the caudate-putamen of the basal ganglia. This is the major DA system in the brain as it accounts for about 70% of the total DA in the brain and its degeneration makes a major contribution to the pathophysiology of Parkinson's disease; (ii) *the mesolimbic system* that originates in the midbrain tegmentum and projects to the nucleus accumbens septi and lateral septal nuclei of the basal forebrain as well as the amygdala, hippocampus and the entorhinal cortex. They are all considered components of the limbic system and hence of particular interest for the pathophysiology of idiopathic psychiatric disorders; (iii) the *mesocortical* system, which also arises from neuronal cell bodies in the tegmentum which project their axons to the cerebral cortex, particularly the medial prefrontal regions; (iv) the *tuberinfundibular* pathway, which is a neuroendocrinological pathway arising from the arcuate and other nuclei of the hypothalamus and ending in the median eminence of the inferior hypothalamus. DA released in this system exerts regulatory effects in the anterior pituitary and inhibits the release of prolactin. DA is involved in the control of both motor and emotional behaviour. Despite the large number of crucial functions it performs, DA is found in a relatively small number of brain cells. In fact, while there are a total of 10 billion cells in the cerebral cortex alone, there are only one million dopaminergic cells in the entire brain (Missale *et al*., 1998).

Biosynthesis of dopamine

DA is synthesized from the amino acid L-tyrosine. L-tyrosine is hydroxylated by the enzyme tyrosine hydroxylase (TH) to give L-3, 4-dihydroxyphenylalanine (L-DOPA) which is the rate limiting step. L-DOPA is subsequently decarboxylated to DA by the enzyme aromatic L-amino acid decarboxylase. Therefore, it is not possible to enhance the levels of DA by providing L-tyrosine. The activity of tyrosine hydroxylase is regulated by several endogenous mechanisms. For example, the enzyme is activated by increased neuronal impulse flow, but is inactivated either by

DA itself as an end product inhibitor or by activation of presynaptic DA receptors. On the other hand, the enzyme aromatic L-amino acid decarboxylase converts L-DOPA to DA instantaneously. Therefore, providing L-DOPA creates a possibility to enhance the formation of DA.

Dopamine reuptake and metabolism

DA exerts its functions mediated through various receptors and these actions are terminated to prevent continuous stimulation of the receptors. This inactivation is brought about by reuptake mechanisms and metabolism of DA. Reuptake of DA is accomplished by a high affinity carrier present in the membrane, the dopamine transporter (DAT). The DA transporter recycles extracellular DA by actively pumping it back into the nerve terminal. The DA content which is about 70 to 80 % in the striatal synaptic cleft is inactivated by this process. Drugs, such as cocaine are able to block the action of the DA transporter, thereby sustaining the presence of DA in the synaptic cleft and its action on DA receptors. Part of the DA is inactivated by conversion to inactive compounds by metabolic enzymes, which are present both intra and extraneuronally. Monoamine oxidase (MAO), aldehyde dehydrogenase (ALDH) and catechol-*O*-methyltransferase (COMT) are responsible for the metabolism of DA. DA after reuptake is intraneuronally deaminated by MAO to give dihydroxyphenyl acetaldehyde which subsequently is converted to 3, 4-dihydroxyphenylacetic acid (DOPAC) by ALDH. DOPAC is then methylated by COMT to give homovanillic acid (HVA). Extraneuronally, DA is metabolized by an alternative route in which it is first *O*-methylated to 3-methoxytyramine (3-MT) through the action of COMT and subsequently oxidized by MAO and ALDH to HVA.

Dopamine receptors

DA mediates its actions *via* membrane receptor proteins. DA receptors are found on postsynaptic neurons in brain regions that are DA enriched. In addition,

they reside presynaptically on DA neuronal cell bodies and dendrites in the midbrain as well as on their terminals in the forebrain. DA receptors belong to a family of large peptides that are coupled to G-proteins which are modified by attached carbohydrate, lipid-ester or phosphate groups. The topologies of the five DA receptors are predicted to be the same as all the other G-protein-coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions. The third intra cytoplasmic loop is functionally critical and interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects (Royh *et. al*., 1991; Carlsson, 1993; Tarazi *et al*., 1997 a, b, 1998 a, b; Tepper *et. al*., 1997). In their putative transmembrane domains, the DA D_1 and DA D_5 receptors are 79% identical to each other, while they are only 40–45% identical to the DA D_2, DA D_3, and DA D_4 receptors. Conversely, the DA D_2, DA D_3, and DA D_4 receptors are between 75% and 51% identical to each other. They contain seven putative membrane spanning helices which would form a narrow dihedral hydrophobic cleft surrounded by three extracellular and three intracellular loops. The receptor polypeptides are probably further anchored to the membranes through palmitoylation of a conserved Cys residue found in their carboxy tails, 347 in DA D_1, the C-terminus in DA D_2 like receptors. The DA receptors are glycosylated in their N-terminal domains. DA D_1 like subtypes have potential glycosylation sites in their first extra cytoplasmic loop.

DA receptors are divided into two families on the presence or absence of ability of DA to stimulate adenylyl cyclase and produce the second-messenger molecule cyclic AMP (cAMP) (Kebabian & Calne, 1979; Schwartz *et al*., 1992; Civelli *et al.*, 1993; O'Dowd, 1993; Jackson & Westlind, 1994; Ogawa, 1995; Strange, 1996). This classification is based on similarities in structure, pharmacology, function and distribution. DA D_1 like receptors are characterized initially as mediating the stimulation of cAMP production. DA D_2 like receptors inhibit the production of cAMP. This pharmacological characterization is based on the ability of some DA

agents to block adenylyl cyclase activity to inhibit the release of prolactin *in vivo* and *in vitro* in a cAMP independent fashion (Seeman, 1980). Applications of recent technical advances in molecular genetics have greatly facilitated the isolation and characterization of novel DA receptors, DA D_3, DA D_4 and DA D_5, with different anatomical localization from traditional DA D_1 or DA D_2 receptors. Based upon their pharmacological profiles, including their effects on different signal transduction cascades, these receptors are currently divided into two families: the DA D_1 like family which includes DA D_1 and DA D_5 receptors. The DA D_2 like family includes DA D_2, DA D_3 and DA D_4 receptors (Schwartz *et al*., 1992; Grandy *et al*., 1993; Sibley *et al*., 1993). The genomic organizations of the DA receptors demonstrate that they are derived from the divergence of two gene families that mainly differ in the absence or the presence of introns in their coding sequences. DA D_1 like receptors genes do not contain introns in their coding regions, a characteristic shared with most G-protein coupled receptors. The genes encoding the DA D_2 like receptors are interrupted by introns (Gingrich & Marc, 1993). Furthermore, most of the introns in the DA D_2 like receptor genes are located in similar positions.

Dopamine D_1 like family

The DA D_1 receptor is the most abundant DA receptor in the CNS. The DA D1 like receptors are characterized by a short third loop as in many receptors coupled to Gs protein (Civelli *et al*., 1993; Gingrich & Canon, 1993; O'Dowd, 1993). The DA D1 like receptors have short third intracellular loops and long carboxy terminal tails. The DA D1 like receptors are classified into DA D_1 and DA D_5. In the DA D_1 and DA D_5 receptor third intracellular loop and the carboxy terminus are similar in size but divergent in their sequence. In contrast, the small cytoplasmic loops 1 and 2 are highly conserved so that any difference in the biology of these receptors is related to the third cytoplasmic loop and the carboxy terminal tail (Civelli *et al*., 1993; Gingrich & Canon, 1993; O'Dowd, 1993). The external loop between transmembrane domain

(TM) TM4 and TM5 is considerably different in the two receptor subtypes, being shorter (27 amino acids) in the DA D_1 receptor than in the DA D_5 receptor (41 amino acids). The amino acid sequence of this loop is divergent in the DA D_5 receptor (Marc *et al*., 1998).

Dopamine D_1 receptor

DA D_1 receptors are found at high levels in the typical DA regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercles. DA D_1 receptor seems to mediate important actions of DA to control movement, cognitive function and cardiovascular function. The DA D_1 receptor gene, which lacks introns, encodes a protein that extends for 446 amino acids (Dohlman *et al*., 1991). In humans DA D_1 receptor gene has been localized to chromosome 5 (Sunahara *et al.,* 1990). The DA D_1 receptors show characteristic ability to stimulate adenylyl cyclase and generate inositol 1, 4, 5-trisphosphate (IP3) and diacylglycerol (DAG) *via* the activation of phospholipase C (Monsma *et al.,* 1990; Sibley *et al.,* 1990). DA D_1 receptors are highly expressed in basal ganglia followed by cerebral cortex, hypothalamus and thalamus. DA D_1 receptors messenger ribonucleic acid (mRNA) is colocalized in striatal neurons of the basal ganglia with mRNA for DA receptor phosphor protein (DARPP-32; 32 kDa) which is a DA and cAMP-regulated phosphoprotein. DA Receptor Phosphor Protein contributes to the actions of DA D_1 receptor (Hemmings & Greengard, 1986; Greengard *et al*., 1987). The DA D_1 receptors in the brain are linked to episodic memory, emotion and cognition.

Classical pathway contributes to DA D_1 enhancement of NMDA receptor currents; however, they have also shown different downstream effectors (Blank *et al*., 1997; Cepeda *et al*., 1998a; Flores-Hernandez *et al.,* 2002). DA D_1 activation has been shown to enhance NMDA responses *via* the adenylate cyclase–protein kinase-A and DARPP-32 (dopamine and cyclic adenosine 3′,5′-monophosphate-Regulated PhosphoProtein, 32 kDa) cascade (Levine *et al.,* 1996; Blank *et al.,* 1997; Flores-

Hernandez *et al.*, 2002). In addition Dunah & Standaert (2001) have shown that DA D_1 receptor activation enhances the abundance of NR1, NR2A and NMDA 2b subunits. There is a developmental switch in DA D_1 receptor modulation between early in development when NMDA 2b receptors predominate and DA D_1 receptor activation down regulates NMDA receptors, to later in development when a mixed NMDA 2a/NMDA 2b receptor population is likely to be present and DA D_1 receptor activation stabilizes NR2A-containing receptors at the synapse (Tong & Gibb, 2008).

Dopamine D_5 receptors

DA D_5 receptors are localized in the substantia nigra pars compacta, hypothalamus, striatum, cerebral cortex, nucleus accumbens and olfactory tubercle (Khan *et al.*, 2000). The DA D_5 receptor gene is intronless and encodes a protein that extends for 47 amino acids (Tarazi & Kaufman, 2005). This protein has an overall 50% homology with DA D_1 receptor and 80% if only the seven transmembrane segments are considered. The gene encoding the human DA D_5 protein is located at the short arm of chromosome 4, the same region where the Huntington's disease gene has been located (Gusella, 1989). Two DA D_5 receptor pseudogenes having 154 amino acids have been identified with 90% homology (Gusella, 1989). These pseudogenes, however, contain stop codons in their coding regions that prevent them from expressing functional receptors. The functions of these pseudogenes, which appear so far to be specific to humans, are not yet known (Grandy *et al*., 1991).

DA D_5 receptor mRNA expression is unique and limited to the hippocampus and parafascicular nucleus of the thalamus (Civelli *et al*., 1992). It is involved in the thalamic processing of painful stimuli (Giesler *et al*., 1979). DA D_5 receptors appear to interact with G-proteins and can stimulate adenylyl cyclase, with relatively high affinity for DA and DA D_1 selective agonists (Tarazi & Kaufman, 2005). Studies by Holmes *et al*., (2001) concluded that DA D_5 contributes to the pharmacological

activation of dopaminergic pathways relevant to exploratory locomotion, startle, and prepulse inhibition.

Dopamine D_2 like family

DA D_2 like receptors belong to the G-protein coupled receptors and has 400 amino acid residues. DA D_2 like receptors are characterized by a long extracellular amino terminus which has several glycosylation sites and a shorter carboxy terminal tail with putative phosphorylation sites. The function of sugar moieties is not clear (Marc *et al*., 1998; Sibley, 1999). It is generally believed that the membrane enclosed part of the amino acid chain of G-protein coupled receptors is folded into seven α-helices. The transmembrane helices consist primarily of hydrophobic amino acid residues. The unique feature of DA D_2 like receptors family is that they posses a bigger third cytoplasmic (intracellular) loop in common, which is thought to be the site where the G-protein couples (Marc *et al*., 1998). Between the different DA receptors, the third loop also displays the greatest variability in amino acid sequence. This has consequences for their respective second messenger systems. The DA D_2 like receptors are coupled to G_i protein and inhibit the formation of cAMP. The DA D_2 receptors tertiary structure is stabilized by two cysteine disulphide bridges.

Dopamine D_2 receptors

The DA D_2 receptor gene encodes a protein that extends for 415 amino acids. Similar to other G-protein coupled receptors, the DA D_2 receptor has seven transmembrane segments, but in contrast to DA D_1 like receptors, the third cytoplasmic domain is long and the carboxy terminus is short. Unlike the DA D_1 like receptor genes, the DA D_2 receptor gene contains seven introns that are spliced out during mRNA transcription (Fischer *et al*., 1989). The gene encoding this receptor was found to reside on q22-q23 of human chromosome 11 (Makam *et al.*, 1989). The DA D_2 receptor was the first receptor to be cloned (Chrisre *et al*., 1988). The DA D_2

receptors are involved in several signal transduction cascades, including inhibition of cAMP production (Vallar & Meldolesi, 1989), inhibition of phosphoinositide turnover (Epelbaum *et al.,* 1986), activation of potassium channels and potentiation of arachidonic acid release (Axelrod *et al*., 1991). The DA D_2 receptors are highly expressed in basal ganglia, nucleus accumbens septi and ventral tegmental area (Schwartz *et al.,* 1992).

The DA D_2 receptors exist as two alternatively spliced isoforms differing in the insertion of a stretch of 29 amino acids in the third intracellular loop and are designated as DA D_{2S} and DA D_{2L} (Seeburg *et al*.,1989; Marc *et al*., 1998). Because this loop seems to play a central role in receptor coupling, the existence of a splicing mechanism at this level could imply functional diversity. However, in spite of the efforts of several groups, no obvious differences have emerged so far between the two DA D_2 receptor isoforms. The two isoforms are derived from the same gene by alternative RNA splicing which occurs during the maturation of the DA D_2 receptor pre-mRNA (Schwartz *et al*., 1989). DA D_2 receptor isoforms, DA D_{2S} and DA D_{2L} vary within each species by the presence or absence of a 29-amino acid sequence in the third cytoplasmic domain of the DA D_2 receptor peptide chain. Both variants share the same distribution pattern. The shorter form is less abundantly transcribed and they appear to differ in their mode of regulation (Marc *et al*., 1998). Pharmacologically, both isoforms exhibit nearly similar profiles in terms of their affinities to different DA D_2 selective agents and inhibit adenylyl cyclase activity. However, these isoforms display an opposite regulatory effect (Sibley *et al*., 1993). These isoforms have the same pharmacological profile, even though a marginal difference in the affinity of some substituted response to DA treatment is reported. DA induces the up regulation of DA D_{2L} isoform of DA D_2 receptors (Castro & Strange, 1993). When expressed in host cell lines, both isoforms inhibited adenylyl cyclase (Marc *et al*., 1998; Sibley, 1999). However, the DA D_{2S} receptor isoform displayed higher affinity than the DA D_{2L} in this effect (Seeburg *et al*., 1993). The

isoforms of DA D_2 mediate a phosphatidylinositol-linked mobilization of intracellular calcium in mouse Ltk [-] fibroblasts. Protein kinase C (PKC), however, differentially modulates DA D_{2S} and D_{2L} activated transmembrane signalling in this system with a selective inhibitory effect on the DA D_{2S} mediated response.

Dopamine D_3 receptors

DA D_3 receptor gene contains five introns and encodes a 446 amino acid protein (Schwartz *et al*., 1992). The gene encoding this receptor resides on chromosome 3 (Giros *et al*., 1990). The DA D_3 receptors bear close structural and pharmacological similarities to the DA D_2 receptors. DA D_3 mRNA occurs in longer and shorter spliced forms generated from the same gene (Schwartz *et al*., 1992). Distribution of DA D_3 receptor mRNA are distributed and expressed mainly in subcortical limbic regions including islands of Calleja, nucleus accumbens septi and olfactory tubercle, with low levels of expression in the basal ganglia. DA D_3 receptor mRNA has also been found in neurons of the cerebellum, which regulate eye movements (Levesque *et al*., 1992). The status of the DA D_3 molecular entity as a functional receptor remains uncertain since it neither couples to G-proteins nor consistently transduces an effector mechanism. However, the structural similarity with DA D_2 receptor raises the possibility that DA D_3 receptor also inhibit adenylyl cyclase activity in its normal cellular setting. Studies have reported that DA D_3 receptors mediate positive regulatory influences of DA on production of the peptide neurotensin (Schwartz *et al*., 1992; Sokoloff *et al*., 1990).

Dopamine D_4 receptors

DA D_4 receptor gene contains four introns and encodes a 387 amino acid protein (Van Tol *et al*., 1991). The overall homology of the DA D_4 receptor to the DA D_2 and DA D_3 receptors is about 41% and 39% respectively, but this homology increases to 56% for both receptors when only the transmembrane spanning segments

are considered. The gene encoding the human DA D_4 protein is located at the tip of the short arm of chromosome 11 (Civelli & Bunzow, 1993; Missale *et al*., 1998). DA D_4 receptor gene has been localized in brain regions like hippocampus and frontal cortex using specific histoprobes. The stimulation of DA D_4 receptor inhibits adenylyl cyclase activity and release arachidonic acid in brain neurons (Huff *et al*., 1994; Misalle *et al*., 1998). In humans, DA D_4 receptor occurs in several genomic polymorphic variants that contain two to eleven repeats of a 48 base pair segment that is expressed in the third cytoplasmic domain (Van Tol *et al*., 1992; Misalle *et al*., 1998). These are called the DA D_4 alleles which are represented as DA $D_{4.2}$, DA $D_{4.4}$ and DA $D_{4.7.}$ These contribute to the pathophysiology of certain neuropsychiatric disorders (Jackson & Westlind-Danielsson, 1994).

Most dorsal hypothalamic spinal projection neurons are dopaminergic and appear to be involved in autonomic function (Cechetto *et al*., 1988). DA terminal axon density is highest in the intermediolateral cell columns of the spinal cord, where preganglionic sympathetic nervous system neurons originate and microelectrophoretic DA application there inhibits sympathetic preganglionic neurons (Lindvall *et al*., 1983). Retrograde labeling has identified caudal lateral hypothalamic area neurons as likely sympathetic nervous system "central command neurons" (Jansen *et al*., 1995). In humans, DA D_2 agonists cause inhibition of sympathetic output that is abolished by DA D_2 antagonists but only at higher degrees of sympathetic stimulation (Mannelli *et al*., 1997). These data are consistent with previously discussed anatomical evidence for dopaminergic inhibition of sympathetic nervous system function and indicate that state dependent factors mediate DA D_2 antagonist effects.

Activation of these presynaptic receptors inhibits the release from their respective nerve terminals of other neurotransmitters such as NE, ACh, and GABA (Hársing & Zigmond, 1997) from the striatum. Dopaminergic innervation of the medial and dorsolateral prefrontal cortex appears to be particularly vulnerable to stress and relatively low intensity levels of stress are capable of promoting significant

responses. The prefrontal dopaminergic neurons have a number of higher functions including attention, 'working' memory and the acquisition of coping patterns in response to stress (Castellano *et al*., 1999). Amphetamines and cocaine agonise these receptors and have a similar effect as stress, resulting in symptoms such as anxiety, panic, hypervigilence, exaggerated startle reflexes and paranoia (Horger *et al*., 1999). Malondialdehyde (MDA) and opiate receptors are plentiful in this area and stress-induced innervation of the fronto-cortical neurons is prevented if these receptors are selectively blocked. This increase of DA from the dendrites of DA neurons is due to an alteration in GABA regulation of the DA neurons. As with noradrenergic systems, single or repeated exposures to stress potentiates the capacity of a subsequent stressor to increase DA function in the forebrain without altering basal DA turnover, suggesting that the receptors have been hyper-sensitized (Basso *et al*., 1999). An uncontrolled rise in extracellular DA has also been implicated as an important cause of pathogenesis in the hypoxic/ischemic brain (Globus *et al*., 1988). The consequences of severe hypercapnic hypoxia (H/H) combined with brain ischemia, mostly as a result of secondary hypotension (Volpe *et al*., 1985), are well documented to be associated with neuronal damage as a frequent cause of the chronic handicapping conditions of cerebral palsy, mental retardation, and epilepsy (Vannucci, 1997). Evidence exists that in the newborn brain, specific structures and/or tissues are especially vulnerable to injury, creating syndromes of functional disabilities. In term newborns, a specific pattern of symmetric basal ganglia and adjacent cortex injury has been revealed as the structural substrate for extrapyramidal cerebral palsy (Menkes & Curran, 1994; Hoon *et al*., 1997; Roland *et al*., 1998). It has been proposed that neurons that are connected in already established neuronal circuits seem to be especially vulnerable to excitotoxic damage based on a hyperactivity of the major excitatory glutamatergic input (Johnston *et al*., 2001). However, the dopaminergic system is also sensitive to O_2 deprivation in the immature brain (Gordon *et al*., 1990; Pastuszko *et al*., 1993; Nakajima *et al*., 1996). Obviously, there

is no "oxygen reserve" that protects DA release and metabolism from decrease in O_2 pressure, because in the newborn piglet brain, even a small reduction of the brain tissue pO2 causes a significant increase in the striatal extracellular DA concentration in a dose-dependent relationship. An increase of aromatic amino acid decarboxylase (AADC) activity, indicating an increase of mesostriatal dopaminergic activity in newborn piglets (Huang *et al*., 994) is known to be associated with pronounced neuronal injury as a result of hypoxic-ischemic brain (Globus *et al*., 1987, Ren *et al*., 1997).

Sensory and cognitive dissociations resulting from dopaminergic hyperfunction produce a state of fear and anxiety *via* direct anatomic connections from cortical brain structures to the limbic system principally through mesolimbic pathways (Iturriaga *et al*., 1996). This disinhibition of mesolimbic dopaminergic neurons cause the bizarre behavioural and cognitive symptoms experienced by patients in schizophrenia and by extension, with delirium (Harrison, 1999). Delirium resulting from dopaminergic hyperfunction is characterized by global disorders of cognition and wakefulness by impairment of psychomotor behavior (Miller *et al*., 1991). Major cognitive functions such as perception, deductive reasoning, memory, attention and orientation are all globally disordered. Excessive motor activity frequently accompanies severe cases of delirium and, when this occurs, the resulting constellation of symptoms is called 'agitated delirium' (Crippen, 1994). If excessive responses to dopaminergic systems contribute to afore mentioned manifestations, the neuroleptic drugs that decrease neural DA activity such as haloperidol should alleviate some of the symptoms, particularly hypervigilence and paranoia. Haloperidol is a butyrophenone structurally similar to droperidol with mechanisms of action similar to piperazine-based phenothiazines (Settle & Ayd, 1983). Haloperidol is a DA antagonist and benzodiazepines are GABA agonists. Theoretically, there should be a synergistic relationship between the two when used in a conjoined fashion. In addition, butyrophenone such as haloperidol suppress spontaneous movements and complex

behaviour patterns which result from disharmonious brain function, with minimal CNS depressive effect (Tesar *et al*., 1985). There is little or no ataxia, incoordination or dysarthria at ordinary doses. Haloperidol appears to exert a diffused depressive effect by inhibiting dopaminergic receptors and reuptake of neural DA in the subcortical, midbrain and brainstem reticular formation (Todd & Grace, 1999). A unique effect of haloperidol is a relatively strong suppression of spontaneous musculoskeletal hyperactivity and behaviour that results from hyperdopaminergic brain function without pronounced sedation or hypotension (Wagner *et al.*, 1997). Haloperidol produces less sedation than other phenothiazines, with very little effect on heart rate, blood pressure and respiration. It appears to have a very wide range between therapeutic doses and the dose which precipitates extrapyramidal reactions (Gerlach & Larsen, 1999). It is thought that haloperidol's molecular structure is changed in some fashion when given orally, increasing the possibility of extrapyramidal reactions (Rosebush & Mazurek, 1999).

Second Messengers

Inositol 1,4,5-trisphosphate

Inositol 1,4,5-trisphosphate (IP3) 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 (Wojcikiewicz, 1995; Taylor *et al.*, 1999) and form homotetrameric or

heterotetrameric channels (Monkawa *et al*., 1995). In previous studies, we constructed a plasmid vector containing full-length rat IP3R3 linked to green fluorescent protein (GFP-IP3R3) and visualized the distribution of GFP-IP3R3 in living cells (Morita *et al.*, 2002; Morita *et al.*, 2004). The confocal images obtained in these studies provided strong evidence that IP3Rs are distributed preferentially on the ER network. 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 (Thomas *et al.*, 1998; Swillens *et al*., 1999; Shuai & Jung, 2003). Recently, 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 (Wilson *et al*., 1998; Chalmers *et al*., 2006), 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 $[Ca^{2+}]i$, is absolutely critical for IP3R clustering. We also found that depletion of intracellular Ca^{2+} stores facilitates the generation of agonist-induced IP3R clustering.

Cyclic Adenosine Monophosphate (cAMP)

The second messenger concept of signalling was born with the discovery of cyclic AMP (cAMP) and its ability to influence metabolism, cell shape and gene

transcription (Sutherland, 1972) *via* reversible protein phosphorylations. cAMP is produced from ATP adenylyl cyclase (AC) 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 (Doskeland *et al*., 1993; Francis & Corbin, 1999; Canaves & Taylor, 2002). 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 (Rooij *et al*., 1998; Kawasaki,*et al*., 1998).

The DA D_1 like receptors were assumed to couple to the adenylate cyclase stimulatory G protein G_s. Because G_s is ubiquitously expressed, the ability of DA D_1 like receptors to stimulate adenylate cyclase in virtually any cell line (Huff, 1997), together with physical and functional coupling of both DA D_1 and DA D_5 receptors to G_s (Sidhu, 1998; Jin *et al.*, 2001), strongly support the notion that G_s mediates the DA D_1 like receptor signaling in some tissues.

DA D_1 receptor stimulation simultaneously activates PKA by stimulating the production of cAMP and disinhibits PKA by phosphorylation dependent activation of protein phosphatase-2A and Thr75 dephosphorylation of DARPP-32. PKA increases the phosphorylation of numerous voltage- and ligand-gated ion channels by various combinations of direct PKAcatalyzed phosphorylation of channel subunits and DARPP-32-mediated inhibition of PP1. DA D_1 receptor stimulation induces the expression of a number of transcription factors (Liu & Graybiel,1996; Zhang *et al.*,

2002) which are dependent on initial activation of the transcription factor CREB (Liu & Graybiel,1996; Konradi *et al.*, 1994).

DA D_2 like receptor signalling is mediated primarily by activation of the heterotrimeric G proteins $G\alpha_{i/o}$, a class of G proteins inactivated by pertussis toxin catalyzed ADP-ribosylation (Kurose *et al.*, 1983; Bokoch *et al.*, 1983). There is considerable disagreement in the literature concerning which G proteins interact with D_{2S} and D_{2L} (Robinson & Caron, 1997, Neve *et al.*, 2003). It seems likely that both receptor isoforms are inherently able to activate multiple $G\alpha_{i/o}$ subtypes, including $G\alpha_{i2}$, $G\alpha_{i3}$, and $G\alpha_{o}$ (Lledo *et al.*, 1992; Liu *et al.*, 1994), but that interactions with particular G proteins are restricted in a cell-type dependent manner due to compartmentalization or the availability of appropriate effectors and scaffolding proteins. DA D_{2S} and DA D_{2L} can also activate the pertussis toxin insensitive G protein $G\alpha_{z}$ (Wong *et al.*, 1992; Obadiah, 1999). The first signaling pathway identified for DA D_2 like receptors was inhibition of cAMP accumulation (De Camilli *et al.*, 1979; Stoof & Kebabian, 1981).

DA D_2 like receptor inhibition of adenylate cyclase is mediated by $G\alpha_{i/o}$, because adenylate cyclase 5 is directly inhibited by $G\alpha_{i}$ and is insensitive to $G_{\beta\gamma}$ (Taussig *et al.*, 1994). $G\alpha_{i}$ binds primarily to the C1 cytosolic domain of $G\alpha_{i}$ inhibited forms of adenylate cyclase and reduces C1/C2 domain interaction (Dessauer *et al.*, 2002). DA D_2 receptor signalling occurs *via* inhibition of adenylate cyclase act in opposition to agents that stimulate adenylate cyclase, decreasing the phosphorylation of PKA substrates.

Group I mGluRs (mGluR1/5 subtypes) are demonstrated to mainly affect intracellular Ca^{2+} mobilization (Conn & Pin, 1997; Bordi & Ugolini, 1999). To sequentially facilitate intracellular Ca^{2+} ($[Ca^{2+}]i$) release, group I receptors activate the membrane-bound phospholipase C (PLC), which stimulates phosphoinositide turnover by hydrolyzing phosphoinositol-4,5-biphosphate to 1,4,5-triphosphate (IP3) and

diacylglycerol. IP3 then causes the release of Ca^{2+} from intracellular Ca^{2+} stores (such as endoplasmic reticulum) by binding to specific IP3 receptors on the membrane of Ca^{2+} stores Berridge, 1993). Altered Ca^{2+} levels could then engage in the modulation of broad cellular activities.

Cyclic Guanosine Monophospahte (cGMP)

cGMP generation has been associated with neurotransmission (Hofmann *et al.*, 2000), vascular smooth muscle relaxation (Fiscus *et al.*, 1985) and 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). 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 may 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 (NO) synthase (NOS) type to produce NO, which stimulates guanylyl cyclase to produce cGMP (Garthwaite, 1991).

Cyclic nucleotide pathways can cross talk to modulate each other's synthesis, degradation, and actions. Increased cGMP can increase the activity of cGMP stimulated PDE2 to enhance hydrolysis of cAMP, or it can 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).

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 behavioral 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 analysis can now 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). Three human subtypes (α_{1A}, α_{1B}, α_{1C}) have been identified but their individual functional roles remain to be identified (Hague *et al.,* 2003). α_2-adrenergic receptors mediate many physiological actions of the endogenous catecholamines, EPI, NE and are targets of several therapeutic agents. α_2-adrenergic receptors mediate reduction in blood pressure following α_{2A}-agonist administration (Macmillan *et al*., 1998). α_2-adrenergic receptors play a critical role in regulating neurotransmitter release from sympathetic nerves and from adrenergic neurons in the CNS (Miller, 1998; Langer, 1997). 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). Gu *et al*., (2000) reported that cell death of thymocytes can be induced after stimulation of β-adrenergic receptors, or by addition of exogenous cAMP. Chronic β-adrenergic stimulation does not increase glutamate production in humans. The acute administration of β-receptor stimulating agent profoundly affects the insulin mediated glucose metabolism; however little is known about the impact of chronic β-receptor stimulation on glucose metabolism and insulin sensitivity

(Scheidegger *et al*., 1984). NE did not amplify the stress hormone induced increase in hepatic glucose production. In the acute setting, NE is considerably less potent than EPI in stimulating hepatic glycogenolysis (Connolly *et al*., 1991; Stevenson *et al*., 1991). Although, acutely, EPI has more potent stimulatory effects on gluconeogenesis than glucagon (Stevenson *et al*., 1987, 1991), its chronic stimulatory effects on this process appear to be less substantive than that of glucagon when multiple stress hormones are increased.

EPI also plays a central role by enhancing hepatic glycogenolysis. Cortisol augments hepatic glycogen stores despite marked increases in other counter regulatory hormones. In addition, it maintains the gluconeogenic precursor supply, thus supporting the glucagon and to a lesser extent, EPI-mediated increase in gluconeogenesis. Circulating NE does not play a major role in augmenting hepatic glucose metabolism during chronic stress; no hormone plays the central role in the chronic enhancement in glucose metabolism during stress. Rather, these hormones complement one another to allow an efficient stimulation of hepatic metabolism. The relative importance of a given stress hormone in a particular stress cannot be addressed, because the impact of an individual hormone will depend on the specific stress and the functions of endocrine system (Owen *et al*., 1997). The β-adrenergic receptors showed an up regulation during hypoxia while α_2-adrenergic receptors were down regulated. The up regulation is through the activation of cAMP pathway (Finla, 2007).

Glutamate Receptors

The majority of excitatory synapses are glutamergic, in which Glutamate (Glu) 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. Eight receptors have been discovered and classified into three groups based on their linkage to second messenger systems and their pharmacology: group I acts *via* the phosphoinositol system and groups II and III inhibit adenylyl cyclase (Conn & Pin, 1997). In addition, the stimulation of receptors of these three groups directly influence voltage-gated Ca^{2+} and K^{+} channels through their G-proteins, but their physiological correlate has not yet defined. There is dense expression of mGlu1 receptors throughout the cerebellum (Berthele *et al.*, 1998; 1999), where motor learning is dependent on coincident activation of mGlu1 and an increase in intracellular Ca^{2+} (Conquet *et al.*, 1994; Hansel & Linden, 2000). Cerebellar Long Term Depression (LTD) is mediated through activation of postsynaptic mGlu1 receptors (Aiba *et al.*, 1994; Shigemoto *et al.*, 1994) and a decrease in AMPA receptor expression on the plasma membrane (Wang & Linden, 2000). Mice deficient in mGlu1 receptors have both severe motor and spatial learning deficiencies (Conquet *et al.*, 1994), underscoring the important role of these receptors in motor control. LTD in the striatum is dependent on an increase in intracellular Ca^{2+} and activation of Ca^{2+}-dependent protein kinases (Calabresi *et al.*, 1994). In addition, induction of LTD in the striatum also appears to require coincident activation of DA D_1 and DA D_2-receptors (Calabresi *et al.*, 1992). It is interesting that induction of corticostriatal LTP involves activation of both mGlu1 and mGlu5 receptors (Gubellini *et al.*, 2003). Group I mGlu receptors, particularly mGlu5, activate CREB phosphorylation through IP3 dependent intracellular Ca^{2+} release (Mao & Wang, 2002).

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. For example, activation of DA D_1 receptors and mGlu5 receptors synergistically increases phosphorylation of ERK2 and CREB in cultured striatal neurons (Voulalas *et al.*, 2005) and activation of mGlu5 receptors potentiates the effects of adenosine A2A receptor signalling through the ERK pathway (Ferré *et al.*, 2002; Nishi *et al.*, 2003). In addition, amphetamine is an indirect DA receptor agonist and increases glutamate release in the striatum. Group I mGlu receptors modulate calcium channels. Activation of these receptors decreases N-type Ca^{2+} channel (Sahara *et al.*, 1993) and NMDA receptor (Yu *et al.*, 1997) activation in a fast, membrane–delimited manner, suggesting that modulation occurs through a direct G protein interaction. Group I mGlu receptor activation also inhibits L-type Ca^{2+} channels, but this modulation develops much more slowly and is likely mediated by increased intracellular Ca^{2+} (Sayer *et al.*, 1992; Chavis *et al.*, 1995a). There is evidence for facilitation of L-type Ca^{2+} channels in cerebellar granule cells through IP3 mediated activation of PKC (Chavis *et al.*, 1995b). Group I mGlu receptors also modulate nonselective cation channels. Ca^{2+} dependent nonselective cation channels are activated by group I mGlu receptors in hippocampal CA1 pyramidal cells (Crepel *et al.*, 1994; Gereau & Conn, 1995; Congar *et al.*, 1997;), CA3 pyramidal cells (Caeser *et al.*, 1993) and cerebellar Purkinje cells (Knopfel *et al.*, 1991). However, in some cases the group I mGlu receptors modulate Ca^{2+} dependent nonselective cation currents via PLC mediated modulation of the Na^{+}/Ca^{2+} exchanger (Staub *et al.*, 1992; Keele *et al.*, 1997; Lee & Boden, 1997; Netzeband *et al.*, 1997;). There is also evidence for activation of Ca^{2+} independent nonselective cation channels in hippocampal slice cultures by group I mGlu receptors (Guerineau *et al.*, 1995). Group I mGlu receptors play a prominent role in the regulation of synaptic plasticity in many areas of the brain (Spooren *et al.*, 2003; Simonyi *et al.*, 2005; Riedel, 1996; Riedel &

Reymann, 1996). Both mGlu1 and mGlu5 receptors are abundantly expressed throughout the hippocampus (Spooren *et al.*, 2003), where they are important in LTP, LTD, and memory formation (Riedel & Reymann, 1996; Riedel, 1996; Spooren *et al.*, 2003). Co-application of inactive doses of inhibitors of mGlu5 and NMDA receptors synergistically impairs working memory and instrumental learning (Homayoun *et al.*, 2004), spatial learning (Campbell *et al.*, 2004) and aversive learning paradigms (Gravius *et al.*, 2006). mGlu5 knockout mice have impaired NMDA-dependent LTP in hippocampal CA1 and are deficient in memory tasks (Lu *et al.*, 1997). mGlu5 and mGlu5-dependent facilitation of NMDA currents (Miserendino *et al.*, 1990; Rodrigues *et al.*, 2002) are important for mediating the morphologic changes involved in the memory (Lamprecht *et al.*, 2002). Activation of mGlu receptors is associated with perception and the emotional aspects of pain. mGlu5 receptor function in the amygdala is increased in postsynaptic neurons with a simultaneous up regulation of functional presynaptic mGlu1 receptors (Neugebauer *et al.*, 2003). Activation of group I mGlu receptors causes presynaptic inhibition of GABA release (de Novellis *et al.*, 2003; Drew & Vaughan, 2004) and increases excitability of GABA and glutamate-containing neurons *via* a postsynaptic mechanism (Drew & Vaughan, 2004) since group I mGlu receptors are primarily postsynaptic.

There are several reports of presynaptic localization of GluRs and their involvement in transmitter release. NMDAR plays a central role in the pathologies that can affect the brain development. These range from trauma (Ikonomidou *et al*., 2000; Lea & Faden, 2002), hypoxic-ischemic injury (Mishra *et al.*, 2002), susceptibility to neurotoxicity (Haberny *et al.*, 2002) and neurological disorders such as temporal lobe epilepsy (Mathern *et al.*, 1999). The postnatal development of the human hippocampal formation is accompanied by changes in NMDAR subunit mRNA expression that are similar but not identical to those seen in rat with an increase in NR1 mRNA and decline in NMDA 2b mRNA. Animal and *in vitro* studies indicate that these alterations likely have functional and pathophysiological

implications although further studies are required to confirm this (Law *et al.*, 2003). The fact that NMDA releases Glu (Pittaluga *et al*., 1996), DA (Kuo *et al*., 1998) and NE (Pittaluga & Raiteri, 1992) from axon terminals and facilitate transmitter release *via* NMDA receptors. In addition, presynaptic AMPA receptor activation results in an increase of Glu release, provided that the receptor's fast desensitization was prevented by cyclothiazide (Barnes *et al*., 1994; Desai *et al*., 1994). Montague *et al*. (1994) suggested that Glu and NE release from cortical synaptosomes was in correlation with NMDA induced production of nitric oxide (NO), an endogenous chemical that is able to inhibit basal membrane transporters, thereby increasing the concentration and life-span of transmitters (*e.g*., Glu and NE) released into the extracellular space. The inhibition of neuronal NO synthase by 7-nitroindazole protects against NMDA-mediated excitotoxic lesions but not against those evoked by AMPA or KA (Schulz *et al*., 1995). 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).

NMDA receptor NMDA 2b subunits have one PDZ-binding motif and one tyrosine phosphorylation motif (YEKL 1472–1475) at the C-terminal, which influence receptor internalization (Wenthold *et al.*, 2003). The main site of tyrosine phosphorylation of NMDA 2b is Y1472 (Nakazawa *et al.*, 2001). Roche *et al.*, (2001) demonstrated that the YEKL motif regulates a robust endocytosis of NMDA receptors

in cultured neurons and there was a developmental decline in NMDA receptor endocytosis as neurons mature. This is consistent with a decrease of NMDA 2b subunits together with an increase of NR2A subunits while neurons are maturing (Watanabe *et al.*, 1993; Monyer *et al.*, 1994; Laurie *et al.*, 1997; Wenzel *et al.*, 1997). PSD-95 is abundant in the postsynaptic density (PSD) and is a membrane associated guanylate kinase (MAGUKs), contributing to anchoring NMDA receptors at the synapse (Wenthold *et al.*, 2003; Chung *et al.*, 2004; Lin *et al.*, 2004). PSD-95 associates with the last four amino acids (ESDV) of the NR2 subunit (Kornau *et al.*, 1995; Niethammer *et al.*, 1996; Cousins *et al.*, 2008) and this binding site is very close to Y1472 (Nakazawa *et al.*, 2001), the main site of tyrosine phosphorylation. Roche *et al.*, (2001) suggest that phosphorylation of the NMDA receptor could inhibit the receptor's interaction with PSD-95. They concluded that the disruption of the NMDA receptor–PSD-95 complex destabilized the NMDA receptor, thereby allow receptor internalization. In addition the tyrosine motif binds to AP-2 adaptor complexes allowing a rapid internalization of surface NMDA receptors (Bonifacino & Dell'Angelica, 1999; Lavezzari *et al.*, 2003). Such a mechanism is consistent with the results we have observed here in neonatal rat striatum and with the results of Gu *et al.*, (2007) showing that in HEK cells coexpressing DA D_1, NR1, NMDA 2b and PSD-95, in the presence of the PKA blocker, H89, dopamine caused an inhibition of the NMDA receptor response that is dependent on the expression of PSD-95 (Fiorentini *et al.*, 2003, 2006) who used BRET to show that DA D_1 and NMDA receptors directly interact in transfected HEK cells and that in cells coexpressing DA D_1, NR1, NMDA 2b and PSD-95, coactivation of DA D_1 and NMDA receptors is necessary to observe DA D_1-evoked NMDA receptor internalization.

The excitatory amino acid Glu is the most prevalent transmitter in the brain; its effect on postsynaptic receptors is limited by uptake process (Erecinska, 1987) and by diffusion of Glu from the cleft. The removal of Glu from the extracellular fluid, limitation of its action occurs by uptake and by diffusion (Tong & Jahr, 1994). This is

accomplished by a transporter in the plasma membrane of both neurons and astrocytes (Brooks-Kayal *et al*., 1998; Gelagashvili & Schousboe, 1998). Electrophysiological evidence showed that the block of Glu transporters potentiates postsynaptic excitation of Glu receptors (Tong & Jahr, 1994). The cellular uptake of Glu is driven by the electrochemical gradients of Na^+ and K^+ and is accompanied by voltage and pH changes. 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, also known as 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 (Wolff *et al*., 1986; Kasa *et al*., 1988) 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. 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). Tissue, perfused with artificial cerebrospinal fluid (aCSF) 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). It is reported that prolonged exposure to hypobaric hypoxia transiently reduces GABAA receptor number in mice cerebral cortex (Viapiano *et al.*, 2001).

The ventilatory response to hypoxia is influenced by the balance between inhibitory (GABA, glycine, and taurine) and excitatory (glutamate and aspartate) amino acid neurotransmitters. γ- aminobutyric acid (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. 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).

Adenosine Receptors

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 increase the release of different transmitters (Sebastiao & Ribeiro, 1992; Cunha *et al*., 1994; Gu & MacDermott, 1997) 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).

Integrative brain failure in the ICU environment is almost always associated with a haemodynamic or metabolic decompensation, either intra or extra cranial. The ICU environment provides a repository of typical predisposing factors of a haemodynamic or metabolic nature, including acute or chronic organic brain vascular insufficiency, endocrine insufficiency, acute or chronic cardiopulmonary decompensations, multiple organ system insufficiency, relative hypoxia, poor tissue perfusion, multiple medications and finally sleep–wake cycle disruption caused by immobilization, anxiety and pain (Crippen, 1995).

Serotonin Receptors

During brain development, serotonin (5-hydroxytryptamine; 5-HT) 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).

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 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 M_2 receptors expressed on cholinergic (Aubert *et al*., 1995; Allen & Brown, 1996) 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 M_2 subtype in the periphery and CNS. In contrast, there are muscarinic receptors, apparently of the M_1 subtype, that increase the release of NE (Raiteri *et al*., 1990 a,b) expressed on noradrenergic axon terminals in the periphery. The M_1 receptor is generally coupled to PTX-insensitive G-protein. Its activation results in formation of inositol trisphosphate and diacylglycerol. In contrast, the 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.

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.

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 (Burchfield *et al*., 1993; Berg *et al*., 1996). 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 (CPR) during a nonshockable 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 randomised 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 may 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).

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 [hypoxia-induced factor (HIF)], 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).

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 are 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).

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 (Semenza, 1999; Lopez-Barneo *et al*., 2001). 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 (Gibson & Peterson, 1981; Freeman *et al*., 1987) 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 (Fidone *et al*., 1982; Donnelly, 1993), in tissue slices (Pardal *et al*., 2000) or as isolated cells or cell clusters *in vitro* (Urena *et al*., 1994; Montoro *et al*., 1996; Jackson & Nurse, 1997). Hypoxia also stimulates catecholamine release from neonatal adrenal chromaffin cells (Mojet *et al*., 1997; Thompson *et al*., 1997) and from PC-12 cells (Kumar *et al*., 1998; Taylor *et al*., 2000), an O_2-sensitive cell line derived from the adrenal medulla. 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 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).

Free Radical Release and Toxicity

Free radicals and reactive oxygen species (superoxide and hydrogen peroxide) 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.

Neuronal membranes of the brain constitute high amount of polyunsaturated fatty acids (PUFA). About 20% of the dry weights of the brain constitute essential fatty acids. Hence any change in the relative content of fatty acids affects cognitive function and behaviour (Yehuda *et al*., 1997). 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 can 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 (Coyle & Puttfarcken, 1993; Chan, 1994). 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 oxidant 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.

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*., 1983; 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). 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 CP. 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.

Calcium Imaging

Intracellular free Ca^{2+} concentration plays a pivotal role in the regulation of various cellular functions as an intracellular messenger system. Since the development of digital video imaging of Ca^{2+} novel findings including Ca^{2+} oscillations (Berridge & Galione, 1988; Berridge, 1990) and Ca^{2+} waves (Berridge, 1993) have been

described in many different cultured cell types. Ca^{2+} spots were reported as an elementary Ca^{2+} influx event through mechanosensitive channels directly coupled with the initial step in mechanotransduction in cultured endothelial (Ohata *et al*., 2001a,b; Tanaka & Takamatsu, 2001) and cultured lens epithelial cells (Ohata *et al*., 2001b,c). The Ca^{2+} spots which develop sporadically, exhibit a spatiotemporal pattern distinct from Ca^{2+} sparks, the elementary Ca^{2+} release events from intracellular stores (Cheng *et al*., 1993; Nelson *et al*., 1995).

In the present study, we investigated the role of glucose, epinephrine and oxygen supplementation in dopaminergic receptor regulation in the brain regions of hypoxic neonatal rats. Gene expression studies using Real-Time PCR were done to confirm the receptor data. Ca^{2+} release experiments using confocal microscopy was done to confirm the 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 is the first molecular study on hypoxic neonates showing that glucose supplementation has significant impact in controlling hypoxia induced functional damage at the dopaminergic receptors which has immense therapeutic application in the neonatal care.

Materials and Methods

Chemicals used and their sources

Biochemicals

Dopamine, homovanillic acid, sodium octyl sulfonic acid, ethylene diamine tetra acetic acid-EDTA, HEPES - [n′ (2-hydroxy ethyl)] piperazine-n′-[2-ethanesulfonic acid], ascorbic acid, pargyline, D-glucose, calcium chloride, butaclamol, (-) sulpiride, SCH 23390, (+)MK-801 [(+)5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate, Trypsin and bovine serum albumin fraction V. (±)Norepinephrine, (±)epinephrine, 5-hydroxy tryptamine, 5-hydroxy indole acetic acid, citric acid, ascorbic acid, were purchased from Sigma Chemical Co., St. Louis, MI, USA. All other reagents were of analytical grade purchased locally. Tissue freezing medium Jung was purchased from Leica Microsystems Nussloch GmbH, Germany. HPLC solvents were of HPLC grade obtained from SRL, India.and Sigma, Chemical Co., St. Louis, MI, USA.

Radiochemicals

[^{3}H]Dopamine (Sp. activity- 45.1Ci/mmol), [^{3}H]SCH 23390 (Sp. activity 83Ci/mmol) and [^{3}H]YM-09151-2 (*cis–N-(1-benzyl-2-methylpyrrolidine-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide* Sp. activity - 85.0Ci/mmol) were purchased from NEN Life Sciences Products, Inc. Boston, USA., (+)-[3-^{3}H]- MK-801 (Sp. activity- 27.3Ci/mmol) was purchased from Perkin Elmer Life and Analytical Sciences, Bostaon, MA. 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, MI, USA. ABI PRISM High Capacity cDNA Archive kit, Primers and Taqman probes for Real-Time PCR - Dopamine D_1 (Rn_02043440) Dopamine D_2 (Rn_00561126), mGLU5 gene (Rn_00566628_m1) and glutamergic- NMDAR 2b (Rn_00561352_m1) gene, endogenous control (β-actin) were purchased from Applied Biosystems, FosterCity, CA, USA. Ca^{2+} fluorescent dye, fluo 4-AM and nuclear stain TO-PRO-3 was purchased from Molecular Probes, Eugene, Oregon, USA.

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, 10% dextrose (500mg/ Kg body wt) and epinephrine (0.1µg/ Kg body wt) were injected i.p. and then treated with 100%

oxygen for 30 minutes (Hx+G+E+O). The experimental animals were maintained at 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. The brain regions and body parts were dissected out quickly over ice according to the procedure of Glowinski & 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.

Quantification of brain monoamines and their metabolites in the experimental groups of neonatal rats

The monoamines were assayed according to the modified procedure of Paulose *et al*., (1988). The brain stem (BS) and Cerebellum (CB) of experimental neonatal grouos of rats was homogenised in 0.4N perchloric acid. The homogenate was then centrifuged at 5000 x g for 10 minutes at 4°C in a Sigma 3K30 refrigerated centrifuge and the clear supernatant was filtered through 0.22μm HPLC grade filters and used for HPLC analysis.

Epinephrine (EPI), norepinephrine (NE), dopamine (DA) Homovanillic Acid (HVA) and serotonin (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, 15% methanol. The pH was adjusted to 3.25 with orthophosphoric acid, filtered through 0.22μm 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 detection. 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.

DOPAMINE RECEPTOR BINDING STUDIES USING [^{3}H]RADIOLIGANDS IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS

Dopamine receptor binding studies using [^{3}H]Dopamine

DA receptor assay was done using [^{3}H]DA according to Madras *et al.*, (1988) and Hamblin & Creese, (1982). Brain tissues were homogenised in a polytron homogeniser with 20 volumes of cold 50mM Tris-HCl buffer, along with 1mM EDTA, 0.01%ascorbic acid, 4mM $MgCl_2$, 1.5mM $CaCl_2$, pH. 7.4 and centrifuged at 38,000 x g for 30min at 4°C. The pellet was washed twice by rehomogenization and centrifuged twice at 38,000 x g for 30min at 4°C. This was resuspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.25nM-1.5nM of [^{3}H]DA in 50mM Tris-HCl buffer, along with 1mM EDTA, 0.01% ascorbic acid, 1mM $MgCl_2$, 2mM $CaCl_2$, 120mM NaCl, 5mM KCl, pH.7.4 in a total incubation volume of 250µl containing 200-300µg of proteins. Specific binding was determined using 100µM unlabelled dopamine. Competition studies were carried out with 0.25nM [^{3}H]DA in each tube with unlabelled ligand concentrations varying from 10^{-9} - 10^{-4}M of DA.

Tubes were incubated at 25°C for 60min. and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 5.0ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Dopamine D_1 receptor binding studies using [^{3}H]SCH 23390

Dopamine D_1 receptor binding assay using [^{3}H]SCH 23390 in the brain regions were done according to the modified procedure of Mizoguchi *et al.,* (2000). The tissues were weighed and homogenized in 10 volumes of ice cold 50mM Tris-HCl buffer, along with 1mM EDTA, 4mM $MgCl_2$, 1.5mM $CaCl_2$, 5mM KCl, pH. 7.4. The homogenate was centrifuged at 40,000 x g for 30min. The pellet was washed and centrifuged with 50 volumes of the buffer at 40,000 x g for 30min. This was suspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.5 - 5.0nM of [^{3}H]SCH 23390 in 50mM Tris-HCl buffer, along with 1mM EDTA, 4mM $MgCl_2$, 1.5 mM $CaCl_2$, 5mM KCl with 12µM pargyline and 0.1% ascorbic acid in a total incubation volume of 250µl containing 150-200µg protein. Specific binding was determined using 50µM unlabelled SCH 23390. Competition studies were carried out with 1.0nM [^{3}H]SCH 23390 in each tube with unlabelled ligand concentrations varying from 10^{-9}- 10^{-4} M of SCH 23390.

Tubes were incubated at 25°C for 60 min. and filtered rapidly through GF/B filters. The filters were washed quickly by three successive washing with 5.0ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Dopamine D_2 receptor binding studies using [^{3}H]YM-09151-2

Dopamine D_2 receptor binding assay was done according to the modified procedure of Unis *et al*., (1998) and Madras *et al*., (1988). The dissected brain tissues were weighed and homogenized in 10 volumes of ice cold 50mM Tris-HCl buffer, along with 1mM EDTA, 5mM $MgCl_2$, 1.5mM $CaCl_2$, 120mM NaCl, 5mM KCl, pH, 7.4. The homogenate was centrifuged at 40,000 x g for 30min. The pellet was washed and centrifuged with 50 volumes of the buffer at 40,000 x g for 30min. This was suspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.1 - 2.0nM of [^{3}H]YM-09151-2 in 50mM Tris-HCl buffer, along with 1mM EDTA, 5mM $MgCl_2$, 1.5mM $CaCl_2$, 120mM NaCl, 5mM KCl with 10µM pargyline and 0.1% ascorbic acid in a total incubation volume of 300µl containing 200-300µg of protein. Specific binding was determined using 5.0µM unlabelled sulpiride. Competition studies were carried out with 0.5nM [^{3}H]YM-09151-2 in each tube with unlabelled ligand concentrations varying from 10^{-9}- 10^{-4}M of sulpiride.

Tubes were incubated at 25°C for 60 min. and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 5.0ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

NMDA receptor binding studies using [^{3}H]MK-801

The membrane fractions were prepared by a modification of the method described by Hoffman *et al.*, (1996). Brain regions were homogenized in a 0.32M sucrose buffer solution containing 10mM HEPES/1mM EDTA buffer, pH 7.0. The homogenate was centrifuged at 1,000 x g for 10min, and the supernatant was centrifuged at 40,000 x g for 1h. The pellet was resuspended and homogenized in 10mM HEPES buffer containing 1.0mM EDTA, pH 7.0 and centrifuged at 40,000 x g

for 1h. The final pellet was suspended in HEPES/EDTA buffer and stored at -80°C until binding assays were performed. The [^{3}H]MK-801 binding saturation assay was performed in a concentration range of 0.5 to 50nM at 23°C in an assay medium containing 10mM HEPES, pH 7.0, 200 - 250µg of protein, 100µM glycine and 100µM glutamate. Specific [^{3}H]MK-801 binding was obtained by subtracting nonspecific binding in the presence of 100µM unlabeled MK-801 from the total binding. After 1h of incubation, the reaction was stopped by filtration through GF/B filters and washed with additional buffer. Bound radioactivity was counted with cocktail-T in a liquid scintillation counter (Wallac 1409). The nonspecific binding determined showed 20-30% 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.

GENE EXPRESSION STUDIES OF DA D_1, DA D_2, mGLU5 and NMDA 2b RECEPTOR IN DIFFERENT BRAIN REGIONS OF CONTROL AND EXPERIMENTAL NEONATAL RATS

Preparation of RNA

RNA was isolated from the different brain regions of control and experimental neonatal rats using the Tri reagent from Sigma Aldrich.

Isolation of RNA

Tissue (25-50mg) 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 were 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, Fosterity, 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 probe for Dopamine DA D_1 (Rn_02043440_s1) and DA D_2 (Rn_00561126_m1) gene, mGLU5 gene (Rn_00566628_m1) and glutamergic- NMDAR 2b (Rn_00561352_m1) gene, 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 ($2^{-\Delta\Delta CT}$).

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

Brain tissues -CC, BS and CB were 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:

$$\frac{\text{(Standard or sample cpm} - \text{NSB cpm)}}{\text{(}B_0\text{ cpm} - \text{NSB cpm)}} \times 100$$

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 tissues- CC, 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 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 is 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 is 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 is calculated.

Rotarod Test

Rotarod has been used to evaluate motor coordination by testing the ability of rats to remain on revolving rod (Dunham, 1957). The apparatus has a horizontal rough metal rod of 3cm diameter attached to a motor with variable speed. This 70cm long rod was divided into four sections by wooden partitions. The rod was placed at a height of 50cm to discourage the animals to jump from the rotating rod. The rate of rotation was adjusted to allow the normal rats to stay on it for five minutes. Each rat

was given five trials before the actual reading was taken. The readings were taken at 10, 15 and 25rpm after 15 days of treatment in all groups of rats

Calcium Imaging

Cortical neuron isolates were prepared from 3 day old rats by Trypsin digestion method. After incubation cells were washed twice in indicator free RPMI medium to remove excess dye that was non specifically associated with the cell surface and then incubated for further 30 minutes to allow complete deesterification of intracellular AM esters. The 35mm plates, containing neuronal cells were placed on the stage of a Leica TCS SP5 laser scanning confocal microscope equipped with a HC PL FLUOTAR 20.0 X 0.50 dry objective (NA 0.5). Fluo 4-AM was excited with 514nm laser lines from an argon laser, with laser intensity set at 38% of available power. For visualization of Fluo 4-AM, the emission window was set at 508.4nm – 571.5nm. The images were continuously acquired before and after the cells underwent hypoxia, addition of 4mM glucose, 10^{-8}M DA and 10^{-6}M glutamate at time intervals of 1.318 seconds, for a total of 600 seconds. Time series experiments were performed collecting 512x512 pixel images at 400Hz. Fluorescence intensity was analysed using the quantitation mode in LAS-AF software from Leica Microsystems, Germany. A region of interest (ROI) was drawn within a field of view. For each ROI, the pixel intensity was calculated for each image in the 600 seconds sequence to analyse the intracellular Ca^{2+} release from the pancreatic islet cells in experimental conditions.

TO-PRO-3 Staining

Whole brain sections of control and hypoxic rats were used for the staining. The anaesthetized experimental rats were transcardially perfused with lukewarm PBS and 4% paraformaldehyde in PBS, pH 7.4. The brains were removed and placed in sucrose solution in the following order, 5% sucrose for 1h, 15% sucrose overnight and 30% sucrose overnight at 4°C. The embedding was done. The brains were thoroughly

rinsed in PBS. The brain was placed in a mould and was embedded with tissue freezing medium Jung (Leica Microsystems Nussloch GmbH, Germany). It was kept in cryostat for 10-20min until the medium hardened. The blocks thus formed were removed and mounted in Leica CM 1510 S cryostat and serial sagittal sections were taken at 20μ and directly mounted on the slides. TO-PRO-3 stain diluted 1:1000 in 1X PBS was added and kept for 10min at room temperature (Suzuki et al., 1997). The sections were placed on the stage of a Leica TCS SP5 laser scanning confocal microscope equipped with a HC PL APO CS 63.0 X 1.40 OIL UV objective (NA 1.40). TO-PRO-3 was excited with 633nm laser lines from HeNe laser, with laser intensity set at 38% of available power. For visualization of TO-PRO-3 the emission window was set at 650nm – 727.1nm. The images were collected at 1024X1024 pixel size.

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).

Dopamine and Homovanillic Acid Content (nmoles/g wet wt.) in the Brainstem of Control and Experimental Groups of Neonatal Rats

DA content in the brainstem showed a significant decrease ($p<0.001$) in Hx; Hx+G; Hx+O; Hx+G+O and Hx+G+E+O compared to C. Glucose treatment to Hx- Hx+G+O significantly ($p<0.01$) reversed the DA contents near to C. HVA content significantly decreased in Hx ($p<0.01$); Hx+G ($p<0.001$); Hx+O ($p<0.01$), Hx+G+O ($p<0.001$) and Hx+G+E+O ($p<0.001$) compared to C. DA/HVA ratio decreased significantly in Hx ($p<0.001$); Hx+O ($p<0.001$) and Hx+G+E+O ($p<0.01$). Glucose treatment to Hx- Hx+G ($p<0.001$) and Hx+G+O ($p<0.01$) significantly reversed the DA/HVA ratio towards control (Table 2).

Dopamine and Homovanillic Acid Content (nmoles/g wet wt.) in the Cerebellum of Control and Experimental Groups of Neonatal Rats

DA content in the cerebellum showed a significant decrease ($p<0.001$) in Hx; Hx+O; and Hx+G+E+O compared to C. Glucose treatment to Hx - Hx+O and Hx+G+O significantly reversed ($p<0.001$) the DA contents near to C. There was no significant change in HVA content in Hx; Hx+G; Hx+O and Hx+G+O groups. The HVA content significantly decreased in Hx+G+E+O ($p<0.001$, $p<0.01$) compared to C and Hx respectively. DA/HVA ratio decreased significantly in Hx ($p<0.01$); Hx+O ($p<0.01$) and Hx+G+E+O ($p<0.05$). Glucose treatment to Hx- Hx+G and Hx+G+O significantly reversed ($p<0.01$) the DA/HVA ratio towards control (Table 3).

Dopamine and Homovanillic Acid Content (nmoles/g wet wt.) in the Plasma of Control and Experimental Groups of Neonatal Rats

NE content in plasma showed no significant change in all the experimental groups compared to C. There was a significant increase in the EPI content in Hx ($p<0.01$); Hx+G ($p<0.05$); Hx+O ($p<0.05$); Hx+G+O ($p<0.05$) and Hx+G+E+O ($p<0.001$) groups. The EPI content remained significantly higher in Hx+G+E+O ($p<0.01$) compared to Hx. There was no significant change in plasma DA levels in all the experimental groups of neonatal rats. The HVA content increased in HX+O ($p<0.05$); Hx+G+O ($p<0.05$) and decreased in Hx+G+E+O ($p<0.01$) compared to both C and Hx. There was no significant change in the DA/HVA ratio. (Table 4)

BRAIN DOPAMINE RECEPTOR ALTERATIONS IN THE CONTROL AND EXPERIMENTAL GROUPS OF NEONATAL RATS

Cerebral Cortex

Scatchard analysis using [^{3}H]DA against DA

Scatchard analysis of [^{3}H]DA against DA in cerebral cortex showed a significant increase ($p<0.001$) in B_{max} in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to Hx- Hx+G and Hx+G+O reversed the DA binding parameter near to C. K_d showed no significant change in all the experimental groups compared to C (Fig 1-5; Table 5-9).

Scatchard analysis using [^{3}H]SCH 23390 against SCH 23390

Binding studies of [^{3}H]SCH 23390 against SCH 23390 for DA D_1 receptors showed a significant decrease in B_{max} ($p<0.05$) in the Hx groups. Glucose treated groups - Hx+G ($p<0.001$), Hx+G+O ($p<0.01$) and HX+G+E+O ($p<0.001$) groups showed a significant increase in the B_{max}. There was a significant increase ($p<0.05$) of K_d in Hx, Hx+O and Hx+G+E+O compared to C (Fig 6-10; Table 9-14).

Real-Time PCR analysis of DA D_1 Receptors

The gene expression studies by real-time PCR analysis showed that DA D_1 receptor mRNA was significantly up regulated in Hx ($p<0.001$), Hx+O ($p<0.01$) and Hx+G+E+O ($p<0.001$) groups compared to C. Glucose treatment to Hx - Hx+G and Hx+G+O significantly ($p<0.001$) reversed the up regulation compared to hypoxic groups (Fig 11; Table 15).

Scatchard analysis using [^{3}H]YM-09151-2 against sulpiride

Binding studies of [^{3}H]YM-09151-2 against sulpiride for DA D_2 receptors in cerebral cortex showed that the B_{max} decreased significantly ($p<0.001$) in Hx and Hx+O groups while in Hx+G and Hx+G+O groups the receptor numbers reversed to near control values. The K_d increased significantly in Hx ($p<0.05$) and Hx+G+E+O ($p<0.001$) when compared to control (Fig 12-16; Table 16-20).

Real-Time PCR analysis of DA D_2 Receptors

The gene expression studies by real-time PCR analysis showed that DA D_2 receptor mRNA was significantly ($p<0.001$) down regulated in Hx, Hx+O and Hx+G+E+O groups. Glucose treatment to hypoxic rats - Hx+G and Hx+G+O significantly ($p<0.001$) reversed the down regulation compared to Hx (Fig 17; Table 21).

Brainstem

Scatchard analysis using [^{3}H]DA against DA

Scatchard analysis of [^{3}H]DA against DA in brainstem showed a significant increase in B_{max} of Hx ($p<0.001$), Hx+O ($p<0.05$) and Hx+G+E+O ($p<0.001$) groups compared to C. Glucose treatment to Hx- Hx+G and Hx+G+O reversed the DA binding parameter near to C. Hx+G+E+O group showed a significant decrease

($p<0.001$) in K_d compared to C. There was no change in K_d in other experimental groups compared to C (Fig 18-22; Table 22-26).

Scatchard analysis using [^{3}H]SCH 23390 against SCH 23390

Binding studies of [^{3}H]SCH 23390 against SCH 23390 for DA D_1 receptors showed a significant increase in B_{max} in the Hx ($p<0.01$) and Hx+G+E+O ($p<0.001$) groups compared to C. B_{max} of Glucose treated groups - Hx+G, Hx+G+O reversed towards control level. There was a significant increase in K_d for Hx ($p<0.01$), Hx+O ($p<0.01$), Hx+G+O ($p<0.01$) and Hx+G+E+O ($p<0.001$) compared to C (Fig 23-27; Table 27-31).

Real-Time PCR analysis of DA D_1 Receptors

The gene expression studies by real-time PCR analysis showed that DA D_1 receptor mRNA was significantly up regulated in Hx ($p<0.001$) and Hx+O ($p<0.01$) and Hx+G+E+O ($p<0.001$) groups. Glucose treatment to hypoxic (Hx+G, Hx+G+O) significantly ($p<0.001$) reversed the up regulation compared to Hx groups (Fig 28; Table 32).

Scatchard analysis using [^{3}H]YM-09151-2 against sulpiride

Binding studies of [^{3}H]YM-09151-2 against sulpiride for DA D_2 receptors in brainstem showed that the B_{max} decreased significantly in Hx ($p<0.01$) and Hx+O ($p<0.05$) groups while in Hx+G and Hx+G+O groups the receptor numbers reversed to near control values. The K_d increased significantly in Hx ($p<0.001$). All other groups did not show any significant change in K_d compared to C. (Fig 29-34; Table 32-37).

Real-Time PCR analysis of DA D_2 Receptors

The gene expression studies by real-time PCR analysis showed that DA D_2 receptor mRNA was significantly ($p<0.001$) up regulated in Hx, Hx+O and Hx+G+E+O groups. Glucose treatment to hypoxic rats - Hx+G and Hx+G+O significantly ($p<0.001$) reversed the up regulation compared to Hx (Fig 34; Table 38).

Cerebellum

Scatchard analysis using [^{3}H]DA against DA

Scatchard analysis of [^{3}H]DA against DA in brainstem showed a significant decrease in B_{max} of Hx ($p<0.01$) and Hx+O ($p<0.05$) groups compared to C. Glucose treatment to Hx- Hx+G and Hx+G+O reversed the DA binding parameters near to C. Hx and Hx+G+E+O groups showed significantly ($p<0.01$) decreased K_d compared to C. There was no change of K_d in other experimental groups compared to C (Fig 35-39; Table 39-43).

Scatchard analysis using [^{3}H]SCH 23390 against SCH 23390

Binding studies of [^{3}H]SCH 23390 against SCH 23390 for DA D_1 receptors showed a significant decrease in B_{max} in the Hx ($p<0.001$), Hx+O ($p<0.05$) and Hx+G+E+O ($p<0.001$) groups compared to C. Glucose treated groups - Hx+G, Hx+G+O groups reversed towards C in the B_{max}. There was a significant increase in K_d of Hx+G ($p<0.01$) and Hx+O ($p<0.001$) compared to C (Fig 40-44; Table 44-48).

Real-Time PCR analysis of DA D_1 Receptors

The gene expression studies by real-time PCR analysis showed that DA D_1 receptor mRNA was significantly down regulated in Hx ($p<0.001$) and Hx+O ($p<0.01$) and Hx+G+E+O ($p<0.001$) groups compared to C. Glucose treatment to hypoxia - Hx+G, Hx+G+O significantly ($p<0.001$) reversed the up regulated mRNA expression compared to Hx (Fig 45; Table 49).

Scatchard analysis using [^{3}H]YM-09151-2 against sulpiride

Binding studies of [^{3}H]YM-09151-2 against sulpiride for DA D_2 receptors in cerebellum showed that the B_{max} decreased significantly in Hx ($p<0.01$) and Hx+O ($p<0.05$) groups while in Hx+G and Hx+G+O groups the receptor numbers reversed to near control values. There was no significant change in K_d compared to C in all the experimental groups (Fig 46-50; Table 50-54).

Real-Time PCR analysis of DA D_2 Receptors

The gene expression studies by real-time PCR analysis showed that DA D_2 receptor mRNA was significantly ($p<0.001$) down regulated in Hx, Hx+O and Hx+G+E+O groups. Glucose treatment to hypoxic rats - Hx+G and Hx+G+O significantly ($p<0.001$) reversed the down regulation compared to Hx (Fig 51; Table 55).

BRAIN NMDA RECEPTOR ALTERATIONS IN THE CONTROL AND EXPERIMENTAL GROUPS OF NEONATAL RATS

Cerebral Cortex

Scatchard analysis using [^{3}H]MK-801 against MK-801

Scatchard analysis of [^{3}H]MK-801 against MK-801 in cerebral cortex showed a significant decrease ($p<0.01$) in B_{max} in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to Hx - Hx+G and Hx+G+O reversed the DA binding parameter near to C. K_d showed significant decrease ($p<0.05$) in Hx while in Hx+O and Hx+G+E+O groups the K_d significantly increased ($p<0.05$) compared to C. In glucose treated groups- Hx+G and Hx+G+O, K_d values reversed to C. (Fig 52-56; Table 56-60).

Real-Time PCR analysis of mGLU5 Receptors

The gene expression studies by real-time PCR analysis showed that mGLU5 receptor mRNA was significantly up regulated ($p<0.001$) in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to hypoxic rats - Hx+G, Hx+G+O significantly ($p<0.001$) reversed the up regulation compared to hypoxic groups (Fig 57; Table 61).

Real-Time PCR analysis of NMDA 2b Receptors

The gene expression studies by real-time PCR analysis showed that NMDA 2b receptor mRNA was significantly down regulated ($p<0.001$) in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to hypoxic rats - Hx+G, Hx+G+O significantly ($p<0.001$) reversed the down regulation compared to hypoxic groups (Fig 58; Table 62).

Brainstem

Scatchard analysis using [^{3}H]MK-801 against MK-801

Scatchard analysis of [^{3}H]MK-801 against MK-801 in brainstem showed a significant increase in B_{max} in Hx ($p<0.001$), Hx+O ($p<0.001$) and Hx+G+E+O ($p<0.01$) groups compared to C. Glucose treatment to Hx - Hx+G and Hx+G+O significantly reversed ($p<0.001$) the DA binding parameters near to C. K_d showed significant decrease ($p<0.05$) in Hx, Hx+O and Hx+G+E+O groups compared to C. In glucose treated groups- Hx+G and Hx+G+O, K_d values reversed to C. (Fig 59-63; Table 63-67).

Real-Time PCR analysis of mGLU5 Receptors

The gene expression studies by real-time PCR analysis showed that mGLU5 receptor mRNA was significantly up regulated ($p<0.001$) in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to hypoxic rats - Hx+G,

Hx+G+O significantly ($p<0.001$) reversed the up regulation compared to hypoxic groups (Fig 64; Table 68).

Real-Time PCR analysis of NMDA 2b Receptors

The gene expression studies by real-time PCR analysis showed that NMDA 2b receptor mRNA was significantly up regulated ($p<0.001$) in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to hypoxic rats - Hx+G, Hx+G+O significantly ($p<0.001$) reversed the down regulation compared to hypoxic groups (Fig 65; Table 69).

Cerebellum

Scatchard analysis using [^{3}H]MK-801 against MK-801

Scatchard analysis of [^{3}H]MK-801 against MK-801 in cerebellum showed a significant increase ($p<0.001$) in B_{max} in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to Hx - Hx+G and Hx+G+O significantly reversed ($p<0.001$) the DA binding parameters near to C. K_d showed significant decrease in Hx+O ($p<0.05$) and Hx+G+E+O ($p<0.01$) groups compared to C. In glucose treated groups- Hx+G and Hx+G+O, K_d values reversed to C (Fig 66-70; Table 70-74).

Real-Time PCR analysis of mGLU5 Receptors

The gene expression studies by real-time PCR analysis showed that mGLU5 receptor mRNA was significantly up regulated ($p<0.001$) in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to hypoxic rats - Hx+G, Hx+G+O significantly ($p<0.001$) reversed the up regulation compared to hypoxic groups (Fig 71; Table 75).

Real-Time PCR analysis of NMDA 2b Receptors

The gene expression studies by real-time PCR analysis showed that NMDA 2b receptor mRNA was significantly up regulated ($p<0.001$) in Hx, Hx+O and Hx+G+E+O groups compared to C. Glucose treatment to hypoxic rats - Hx+G, Hx+G+O significantly ($p<0.001$) reversed the down regulation compared to hypoxic groups (Fig 72; Table 76).

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

The cGMP content in the cerebral cortex decreased significantly ($p<0.001$) in Hx, Hx+O and Hx+G+E+O compared to C. Glucose treatment to hypoxic rats – Hx+G ($p<0.001$), Hx+G+O ($p<0.01$) significantly reversed the cGMP levels to C (Fig 73; Table 77).

cGMP content in the brainstem of experimental groups of neonatal rats

The cGMP content in the brainstem increased significantly ($p<0.05$) in Hx, Hx+O and Hx+G+E+O compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed ($p<0.05$) the cGMP levels to C (Fig 74; Table 78).

cGMP content in the cerebellum of experimental groups of neonatal rats

The cGMP content in the cerebellum increased significantly ($p<0.001$) in Hx, Hx+O and Hx+G+E+O compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed ($p<0.01$) the cGMP levels to C (Fig 75; Table 79).

cAMP content in the brainstem of experimental groups of neonatal rats

The cAMP content in the brainstem increased significantly ($p<0.05$) in Hx, Hx+O and Hx+G+E+O compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed ($p<0.001$) the cAMP levels to C (Fig 76; Table 80).

cAMP content in the cerebellum of experimental groups of neonatal rats

The cAMP content in the cerebellum increased significantly in Hx ($p<0.01$), Hx+O ($p<0.01$) and Hx+G+E+O ($p<0.001$) compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed ($p<0.05$) the cAMP levels to C (Fig 77; Table 81).

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

The IP3 content in the cerebral cortex decreased significantly ($p<0.001$) in Hx, Hx+O and Hx+G+E+O compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed ($p<0.001$) the IP3 levels to C (Fig 78; Table 82).

IP3 content in the brainstem of experimental groups of neonatal rats

The IP3 content in the brainstem decreased significantly ($p<0.001$) in Hx ($p<0.001$), Hx+O ($p<0.001$) and Hx+G+E+O ($p<0.01$) compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed ($p<0.001$) the IP3 levels to C (Fig 79; Table 83).

IP3 content in the cerebellum of experimental groups of neonatal rats

The IP3 content in the cerebellum increased significantly in Hx ($p<0.01$), Hx+O ($p<0.05$) and Hx+G+E+O ($p<0.05$) compared to C. Glucose treatment to hypoxic rats – Hx+G, Hx+G+O significantly reversed ($p<0.01$) the IP3 levels to C (Fig 80; Table 84).

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 ($p<0.01$) and Hx+G+E+O ($p<0.001$) when compared to C. There was

no significant change in Hx+G, Hx+O and Hx+G+O groups compared to C (Table 85).

Rotarod Performance of experimental animals

Rotarod test at 10 ($p<0.01$), 15 ($p<0.001$) and 25 ($p<0.001$) revolutions per minute (rpm) showed a significant decrease in the retention time on the rotating rod in Hx, Hx+O and Hx+G+E+O compared to respective C. Glucose treatment – Hx+G and Hx+G+O significantly reversed the retention time near to control at 10 ($p<0.01$), 15 ($p<0.001$) and 25 ($p<0.001$) rpm compared to Hx group (Fig 81; Table 86).

Calcium Imaging

Calcium imaging studies in neonatal cortical cells showed a significant decrease in extracellular Ca^{2+} during hypoxia compared to control levels. Administration of 4mM glucose reversed the Ca^{2+} levels towards control (Fig 82; Table 87).

Studies on the combination effect of 10^{-8}M DA and 10^{-6}M Glutamate on Ca^{2+} release in control and hypoxic cortical cells showed an increase in Ca^{2+} release during addition of DA and a decrease with glutamate administration (Fig 83,84; Table 88,89).

TO-PRO-3 Staining

TO-PRO-3 staining of neonatal rat brain showed a decrease in the cell numbers in hypoxia compared to control. This is due to the apoptotic processes during hypoxia (Fig 85).

Discussion

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) had previously 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). It was reported that hyperoxia triggers diffuse 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). It is reported that 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). Hence, in our experiments the neonatal rats were subjected to single episode of hypoxia for 30 minutes and sacrificed on day 12 after birth. In the present study, we investigated the dopaminergic and glutamatergic functional regulation of hypoxia in hypoxic neonates and in hypoxic neonates supplemented with glucose, oxygen and epinephrine.

DA plays a critical role in normal cognition throughout the lifespan and has been implicated in the pathophysiology of neuropsychiatric disorders such as schizophrenia and attention deficit disorder. DA is of particular interest in relation to the development of cognitive abilities subserved by the PFC. DA has been shown to be necessary for normal performance on working memory tasks in both human and non-human primates (Williams & Goldman- Rakic, 1995; Dreher *et al.,* 2002; Mattay *et al.,* 2002; Gao & Goldman-Rakic, 2003). Alterations in DA markers in the PFC have been characterized during postnatal development in non-human primates. Considering the involvement of DA system in neurodevelopmental dysfunction, it is important to clarify the effect of DA on synaptic transmission in the developing

hippocampus (Noriyama *et al.*, 2006), cerebral cortex (Weickert *et al.*, 2007), cerebellum (Tranquart *et al.*, 2001) and brainstem (Shiraishi *et al.*, 2008).

Glutamate and aspartate are the dominating excitatory amino acids (EAA) and the primary neurotransmitter in about half of all the synapses in the mammalian forebrain. Of the ionotropic receptors, the NMDA receptors dominate in the immature brain when synaptic transmission is weak and extremely plastic (Herlenius & Lagercrantz, 2004). During critical periods of development and synaptogenesis, NMDA receptors play an essential role in activity-dependent plasticity and synaptic refinement (McDonald & Johnston, 1990; Qu *et al*., 2003). NMDA channels seem to be crucially involved in the appearance of long-term potentiation (LTP) and synaptic plasticity underlying learning and memory storage throughout life. But excess activation of NMDA and metabotropic receptors are implicated in the pathophysiology of brain injury in several clinical disorders to which the developing brain is susceptible, including hypoxia-ischemia and seizures (McDonald & Johnston, 1990; Qu *et al.*, 2003). NMDA receptor stimulation by excessive glutamate release leads to Ca^{2+} influx that induces subsequent neural apoptosis.

Group I mGLU receptors play a prominent role in the regulation of synaptic plasticity in many areas of the brain (Riedel, 1996; Riedel & Reymann, 1996; Spooren *et al.*, 2003; Simonyi *et al.*, 2005). Both mGLU1 and mGLU5 receptors are important in the LTP, long-term depression (LTD) and memory formation. Co application of inactive doses of inhibitors of mGLU5 and NMDA receptors synergistically impairs working memory, instrumental learning (Homayoun *et al.*, 2004), spatial learning (Campbell *et al.*, 2004) and aversive learning paradigms (Gravius *et al.*, 2006). mGLU5 knockout mice have impaired NMDA dependent LTP and are deficient in memory tasks. mGLU5 and mGLU5 dependent facilitation of NMDA currents (Miserendino *et al.*, 1990; Rodrigues *et al.*, 2002) are important for mediating the morphological changes involved in memory (Lamprecht *et al.*, 2002) but not for the maintenance of fear conditioning (Rodrigues *et al*., 2004).

Body weight and blood glucose level in the serum

There was no significant change in body weight of hypoxic rats when compared to control on postnatal day 12. 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.

Neurotransmitters in the cerebellum and brainstem.

Monoamine metabolites have been found to decrease in the extracellular fluid during hypoxia (Sarna *et al.*, 1990; Masuda & Ito, 1993; Richards *et al.*, 1993) and to

increase during the recovery period (Damsma *et al.*, 1990). Exposure of neonatal rats to transient hypoxia induces a prolonged decrease of brain DA as well as DA uptake activity (Hadjiconstantinou *et al*., 1990). Brain hypoxia/anoxia is associated with excessive unregulated release of neurotransmitters, especially Glu (Choi, 1988; Choi & Rothman, 1990) and activation of their receptors. Glu has excitotoxic properties after its induced release. Hypoxic stress in the neonate induces significant changes in neurotransmitter activity and functioning of the hypothalamic- pituitary- adrenal- axis. These alterations cause an impairment of cognition by interfering with working memory capacity, independently of nutritional status. The change in cognitive performance after administration of glucose, depend on the level of sympathetic activation, glucocorticoid secretion and pancreatic β-cell function, rather than simple fuelling of neural activity.

We report a decreased DA and DA/HVA in the cerebellum and brainstem of experimental neonates exposed to hypoxia and supplemented with epinephrine and oxygen (Hx; Hx+O; Hx+G+E+O). Supplementation of glucose to hypoxic rats and hypoxic rats treated glucose and oxygen showed better regulation of altered DA content DA/HVA compared to control. The decreased content of DA in the brain regions is consistent with the investigation of Miwa *et al*., (1986) where the hypoxic insult is reported to cause down regulation of DA synthesis and turnover since DA neurons are most sensitive to hypoxia. DA modulates fast excitatory and inhibitory synaptic transmission in several brain regions. DA is of particular interest in relation to the development of cognitive abilities subserved by the cortical regions. Dopaminergic neurons appear early during development (6-8 weeks) in humans. The DA turnover is relatively high during perinatal period compared to adults. Disturbances of the development of the dopaminergic system lead to dyskinesia, dystonia, tics and abnormal eye movements (Herlenius & Lagercrantz, 2004).

A decrease in the DA content in both cerebellum and brainstem of hypoxic neonates and those supplemented by oxygen and epinephrine will possibly affect

locomotor activity, cognition, emotion, positive reinforcement, food intake and endocrine regulation which are under dopaminergic control (Herlenius & Lagercrantz, 2004) in the developing neonates. Oxygen-free radicals are generated during and after hypoxia. Hundred percent 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 acts as a factor for neurodegeneration (Therade-Matharan *et al.*, 2004). Hypoxemic piglets resuscitated with oxygen have been previously reported to have increased cerebral injury *via* cortical damage. Thus, our results are consistent with earlier reports that hyperoxia with oxygen after hypoxia cause increased damage in the cerebral cortex of the newborn rats (Roberto *et al.*, 2005). During hypoxia, there is an increased epinephrine release in the system (Takahashi *et al.*, 1995). Yagiela (1985) reported that epinephrine altered the distribution of blood by its effects on the cardiovascular system. Sokrab & Johansson (1980) found that epinephrine induced hypertension resulted in increased cerebral blood flow in conscious rats. Epinephrine induces disturbance of the blood-brain-barrier (Johansson & Martinsson, 1979) even functioning as excitatory neurotransmitter (Barchas *et al.*, 1969) and thus decreasing the threshold for convulsions (Yokoyama *et al.*, 1995) which augments an early onset of seizures during hypoxic condition. It would again set in an oxygen stressed cycle in the hypoxic brain leading to decrease in neurotransmitter production and transport including DA (Kranjc *et al.*, 1994). This accounts for the deterioration of the DA content on supplementation with epinephrine during hypoxia.

Glucose metabolism supply NADPH, through the pentosephosphate pathway, aimed at preventing oxidative stress, mitochondrial damage and neurotoxicity during oxygen deprivation to neural cells. A significant amount of the glucose entering the brain during the post hypoxic period appears to be used for metabolite synthesis rather than energy production (Kintner *et al.*, 1983). Koshimura *et al.*, (2003) has reported that decreases in the blood glucose level would affect the brain DA synthesis. This is

consistent with our findings where glucose supplementation alone and along with oxygen brought the DA content to near control level in hypoxic neonates.

Receptor binding parameters in the brain regions of control and experimental neonatal rats

Neuronal communication mediated by the myriads of synapses is mainly mediated by neurotransmitters, although there are also electrical synapses. Expression of transmitters and receptor subtypes are critical for the development of synapses and formation of neuronal networks underlying behavior in the fetus as well as the growing child and adult human. (Herlenius & Lagercrantz, 2004).

Cerebral Cortex

The cerebral cortex is critical to speech, emotion, reasoning, memory, movement and integration of information. We found a decrease in the DA content in the cerebral cortex (Finla, 2007). This is consistent with previous work done by Miwa *et al.,* 1986. In the cerebral cortex total DA receptors and its subtypes - DA D_1 and DA D_2 receptors showed significantly decreased activity in hypoxic rats and on supplementation of oxygen alone to hypoxic rats when compared to control rats. DA D_1 receptor mRNA showed significant decrease and DA D_2 showed significant increase during epinephrine supplementation prior to resuscitation. These dopaminergic receptor alterations were reversed by glucose supplementation. We also observed a down regulation in the DA D_1 and DA D_2 receptors in the hypoxic neonates and those treated with oxygen and epinephrine. This reversed to near control levels with glucose supplementation to hypoxic rats. Hypoxia has profound cellular effects that are mediated by altered activity and expression of proteins (Bandyopadhyay *et al.,* 1999). DA D_2 agonists have been implicated to attenuate pyramidal cell excitability (Gulledge & Jaffe, 1998; Tseng & O'Donnell, 2004), modulating the responses to NMDA and AMPA receptors (Tseng & O'Donnell,

2004). Thus, the decreased DA D_2 receptor in the hypoxic cortex contributes to the neurodegeneration due to increased glutamate mediated calcium influx across the plasma membrane resulting in cell death, apoptosis or necrosis (Sultana *et al.,* 2006). Real time PCR studies were conducted to evaluate the DA functional regulation at the mRNA level during hypoxia and resuscitation by glucose, oxygen and epinephrine. We obtained an up regulation in the DA D_1 receptor mRNA during hypoxia, and those treated with oxygen and epinephrine. Glucose supplemented groups reversed the receptor gene expression towards control level. But the DA D_2 expression was down regulated in the hypoxic condition, oxygen and epinephrine treated groups. Glucose supplementation positively reversed the gene expressions to near control level. The DA D_1 receptor gene expression was not concurrent with the receptor pattern. Earlier studies have also reported discrepancies between protein levels and distribution of mRNAs in DA D_1 and DA D_2 receptors (Mansour *et al.,* 1992; 1990). Study of mechanisms involved in the reduced expression of DA D_1 receptors has shown that there is probably an interval between the reduction in transcription and that of receptor expression. It is well known that transcriptional activity is the first regulatory step, which is not always followed by translational and posttranslational regulation of the proteins. Nevertheless, these changes may influence development, differentiation and neuronal circuitries, contributing to long term abnormalities. Changes during hypoxia do not manifest immediately but cause dysfunction in later stages of life. Our results also confirm the hypothesis that the decrease in DA D_2 receptors is due to reduced transcription. This early and transitory decrease in the expression of DA D_2 receptor mRNA after hypoxic-ischemia episode is supported by findings of Filloux *et al.*, (1996). The present study also supports the idea that therapy aimed at preventing long-term changes induced by hypoxia should be undertaken immediately after hypoxia/ischemia (Bustamante *et al.,* 2003).

Previously we reported that hypoxia causes an increase in the glutamatergic activity through the NMDA receptors, thus causing excitotoxic damage in cerebral

region (Paulose *et al.*, 2008). NMDA receptor activity can be regulated by protein kinases (PKA, PKC and tyrosine kinases) (Blank *et al.*, 1997; Lu *et al.*, 1999; Xiong *et al.*, 1999; Lei *et al.*, 2002). There is substantial evidence showing that G protein coupled receptors such as dopamine receptors modulate NMDA receptor activity (Blank *et al.*, 1997; Chen *et al.*, 2004; Cepeda & Levine, 2006; Surmeier *et al.*, 2007). Colocalization of NMDA receptors and dopamine receptors have been previously reported in brain regions (Fiorentini *et al.*, 2003; Cepeda & Levine, 2006; Scott *et al.*, 2006) and the interaction between glutamatergic and dopaminergic input is crucial for movement and behavioural control (Hallett & Standaert, 2004; Calabresi *et al.*, 2007; Surmeier *et al.*, 2007). It is apparent that an interaction between DA D_1 and NMDA receptors is critical for cognitive functions, including working memory (Goldman-Rakic, 1995; Moghaddam *et al.*, 1997). when DA systems become active, typically in response to salient external stimuli that demand the animal's attention (Schultz, 1997), the ongoing activity in the cortex is reinforced by DA D_1 mediated enhancement of glutamatergic transmission (Jay *et al.*, 1996; Seamans *et al.*, 1998; O'Donnell & Grace, 1994). This may result in an ensemble of neurons maintained in their depolarized ‘up’ state (Lewis and O'Donnell, 2000), facilitating plasticity mechanisms. It has also been shown that hippocampal stimulation-induced LTP in the cortex is facilitated immediately following VTA stimulation (Jay *et al.*, 1995) and blocked by impairing DA transmission (Gurden *et al.* 1999). Thus, the DA D_1 potentiation of NMDA actions that we have observed in the cortex is important to memory and cognitive functions. Our results are consistent with the earlier studies with a decreased DA D_1, DA D_2 and NMDA receptors during hypoxia and those supplemented with oxygen and epinephrine.

The glucose supplementation to hypoxic rats protect the neurons from glutamate neurotoxicity (Ho *et al.*, 1995), stroke and seizure (Lawrence *et al.*, 1996) which are commonly associated with hypoxia/ischemic conditions. Hattori & 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. In response to hypoxia, transient reduction in hepatic glucagon levels too has been reported in order to maintain blood glucose homeostasis which is an acute response to hypoxia, maintaining glucose at nearly normoxic levels (Xue-Qun *et al.,* 2006). Post-hypoxic glucose supplement also reduces an elevated brain lactate level and thereby preventing cerebral infarction occurring during hypoxia (Hattori & Wasterlain, 2004).

Hypoxic rats treated with epinephrine prior to glucose and oxygen resuscitation showed increased receptor numbers compared to control. The exact mechanism through which it deteriorates the hypoxic condition remains is not clear. In our previous study we have already reported that epinephrine decreases the uptake of glutamate in the brain causing persistent activation of glutamate receptors (Paulose *et al.,* 2008) which is capable of causing cholinergic dysfunction (Alkondon & Albuquerque, 2006) leading to a change in DA content, DA D_1 and DA D_2 receptor activity.

We observed a decrease in the NMDA receptors and NMDA 2b gene expression in the cerebral cortex of hypoxic neonates and those treated with oxygen and epinephrine. Supplementation of glucose alone and along with oxygen reversed the NMDA changes to control level. NMDA receptors in the cortical region are involved in memory and cognitive function. Working memory is stored by the maintained firing of a memory- specific subset of NMDA neurons in networks of the prefrontal cortex. Reduced NMDA-receptor-mediated transmission at recurrent synapses leads to a decrease in memory-associated firing (Lisman et al., 1998). Down regulation of the NMDA receptor during the hypoxic condition is supposed to have its effect in cognitive dysfunction as a result of hypoxic stress. Our results suggest that glucose supplementation to hypoxic rats, alone and in combination with oxygen reverts the changes to near control level. 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 increase in AChE activity in glucose supplemented hypoxic rats in our study. Nutritional supplements that enhance the levels of choline in the brain have been recommended for memory enhancement (Klein & Loffelholz, 1998).

Previously we had obtained an increase in the cAMP in the cerebral cortex of hypoxic rats and hypoxic rats treated with oxygen and epinephrine (Finla, 2007) which was reversed to control level by glucose treatment. In the present study we studied the changes in the cGMP content in the control and experimental rats. We observed a significant down regulation in the cGMP content in the cerebral cortex of hypoxic rats and hypoxic rats treated with oxygen and epinephrine. Supplementation of glucose alone and glucose plus oxygen reversed the alterations in cGMP content to near control. NMDA receptors have implicated in cGMP signalling pathway in the cortical regions Suvarna & O'Donnel, (2002).

Thus from our results we conclude that glucose act as a neuroprotective agent in reversing the decreased DA content, DA D_1 and DA D_2 receptor function with hypoxia. To summarize, down regulated DA D_1, DA D_2 receptor subtypes and NMDA receptors led to a decreased activity in the cerebral cortex of hypoxic rats and those supplemented with oxygen and epinephrine suggesting the occurrence of dopaminergic and NMDA mediated functional regulation in the brain of hypoxic rats and are affected by oxygen and epinephrine supplementation. This impaired glutamatergic receptor function will cause behavioural responses during later developmental period in life. The efficient and timely supplementation of glucose reversed DA D_1, DA D_2, NMDA and mGLU5 mediated receptor changes observed in hypoxia, oxygen and epinephrine. Our results suggest that hypoxia causes a significant decrease in dopaminergic function which can be corrected by glucose supplementation in the resuscitation sequence. Oxygen and epinephrine supplementation showed further damages at the molecular level. Thus it is suggested that glucose administration immediately after hypoxia with oxygenated air and if

required epinephrine as a resuscitation programme will be of tremendous advantage especially in neonatal care. This has immense clinical significance in the management of hypoxia in neonatal life which will have role in intellectual and behavioural efficiency at the later stages of the life of the individual.

Brainstem

Hypoxia can cause irreversible damage to the respiratory centres in the individual. The respiratory centre is composed of several groups of neurons located bilaterally in the medulla oblongata and pons of the brain stem. It is divided into three major collections of neurons: (1) a dorsal respiratory group, located in the dorsal portion of the medulla, which mainly causes inspiration; (2) a ventral respiratory group, located in the ventrolateral part of the medulla, which mainly causes expiration; and (3) the pneumotaxic centre, located dorsally in the superior portion of the pons, which mainly controls rate and depth of breathing. The dorsal respiratory group of neurons plays the fundamental role in the control of respiration. Severe protracted hypoxic insults damage the brainstem also causing brain death, a terminal clinical state characterized by loss of cerebral and brainstem function. The clinical criteria for brain death are complete unresponsiveness, absence of brain stem reflexes, electrical silence (flat EEG), and absence of cerebral perfusion. Abnormalities of respiratory rate and pattern occur in patients with brain stem lesions (Lee *et al*., 1976). The regulatory centres for automatic respiration are located in the lower pons and medulla. These centres are constantly influenced by other complex neurogenic as well as metabolic control mechanisms. Thus, the presence of pathological lesions involving these centres are expected to result in changes of respiratory function as reflected by alteration of rates and patterns. The underlying mechanism for the development of respiratory alkalosis in acute stroke is not clear. However, it is probably related to a combination of both neurogenic factors and hypoxic drive.

DA is known to regulate both higher order and autonomic brain functions. DA neurons respond to rewarding cues and are thought to be critical for integrating information necessary for predicting reward (Schultz, 2002). Previous studies suggest that DA is involved in respiratory regulation, the mechanism of dopaminergic respiratory control at the cellular network level. DA application on pons-medulla-spinal cord (PMS) preparation facilitates respiratory rhythm (Murakoshi *et al.*, 1985). Dopamine β-hydroxylase and phenylethanolamine-N-methyltransferase metabolize DA into noradrenaline and adrenaline, which depress respiratory rhythm (Fujii *et al.*, 2004; Errchidi *et al.*, 1991), and DA transporter is suggested to exist at the axonal terminal of noradrenergic and/or adrenergic neurons. Brief application of glutamate on DA neurons induces both depolarization mediated by iGlu receptors and facilitation of burst firing *via* activation of group I mGLU receptors (Mercuri *et al.*, 1992; Shen & Johnson, 1997; Zheng & Johnson, 2002). mGLU1 mediated hyperpolarization induces a pause in DA neuron firing that inhibits NMDA receptor induced burst firing (Morikawa *et al.*, 2003). Murakoshi and colleagues (1985) provided data supporting a stimulatory role of DA on brain stem respiratory motor output in the neonate (Johnson *et al.*, 1998).

We investigated the hypoxic damage to dopaminergic and glutamatergic function in brainstem of neonates and those hypoxic neonates supplemented with glucose. In the brainstem, we observed decrease in the DA content. In brainstem the total DA receptors and DA D_1 receptors showed a significant increase while DA D_2 receptors was down regulated of hypoxic neonates and those supplemented with epinephrine. This was reversed to near control in hypoxic neonates treated with glucose alone and in combination with oxygen. The Real-time PCR analysis showed a significant increase in the DA D_1 receptors while the DA D_2 receptors gene expression showed a significant decrease in hypoxic rats and hypoxic rats treated with epinephrine and oxygen compared to control. These alterations in the DA D_1 and DA D_2 on supplementation of glucose alone and glucose with oxygen to hypoxic rats

compared to control. Hence the decreased DA content and the altered dopaminergic function play a critical role in respiratory functions of the neonate. Glucose supplementation proves beneficial to reverse the hypoxic damage to dopaminergic function in the brainstem.

Accumulation of extracellular glutamate (Benveniste *et al.*, 1984; Choi, 1988; Roettger & Lipton, 1996) is responsible for the ischemia-induced depolarization. Glutamate can accumulate to millimolar concentrations in the synaptic cleft during physiological activity (Clements, 1996). Thus the postsynaptic glutamate receptors might be exposed to even higher concentrations during impairment of metabolism, which causes massive release of this neurotransmitter, often associated with impaired glutamate uptake (Hansen 1985; Szatkowski & Attwell 1994). The N-methyl-D-Aspartate (NMDA) receptor has many functions throughout the central nervous system (CNS) including its role within the centres controlling respiration. Although NMDA receptors are important for normal breathing, they are specifically active under conditions of stress, such as hypoxia. NMDA receptor is also important to the prenatal development of normal neurological pathways for the control of ventilation.

Our experimental findings report an increase in the NMDA receptors and NMDA 2b gene expression in the brainstem of hypoxic neonates and those treated with oxygen and epinephrine. Supplementation of glucose alone and along with oxygen reversed the NMDA changes to control level. Importance of NMDA receptors to both normal breathing and stress responses have been recently demonstrated by observations of antenatal effects of disturbances to the NMDA receptor which disrupts normal breathing as well as causing reduced ventilatory responses during stress in newborns (Waters & Machaalani, 2005). Hence the up regulated NMDA receptor disrupts normal breathing as well as causing reduced ventilatory responses during hypoxic stress in neonates. Glutamatergic excito-cytotoxic cell death through the NMDA receptor over-activation is suggested to be one of the major causes of cell death of dopaminergic neurons which is evident from our investigation. NMDA

receptors mediate their function through the IP3 release. In our study the IP3 content increased in the brainstem of hypoxic rats and those treated with oxygen and epinephrine. IP3 receptor activation leads to excessive Ca^{2+} overload in cells leading to apoptosis. Boehning *et al.,* (2003) demonstrated a small amount of cytochrome C released from mitochondria can bind to and promote Ca^{2+} conductance through IP3 in the endoplamic reticulum membrane. This released Ca^{2+} further triggers mass exodus of cytochrome C from all mitochodria in the cell and thus activating the caspase and nuclease enzyme that finalize the apoptotic process. (Boehning *et al.,* 2003). Supplementation of glucose alone and along with oxygen reversed the NMDA changes to control level. Thus up regulated NMDA receptor functioning causes a down regulatory effect of the total DA receptors, DA D_1 and DA D_2 receptors.

Investigations of the cAMP and cGMP content in the brainstem of hypoxic and those treated with epinephrine revealed a significant increase in the content when compared to control. We observed an increase in the cAMP and cGMP content in the brainstem of hypoxic and hypoxic rats treated with oxygen and epinephrine. Treatment with glucose alone or in combination with oxygen to in brainstem hypoxic rats reversed the alteration to near control. Earlier reports (Yang *et al.*, 2007) suggest the modulation of cAMP by DA D_1 receptors. Here we obtained a significant increase in the cAMP content in the brainstem of hypoxic rats and hypoxic rats treated with oxygen and epinephrine. In the hypoxic condition, DA D_1 is up regulated. Hence the increase in the cAMP is due to DA D_1 subtypes. This rise in cAMP could be also due to the influence of increased mGLU5 (Winder and Conn, 1992) receptors as is seen during hypoxia. Glucose treatment to hypoxic neonates reversed the changes in the cAMP to control.

We found an increased cGMP content in the hypoxic brainstem. It is supposed to be due to the increased NMDA receptors. Earlier works by Suvarna and O'Donnel, (2002) reported the NMDA mediated increase in the cGMP in the neuronal culture studies. We observed that glucose treatment to hypoxic neonates reversed the changes

in the cAMP and cGMP level to near control. Both Ca^{2+} and cAMP are the principal second messengers controlling the phosphorylation of CREB at its regulatory site, Ser^{133} (Montminy *et al.*, 1990; Bito *et al.*, 1996; Hardingham *et al.*, 2002; Johnson *et al.*, 1997). Ca^{2+} and/or diacylglycerol-protein kinase C downstream to group I stimulation, positively interact with NMDA receptors to allow larger Ca^{2+} influx, which adds to the Ca^{2+} response to DHPG (Mao & Wang, 2002). The most extensively studied cGMP signal transduction pathway is that triggered by nitric oxide (NO) (Bredt & Snyder, 1990). cGMP effects are primarily mediated by the activation of cGMP-dependent protein kinases (PKGs).

Cerebellum

The cerebellum is known to play an important role in sensorimotor processings. The major functions range from their role in motor and sensory timing (Cooper *et al.*, 2000; Spencer *et al.*, 2003), calibration of movements and reflexes (Baizer *et al.*, 1999; Martin *et al.*, 2000; Hirata & Highstein 2001; Thach & Bastian 2004; Oulad Ben Taib & Manto 2006; Kolb *et al.*, 2007). Lesions in the cerebellum typically cause hypotonia, dysmetria and dyscoordination (Gowen *and* Miau., 2005). Cerebellar atrophy is a recognized complication of hypoxia. The cerebellar system is also implicated in memory impairment which has been mainly ascribed to hippocampal damage (Morris *et al.*, 1990). The neurons of the deep cerebellar nuclei comprise the main output stage of the cerebellum (Zhang & Linden, 2006). Earlier studies have also established that rhythmic output from the cerebellum contributes to the maintenance of generalized seizures (Kandel & Buzsaki, 1993).

Recent evidence confirms the dopaminergic innervations in the cerebellum and suggests there is a small dopaminergic element, whose properties are similar to the well characterized system of striatum (Giompres & Delis, 2005). In the cerebellum, DA content was decreased whereas the total DA receptors, DA D_1 receptors and DA D_2 receptors showed a decrease in cerebellum of hypoxic neonates

and those supplemented with epinephrine. This was reversed to near control in hypoxic neonates treated with glucose alone and glucose and oxygen. The Real-time PCR analysis showed a significant decrease in the DA D_1 receptors and DA D_2 receptors gene expression in hypoxic rats and those treated with epinephrine and oxygen. These alterations in the DA D_1 and DA D_2 on supplementation of glucose alone and glucose with oxygen to hypoxic rats compared to control rats. These dopaminergic receptor alterations were reversed by glucose supplementation. Hence the decreased DA content and the down regulated total DA receptors, DA D_1 and DA D_2 is suggested to play a critical role in motor and cognitive functions dysfunction if not resuscitated during the critical period. Glucose supplementation proves beneficial to reverse the hypoxic damage to dopaminergic function in the cerebellum.

We observed an increased expression in the mGLU5 expression in the cerebellum of the hypoxic neonates. The cerebellum and the basal ganglia are two brain regions involved in the control of movement, and synaptic transmission in both areas is modulated by group I mGLU receptors. The activity of mGLU5 receptors has been reported to support cell survival and a decline in the expression of mGLU5 receptors gives access to programmed cell death in cerebellar granule cells (Copani *et al.,* 1998). mGLU5 activates CREB phosphorylation through IP3 dependent intracellular Ca^{2+} release (Mao & Wang, 2002). Group I mGLU receptors are also critical for synaptic plasticity in corticostriatal pathways (Gerdeman *et al.,* 2003; Gubellini *et al.,* 2004).

We also observed an increase in the NMDA receptors and NMDA 2b gene expression in the cerebellum of hypoxic neonates. Both mGLU1 and mGLU5 receptors are reported to cause potentiation of the NMDA receptor currents and increased excitability of these neurons, opposing the modulatory effects of DA (Gubellini *et al.,* 2004, Conn *et al.,* 2005, Rouse *et al.,* 2000). NMDA over action leads to increased IP3 dependent intracellular Ca^{2+} release in the cerebellar neurons during hypoxia. Increased Ca^{2+} triggers the release of cytochrome C from mitochodria

in the cell which activates the caspase and nuclease enzyme that initiates the apoptotic process (Boehning *et al.*, 2003). NMDA and mGLU5 receptors oppose the inhibitory actions of DA throughout the basal ganglia, suggesting that group I mGLU receptors are therapeutic targets for drugs that alleviate motor control dysfunction (Canales *et al.*, 2003). Calcium release from internal stores is controlled by various channels including the IP3 receptor and ryanodine receptor families (O'Malley *et al.*, 2003).

Adenylate cyclase catalyzes the conversion of ATP to cyclic AMP, which binds to the regulatory subunits of the PKA holoenzyme to disinhibit the catalytic subunits. Genetic deletion of adenylate cyclase 5 abolishes DA D_2 receptor mediated inhibition of adenylate cyclase in the mouse neostriatum. This null mutation also eliminates the locomotor inhibitory effects of DA D_2 receptor blocking antipsychotic drugs. It shows the behavioural significance of this signalling pathway (Lee *et al.*, 2002). The cyclic nucleotides cAMP and cGMP are involved in a number of intracellular processes such as signal transduction, gene transcription, activation of kinases and regulation of channel function. Stimulation of DA D_1 receptors have been implicated in an increase in the cAMP whereas DA D_2 inhibits the function of cAMP. Moreover NMDA receptor on neurons activates both cAMP and cGMP signalling pathways. This made us to investigate the levels of both cAMP and cGMP in the control and experimental rats. We observed an increase in the cAMP and cGMP content in the cerebellum of hypoxic and hypoxic rats treated with oxygen and epinephrine. Treatment with glucose alone or in combination with oxygen reversed the alteration to near control in hypoxic cerebellum.

Earlier reports (Yang *et al*., 2007) suggest the modulation cAMP by DA D_1 receptors. Here we obtained a significant increase in the cAMP content in the cerebellum of hypoxic rats and hypoxic rats treated with oxygen and epinephrine. But in the hypoxic condition, DA D_1 is down-regulated. Hence the increase in the cAMP is not due to DA D_1 subtypes. There are also reports that DA D_2 inhibits the cAMP function (Yang *et al*., 2007). Thus the down regulated DA D_2 is involved in the

increased cAMP content. Other than this, rise in cAMP is mainly due to the influence of increased mGLU5 (Winder & Conn, 1992) receptors as is seen our studies. mGLURs are referred to as G-protein-coupled receptors. Through G-proteins, eight subtypes of mGLURs are linked to different second messenger systems (Kendall, 1993; Nakanishi & Masu, 1994). Group I regulation of gene expression may be mediated *via* the transcription factor CREB. Through binding to the promoter Ca^{2+} and cAMP response element, phosphorylated CREB (pCREB) relays to transmit the extracellular signal conveyed through the surface receptor and its associative signalling pathways to target DNA transcription (Bito *et al.*, 1996; Silva *et al.*, 1998). Both Ca^{2+} and cAMP are the principal second messengers controlling the phosphorylation of CREB at its regulatory site, Ser^{133} (Montminy *et al.*, 1990; Bito *et al.*, 1996; Hardingham *et al.*, 2002; Johnson *et al.*, 1997). Ca^{2+} and/or diacylglycerol-protein kinase C downstream to group I stimulation, positively interact with NMDA receptors to allow larger Ca^{2+} influx, which adds to the Ca^{2+} response to DHPG (Mao & Wang, 2002).

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 extensively studied cGMP signal transduction pathway is that triggered by nitric oxide (NO) (Bredt & Snyder, 1990). 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 (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 signalling 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 (NO) synthase (NOS) type to produce NO, which stimulates guanylyl cyclase to produce cGMP (Garthwaite, 1991).

In the present study, the increased cGMP content in the hypoxic cerebellum may due to the increased NMDA receptors. Suvarna & O'Donnel, (2002) reported the NMDA mediated increase in the cGMP in the neuronal culture studies. Baltrons *et al*., (1997) and Oh *et al*., (1997) reported an NMDA induced cGMP formation in the cultured cerebellar granule cells. Glucose treatment to hypoxic neonates reversed the changes in the cAMP and cGMP level to near control. Cyclic nucleotide pathways can cross talk to modulate each other's synthesis, degradation, and actions. Increased cGMP can increase the activity of cGMP stimulated PDE2 to enhance hydrolysis of cAMP, or it can inhibit the PDE3 family and decrease the hydrolysis of cAMP (Pelligrino & Wang, 1998). cAMP and cGMP are involved in NMDA receptor-mediated signalling 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).

Thus it is evident that increased IP3 mediated calcium release is involved in the generation of NO in the cerebellum of hypoxic neonates. Increased NO leads to formation of free radicals which hinders cerebellar motor function. Administration of glucose alone and in combination with oxygen to hypoxic rats reversed the free radical mediated damage in the hypoxic neonates.

Thus from our results we conclude that glucose act as a neuroprotective agent in reversing the altered DA content, DA D_1 and DA D_2 receptor function due to

hypoxia. The alterations in DA through DA D_1 and DA D_2 receptor subtypes in hypoxic rats and those supplemented with oxygen and epinephrine suggest the occurrence of dopaminergic functional regulation in the brain of hypoxic rats. This impaired dopaminergic function will cause damage to the brain leading to behavioural changes during later developmental life. The efficient and timely supplementation of glucose reversed DA functional changes through DA D_1 and DA D_2 receptors observed in hypoxia, oxygen and epinephrine supplementation. Our results showed that hypoxia causes a significant modulation in dopaminergic function which is corrected by prior supplementation of glucose to oxygen in the resuscitation sequence. Thus it is suggested that immediate glucose administration during neonatal hypoxia with oxygenated air in the resuscitation programme will reduce the hypoxic damage to the brain cells. This has immense clinical significance in the management of hypoxia in neonatal care which will have role in intellectual and behavioural efficiency at later stages of the life of an individual.

Summary

1) Hypoxia was induced in neonatal rats and was supplemented with glucose, epinephrine and oxygen as a traditional mode of resuscitation during neonatal hypoxia. This experimental model was used to study the dopamine, dopaminergic receptor subtypes and NMDA receptor alterations in the hypoxic neonatal rats.

2) Blood glucose level in the serum was measured to analyse the circulating glucose level changes due to supplementation of glucose, epinephrine and oxygen to hypoxic neonatal rats compared to control.

3) The DA and HVA contents were measured to identify its alteration in the brainstem, cerebellum and serum due to hypoxia using High Performance Liquid Chromatography.

    a. Significant decrease in DA content in the brainstem and cerebellum were observed in hypoxic, hypoxic rats treated with glucose, hypoxic rats treated with oxygen and treated with a combination of glucose, epinephrine and oxygen.
    b. HVA content significantly decreased in hypoxic, hypoxic rats treated with oxygen and treated with a combination of glucose, epinephrine and oxygen in brainstem while there was no change in cerebellum.
    c. DA/HVA ratio decreased significantly in hypoxic, hypoxic rats treated with oxygen and treated with a combination of glucose, epinephrine and oxygen in brainstem and cerebellum. This was

reversed to control levels in hypoxic rats treated with glucose and hypoxic rats treated with glucose and oxygen.

d. Significant increase in EPI content in hypoxic; hypoxic rats treated with glucose; hypoxic rats treated with oxygen; treated with a combination of glucose and oxygen; treated with a combination of glucose, epinephrine and oxygen. DA contents did not show any significant change. HVA contents increased in hypoxic rats treated with oxygen, treated with a combination of glucose and oxygen and treated with a combination of glucose, epinephrine and oxygen. There was no significant change in DA/HVA ratio.

4) Dopaminergic receptor functional status was analysed by Scatchard analysis using [^{3}H]ligands in cerebral cortex, brainstem and cerebellum. Receptor gene expression was confirmed by Real-Time PCR. The total DA receptors in cerebral cortex and brainstem showed a significant increase in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. The total DA receptors showed a decrease in cerebellum in these groups. Glucose supplementation to hypoxic rats showed a reversal to control levels.

Dopamine D_1 binding studies using [^{3}H]SCH 23390 showed a significant decrease in cerebral cortex and cerebellum while there was a significant increase in brainstem in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. Glucose supplementation to hypoxic rats showed a reversal to control levels.

Dopamine D_2 binding studies using [^{3}H]YM-09151-2 showed a significant decrease in cerebral cortex, brainstem and cerebellum. This was reversed to control in glucose treated groups - hypoxic rats treated with glucose, hypoxic rats treated with glucose and oxygen.

The gene expression studies confirmed the mRNA status of the corresponding receptor primers in Real-Time PCR.

5) NMDA receptor functional status was analysed by Scatchard analysis using [^{3}H]MK-801 in cerebral cortex, brainstem and cerebellum. Receptor gene expression was confirmed by Real-Time PCR. The NMDA receptors were decreased in cerebral cortex while it showed a significant increase in brainstem and cerebellum in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. Glucose supplementation reversed the receptor status towards control values.

   The mGLU5 gene was up regulated in cerebral cortex, brainstem and cerebellum in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. This was reversed to control values with glucose administration.

   The NMDA 2b receptor genes showed up regulation in brainstem and cerebellum while it was down regulated in cerebral cortex in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. This was reversed to control values with glucose administration which confirmed the receptor data.

6) The cGMP levels decreased significantly in cerebral cortex while brainstem and cerebellum showed a significant increase in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. Administration of glucose - hypoxic rats treated with glucose; hypoxic rats treated with glucose and oxygen - reversed the cGMP levels to control values.

7) The cAMP levels increased significantly in the brainstem and cerebellum in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. This was reversed to control levels with glucose administration to hypoxic rats - hypoxic rats treated with glucose; hypoxic rats treated with glucose and oxygen.

8) The IP3 levels decreased significantly in cerebral cortex and brainstem while it increased significantly in cerebellum in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. Glucose administration to hypoxic rats - hypoxic rats treated with glucose, hypoxic rats treated with glucose and oxygen - reversed the IP3 to control values.

9) Behavioural studies of the experimental neonatal rats using rotarod test showed a significant decrease in motor activity at varying speeds in hypoxic, hypoxic rats treated with oxygen, hypoxic rats treated with a combination of glucose, epinephrine and oxygen. This was reversed to control values with glucose administration to hypoxic rats - hypoxic rats treated with glucose, hypoxic rats treated with glucose and oxygen.

10) The Ca^{2+} release studies showed decreased extracellular calcium in neonatal cortical cells in hypoxia compared to control cells. Glucose administration reversed the Ca^{2+} levels to control levels. The administration of DA to the cells increased Ca^{2+}. Glutamate addition decreased the extracellular Ca^{2+} which is suggested to be due to increased intracellular transport of Ca^{2+} by glutamate.

11) TO-PRO-3 staining showed a decrease in the cell density in hypoxic neonatal brain compared to control. This is suggested to be due to free radicals' damage and apoptosis in hypoxia.

Thus from our results we conclude that glucose act as a neuroprotective agent in reversing the decreased DA content, DA D_1 and DA D_2 receptor function due to hypoxia. Alteration in DA D_1 and DA D_2 receptor subtypes in hypoxic rats and those supplemented with oxygen and epinephrine suggest the occurrence of dopaminergic functional regulation in the brain of hypoxic rats. This impaired dopaminergic function will cause damage to the brain leading to behavioural changes during later developmental life. The efficient and timely supplementation of glucose reversed DA functional changes through DA D_1 and DA D_2 receptors observed in hypoxia, oxygen and epinephrine supplementation. Our results showed that hypoxia causes a significant modulation in dopaminergic function which is corrected by prior supplementation of glucose to oxygen in the resuscitation sequence. Thus it is suggested that immediate glucose administration during neonatal hypoxia with oxygenated air in the resuscitation programme will reduce the hypoxic damage to the brain cells. This has immense clinical significance in the management of hypoxia in neonatal care which will have role in intellectual and behavioural efficiency at later stages of the life of an individual.

Conclusion

Our findings demonstrated that hypoxia during the neonatal period caused significant impact in the central nervous system both functionally and behaviourally. The evaluation of these damages at molecular level is very important, especially in a critical brain function to reduce the effects of damage during later developmental stages. Even though the body weight or blood glucose level is not altered after seven days of hypoxic insult, it caused alterations in the brain DA and metabolite 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, cerebral palsy and neuro-developmental delay. Structural and functional integrity of brain depends on regular glucose and oxygen supply. The receptor binding studies show alterations in the DA, DA D_1 and DA D_2 receptors in the cerebral cortex, brainstem and cerebellum. The NMDA receptors showed a decrease in cerebral cortex while it was significantly increased in brainstem and cerebellum during hypoxia. Real-Time PCR confirmed receptor data. The second messenger study confirmed that the changes in the receptor levels did percolate through alterations in IP3, cAMP and cGMP levels. The Ca^{2+} release studies showed a decrease in extracellular Ca^{2+} levels during hypoxia. The behavioural studies by rotarod test showed a decrease in motor activity. These studies suggest that DA D_1, DA D_2 receptor potentiates NMDA mediated overactivity leading to increased IP3

dependent Ca^{2+} release which triggers release of Cytochrome C thereby initiating the apoptotic process. This causes cell damage during hypoxic stress in the neonatal rats-hypoxic; hypoxic rats treated with oxygen; epinephrine and oxygen and a combination of glucose, epinephrine and oxygen. The glucose supplementation to hypoxic rats and along with oxygen is able to reverse this damage.

We conclude that glucose act as a neuroprotective agent in reversing the decreased DA content, DA D_1 and DA D_2 receptor function due to hypoxia. Altered DA through DA D_1 and DA D_2 receptor subtypes in hypoxic rats and those supplemented with oxygen and epinephrine suggest the occurrence of dopaminergic functional regulation in the brain of hypoxic rats. This impaired dopaminergic function will cause damage to the brain leading to behavioural changes during later developmental life. The efficient and timely supplementation of glucose reversed DA functional changes through DA D_1 and DA D_2 receptors observed in hypoxia, oxygen and epinephrine supplementation. Our results showed that hypoxia causes a significant modulation in dopaminergic function which is corrected by prior supplementation of glucose to oxygen in the resuscitation sequence. Thus it is suggested that immediate glucose administration during neonatal hypoxia with oxygenated air in the resuscitation programme will reduce the hypoxic damage to the brain cells. This has immense clinical significance in the management of hypoxia in neonatal care which will have role in intellectual and behavioural efficiency at later stages of the life of an individual.

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Papers Published

1. Sulaiman P, **Joseph B**, Kaimal SB, Paulose CS, Decreased Hepatic 5-HT1A Receptors During Liver Regeneration and Neoplasia in Rats ***Neurochemical Research***; 33: 444-449. (**2008**).
2. Pyroja S, **Joseph B**, Paulose CS, Enhanced β- adrenergic receptors in the brain regions and pancreas during pancreatic regeneration in weanling rats ***Journal of Neurological Sciences***; 254: 3-8 (**2007**).

Awards

1. **IBRO 2007 Travel Award** to attend the 11th International Brain Research Organization (IBRO), Associate School of Neuroscience in Kualalumpur, Malaysia (December, 2007).

Abstracts Presented

1. C.S. Paulose and **Binoy Joseph**, "Dopamine D_2 receptor functional regulation in the cerebral cortex of hypoxia induced neonatal rats: Effect of glucose, oxygen and epinephrine treatment". Eight Biennial Meeting of the Asia-Pacific Society of Neurochemistry, Shanghai, People's republic of China (June **2008**).
2. **Binoy Joseph** and C.S. Paulose, "Dopamine D_2 and 5-HT_{2C} receptor functional regulation in the cerebral cortex of hypoxia induced neonatal rats: Effect of glucose, oxygen and epinephrine Treatment". Annual Meeting of Society for Biotechnologists, India & National Symposium on Current Trends in Stem Cell Biology organized by Rajiv Gandhi Centre for Biotechnology, Trivandrum and Society for Biotechnologists, India (December **2007**).

3. **Binoy Joseph,** Reas Khan S and C.S. Paulose, “Decreased acetylcholine esterase in the cerebral cortex of epileptic rats” at National conference on Biotechnology in molecular medicine organised by AIMS, Kochi, Amrita School of Biotechnology, Amritapuri and Society for Biotechnologists, India (January **2007**).

4. **Binoy Joseph**, Reas Khan S and C.S. Paulose, “Decreased glutamate dehydrogenase in the cerebral cortex of epileptic rats” at Third NBRC International Conference, New Delhi organized by NBRC, Manesar, India (December **2006**).

5. **Binoy Joseph,** Jackson James, C. S. Paulose, “Downregulation of nuclear serotonin binding protein in pancreatic islets” International Conference on Biotechnology and Neuroscience jointly organized by CUSAT, Cochin and Society for Biotechnologists (India); (December, **2004**).

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

Animal status	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	120.67 ± 1.74
Hx	7.35 ± 0.54	13.22 ± 0.55	123.17± 0.58
Hx+G	7.12 ± 0.25	13.34 ± 0.62	115.62 ± 1.15
Hx+O	7.33 ± 0.18	13.11± 0.35	122.37 ± 1.38
Hx+G+O	7.85 ± 0.45	13.14 ± 0.32	127.81 ± 2.15
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 pups.

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-2
Dopamine and Homovanillic acid Content (nmol/g wet wt.) in the Brainstem of Control and Experimental Groups of Neonatal Rats

Animal status	DA	HVA	DA/HVA
C	7.65 ± 0.90	6.32 ± 0.38	1.21 ± 0.64
Hx	3.59 ± 0.34***	4.85 ± 0.22**	0.74 ± 0.28***
Hx+G	4.21 ± 0.14 ***	3.81 ± 0.09 ***	1.10 ± 0.12 φφ
Hx+O	3.38 ± 0.20***	4.33 ± 0.11**	0.78 ± 0.15***
Hx+G+O	5.34 ± 0.25*** φφ	2.28 ± 0.27***	1.48 ± 0.26 φφφ
Hx+G+E+O	3.24 ± 0.41***	3.12 ± 0.28***	1.03 ± 0.35**

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
** $p<0.01$, *** $p<0.001$ when compared to Control
φφ $p<0.01$, φφφ $p<0.001$ 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-3
Dopamine and Homovanillic acid Content (nmol/g wet wt.) in the Cerebellum of Control and Experimental Groups of Neonatal Rats

Animal status	DA	HVA	DA/HVA
C	16.38 ± 0.88	15.95 ± 0.52	1.03 ± 0.07
Hx	6.72 ± 0.59***	14.00 ± 0.33	0.48 ± 0.05**
Hx+G	12.80 ± 0.84 φφφ	13.39 ± 0.53	0.96 ± 0.07 φφ
Hx+O	8.06 ± 0.63***	11.95 ± 0.47	0.67 ± 0.06**
Hx+G+O	15.72 ± 0.65 φφφ	13.39 ± 0.39	1.17 ± 0.05 φφ
Hx+G+E+O	6.13 ± 0.32***	8.64 ± 0.84*** φφ	0.71 ± 0.05*

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared to Control
φφ $p<0.01$, φφφ $p<0.001$ 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-4
Dopamine and Homovanillic acid Content (nmol/g wet wt.) in the Plasma of Control and Experimental Groups of Neonatal Rats

Animal status	NE	EPI	DA	HVA	DA/HVA
C	3.46±0.16	2.27±0.14	2.25±0.45	2.27±0.46	0.99±0.45
Hx	3.41±0.83	4.92±0.16**	2.52±0.36	2.69±0.16	0.94±0.26
Hx+G	3.96±0.33	3.36±0.16*φ	2.25±0.18	2.74±0.25	0.82±0.22
Hx+O	3.48±0.16	3.46±0.65*φ	2.33±0.43	3.16±0.44*φ	0.74±0.44
Hx+G+ O	3.90±0.39	3.42±0.50*φ	2.30±0.85	3.24±0.34*φ	0.71±0.60
Hx+G+E+O	3.26±0.52	8.15±026 ***φφ	2.30±0.34	1.46±0.65**φφ	1.58±0.50

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared to Control
φ $p<0.05$, φφ $p<0.01$ 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 –1
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebral Cortex of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

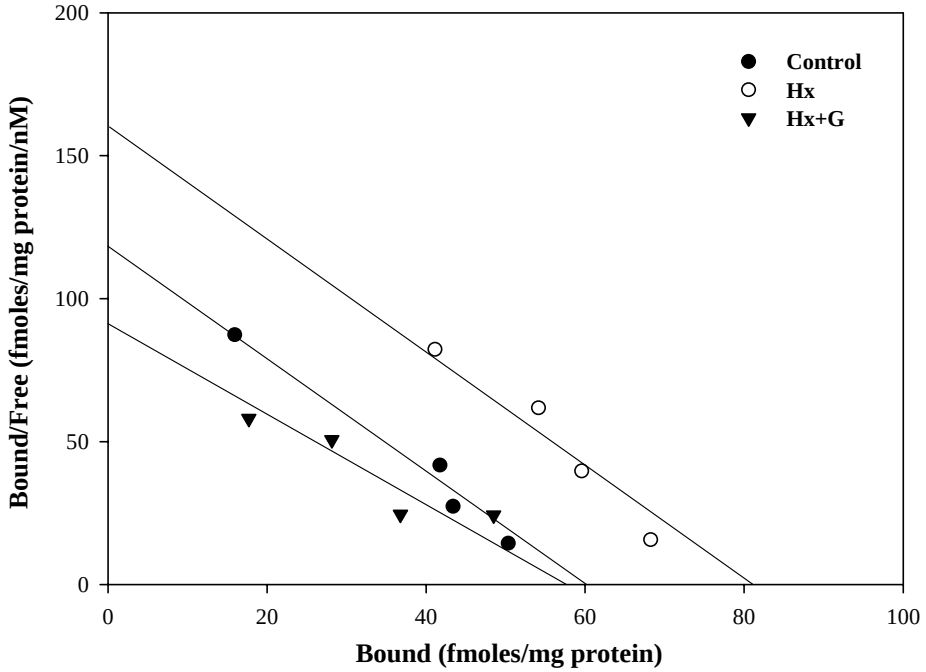


Table -5
Scatchard Analysis of [^{3}H]Dopamine Binding Against Dopamine in the Cerebral Cortex of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	59.00 ± 2.16	1.97 ± 0.04
Hx	82.00 ± 3.24 ***	1.95 ± 0.02
Hx+G	57.00 ± 3.35φφφ	1.50 ± 0.06

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.01$ when compared to Hx
B_{max} – Maximal binding; K_d – Dissociation constant
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose treated – Hx+G

Figure –2
Scatchard Analysis of [^{3}H]Dopamine Binding Against Dopamine in the Cerebral Cortex of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

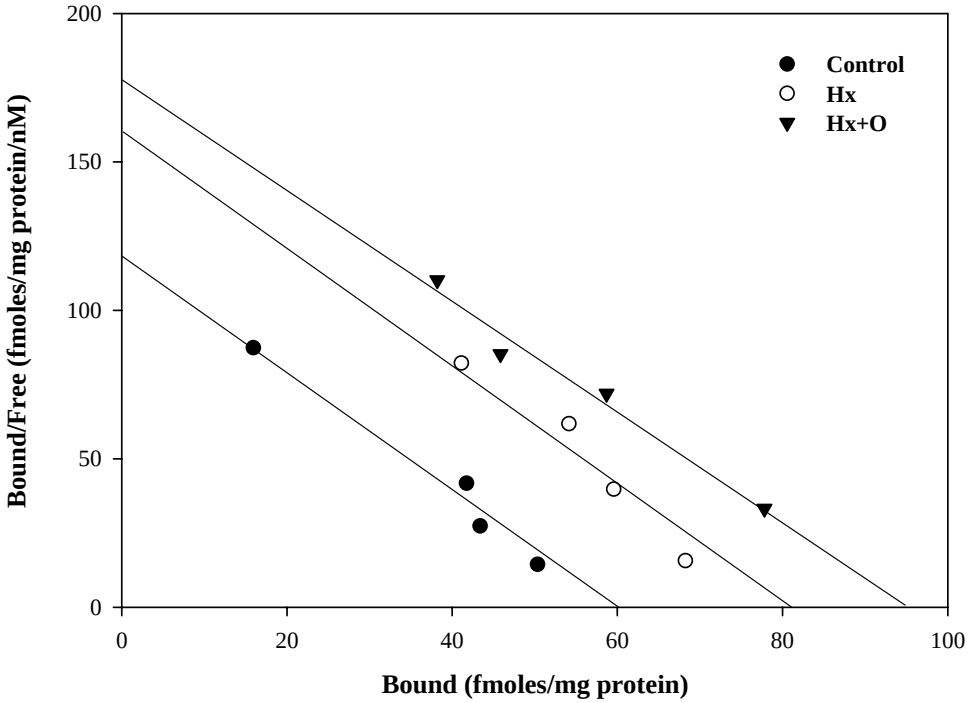


Table -6
Scatchard Analysis of [^{3}H]Dopamine Binding Against Dopamine in the Cerebral Cortex of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	59.00 ± 2.16	1.97 ± 0.04
Hx	82.00 ± 3.24 ***	1.95 ± 0.02
Hx+O	95.00 ± 2.19***	1.86 ± 0.09

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*** $p<0.001$ when compared to Control
Control rats – C; Hypoxic rats – Hx; Hypoxic rats oxygen treated – Hx+O

Figure -3
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebral Cortex of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

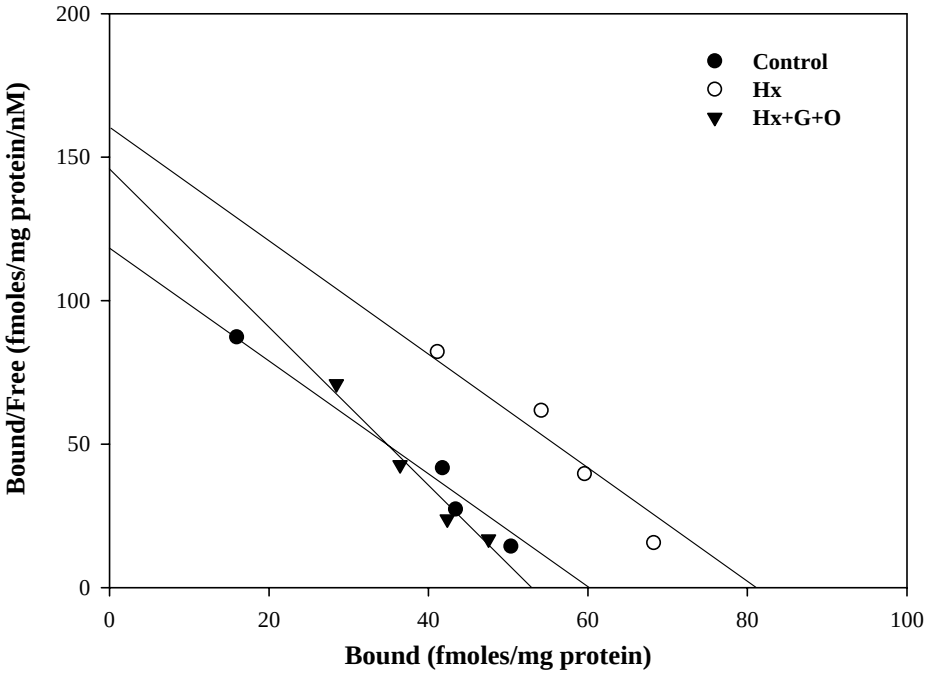


Table -7
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebral Cortex of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	59.00 ± 2.16	1.97 ± 0.04
Hx	82.00 ± 3.24 ***	1.95 ± 0.02
Hx+G+O	52.00 ± 3.23φφφ	2.8 ± 0.06**

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
* $p<0.05$, ** $p<0.01$ when compared to Control
φφφ $p<0.01$ when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose and oxygen treated – Hx+G+O

Figure -4
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebral Cortex of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

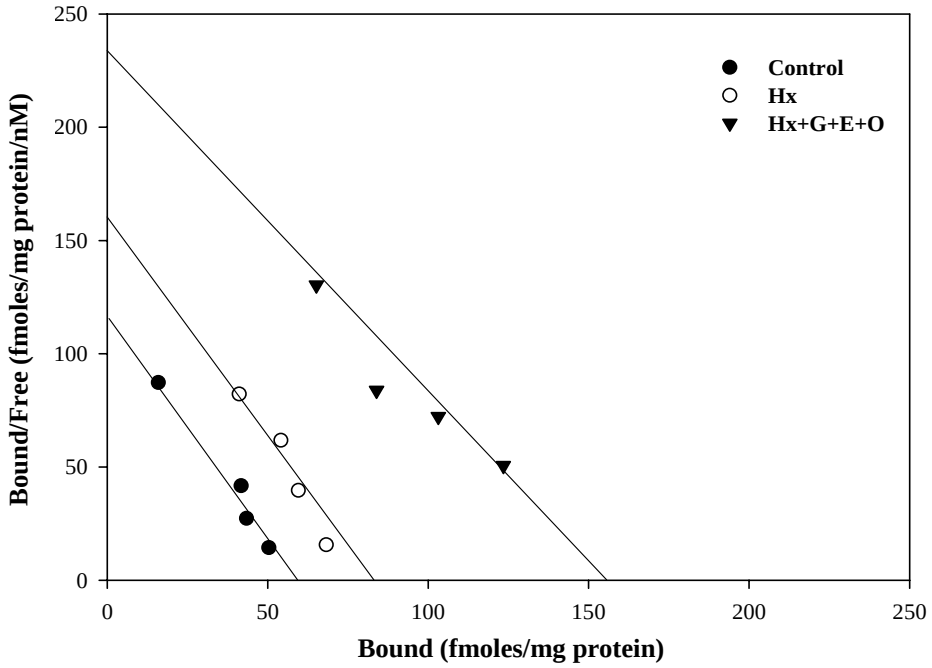
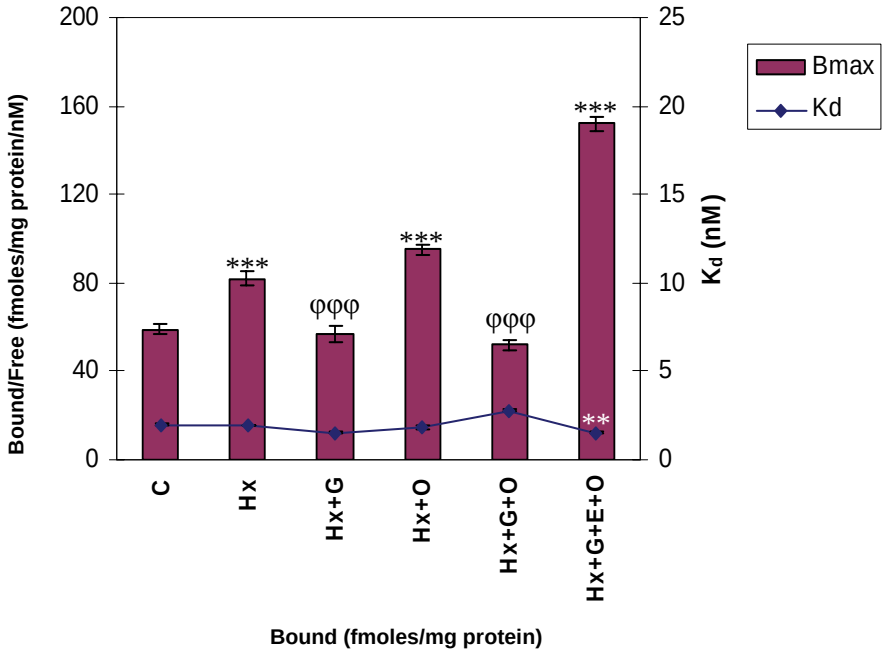


Table -8
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebral Cortex of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	59.00 ± 2.16	1.97 ± 0.04
Hx	82.00 ± 3.24 ***	1.95 ± 0.02
Hx+G+E+O	152.00 ± 2.58*** φφφ	1.50 ± 0.08 *

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
* $p<0.05$, *** $p<0.001$ when compared to Control
φφφ $p<0.01$ when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose, epinephrine and oxygen treated – Hx+G+E+O

Figure -5
Binding Parameters of [^{3}H]Dopamine against Dopamine in the Cerebral Cortex of Control and Experimental groups of Hypoxic Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
** p<0.01, *** p<0.001 when compared to Control
φφφ 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 -9
Binding Parameters of [^{3}H]Dopamine against Dopamine in the Cerebral Cortex of Control and Experimental groups of Hypoxic Neonatal Rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
C	59.00 ± 2.16	1.97 ± 0.04
Hx	82.00 ± 3.24***	1.95 ± 0.02
Hx+G	57.00 ± 3.35 φφφ	1.50 ± 0.06
Hx+O	95.00 ± 2.19***	1.86 ± 0.09
Hx+G+O	52.00 ± 3.23 φφφ	2.8 ± 0.06 **
Hx+G+E+O	152.00 ± 2.58***φφφ	1.50 ± 0.08

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
** $p<0.01$, *** $p<0.001$ when compared to Control
φφφ $p<0.001$ 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 – 6
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Cerebral Cortex of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

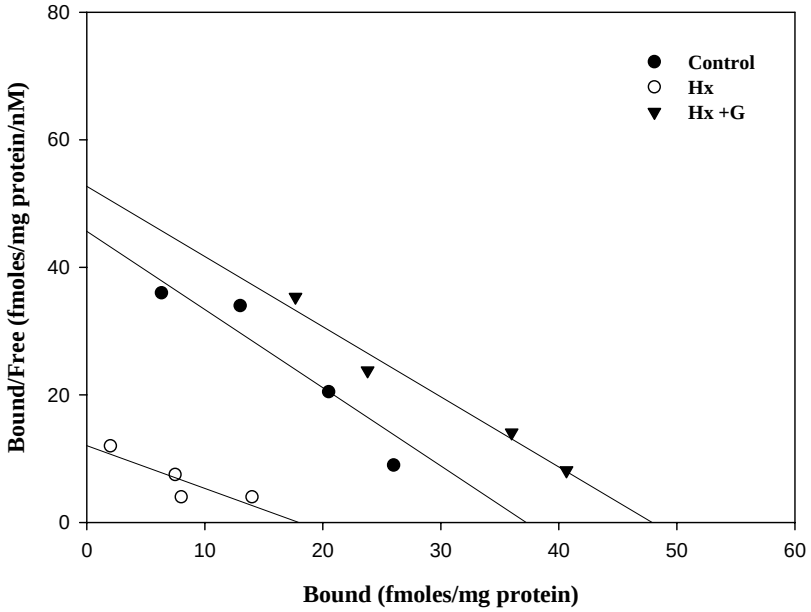


Table -10
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Cerebral Cortex of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	37.00 ± 0.92	1.22 ± 0.07
Hx	18.00 ± 1.14 **	1.51 ± 0.03 *
Hx+G	48.00 ± 1.29 φφ	1.18 ± 0.04 φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
* p<0.05, ** p<0.01when compared to Control
φ p<0.05, φφ p<0.01when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose treated – Hx+G

Figure -7
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Cerebral Cortex of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

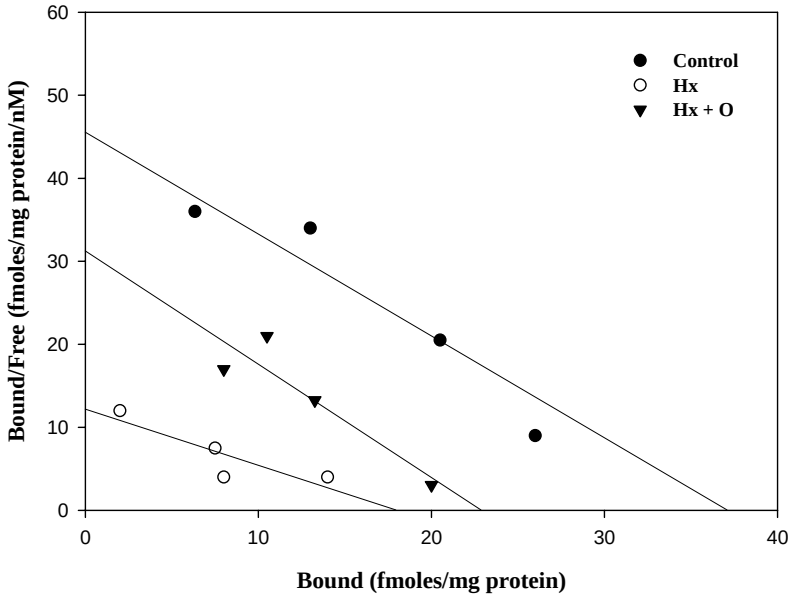


Table -11
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Cerebral Cortex of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	37.00 ± 0.92	1.22 ± 0.07
Hx	18.00 ± 1.14 **	1.51 ± 0.03 *
Hx+O	22.00 ± 1.25 **	1.52 ± 0.03 *

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
* $p<0.05$, ** $p<0.01$when compared to Control
Control rats – C; Hypoxic rats – Hx; Hypoxic rats oxygen treated – Hx+O

Figure –8
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Cerebral Cortex of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

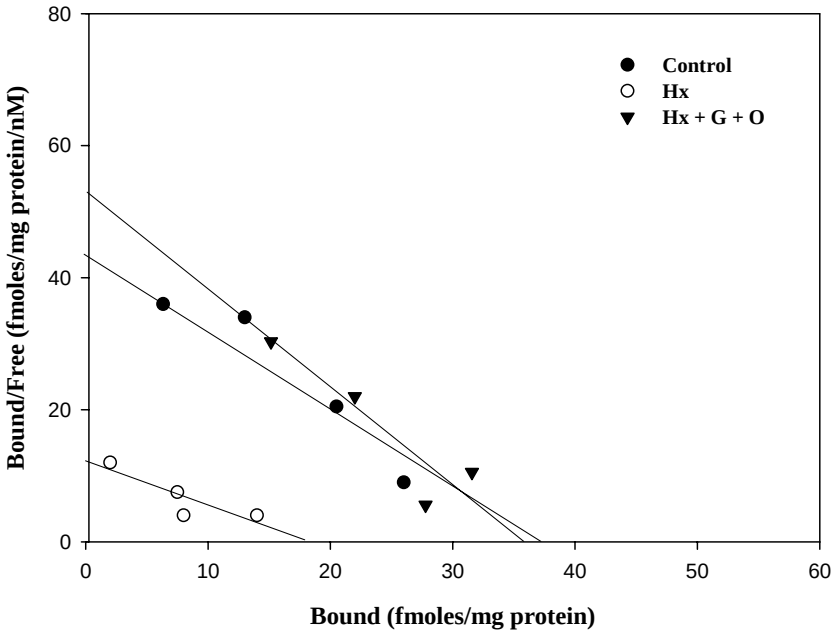


Table -12
Scatchard Analysis of [^{3}H] SCH 23390 Binding against SCH 23390 in the Cerebral Cortex of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	37.00 ± 0.92	1.22 ± 0.07
Hx	18.00 ± 1.14 **	1.51 ± 0.03 *
Hx+G+O	36.00 ±1.22 φφ	1.27 ± 0.02 φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
* p<0.05, ** p<0.01 when compared to Control
φ p<0.05, φφ p<0.01when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + oxygen treated – Hx+G+O

Figure –9
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Cerebral Cortex of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

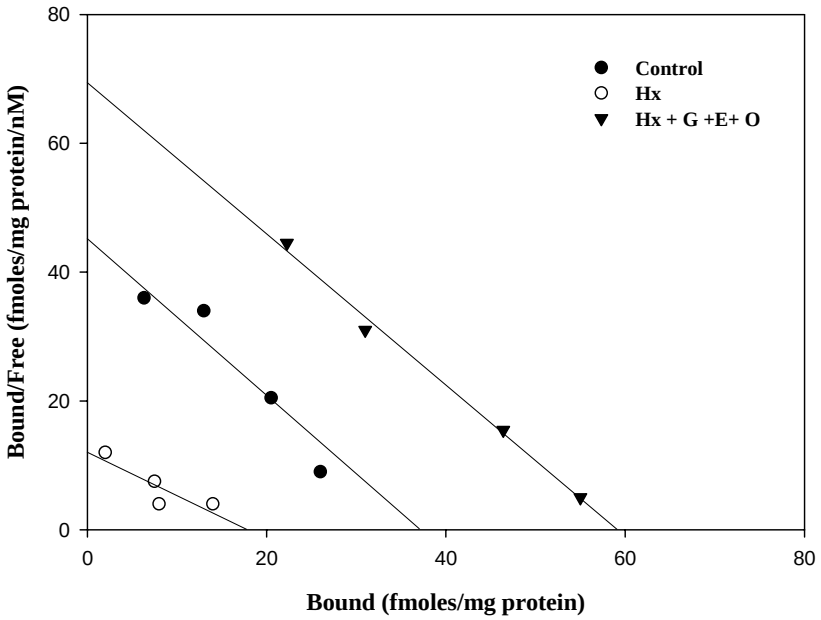
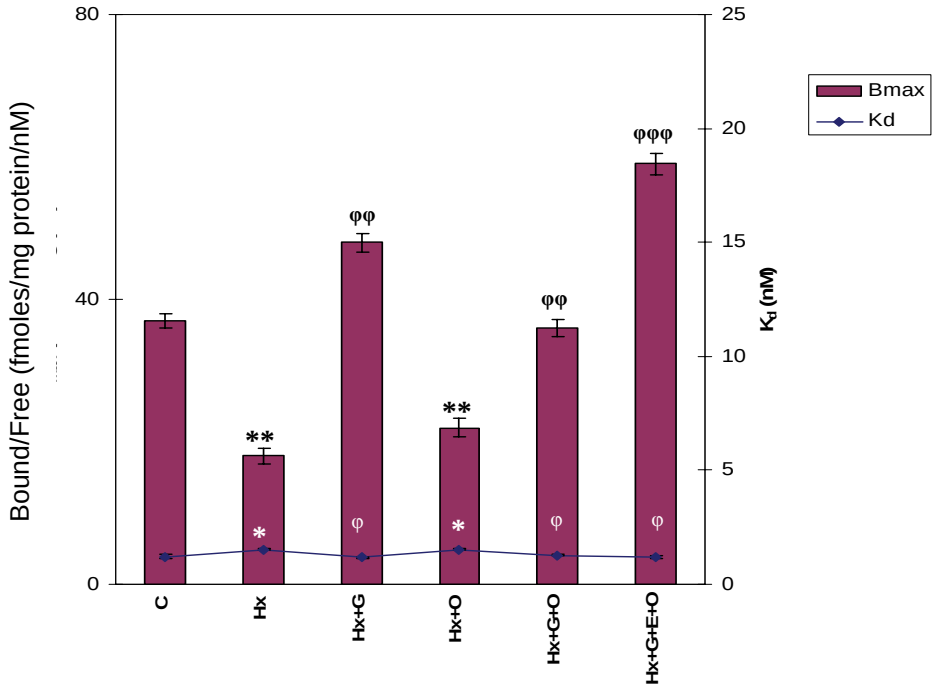


Table -13
Scatchard Analysis of [^{3}H]SCH 23390 Binding Against SCH 23390 in the Cerebral Cortex of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	37.00 ± 0.92	1.22 ± 0.07
Hx	18.00 ± 1.14 **	1.51 ± 0.03*
Hx+G+E+O	59.00 ±1.43 φφφ	1.20 ± 0.02 φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
* p<0.05, ** p<0.001when compared to Control
φ p<0.05, φφφ p<0.001when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose+epinephrine+oxygen treated – Hx+G+E+O

Figure -10
Binding parameters of [^{3}H]SCH 23390 against SCH 23390 in the Cerebral cortex of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
* $p<0.05$, ** $p<0.01$ when compared to Control
φ $p<0.05$, φφ $p<0.01$, φφφ $p<0.001$ 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

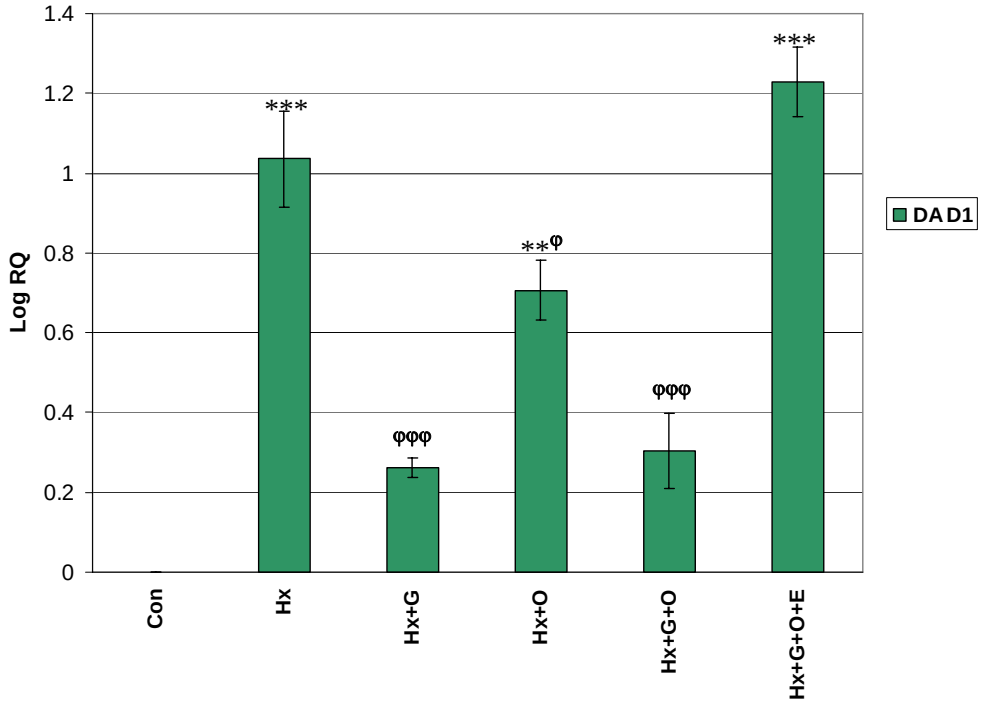
Table -14
Binding parameters of [^{3}H]SCH 23390 against SCH 23390 in the Cerebral cortex of Control and Experimental Groups of Neonatal Rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
C	37.00 ± 0.92	1.22 ± 0.07
Hx	18.00 ± 1.14 **	1.51 ± 0.03 *
Hx+G	48.00 ± 1.29 φφ	1.18 ± 0.04 φ
Hx+O	22.00 ± 1.25 **	1.52 ± 0.03 *
Hx+G+O	36.00 ± 1.22 φφ	1.27 ± 0.02 φ
Hx+G+E+O	59.00 ± 1.43 φφφ	1.20 ± 0.02 φ

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, *** p<0.001 when compared to Control
φ p<0.05, φφ p<0.01, φφφ 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 –11
Real-time PCR amplification of Dopamine D_1 receptor mRNA from the cerebral cortex of control and experimental neonatal rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
**p<0.01, *** p<0.001 when compared to Control
φ p<0.05, φφφ p<0.001 when compared to Hx

Control rats - Con
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 –15

Real-time PCR amplification of Dopamine D_1 receptor mRNA from the cerebral cortex of control and experimental neonatal rats

Animal Status	Log RQ
Con	0
Hx	1.036 ± 0.121***
Hx+G	0.262 ± 0.025φφφ
Hx+O	0.707 ± 0.074**φ
Hx+G+O	0.304 ± 0.094φφφ
Hx+G+E+O	1.228 ± 0.087***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
**p<0.01, *** p<0.001 when compared to Control
φ p<0.05, φφφ p<0.001 when compared to Hx

Control rats - Con
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 –12
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebral Cortex of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

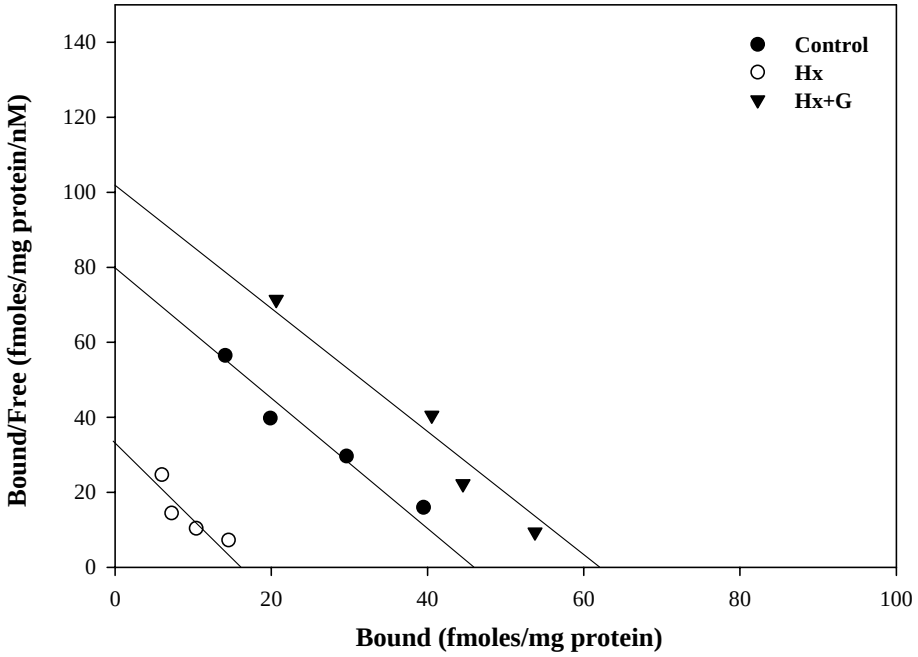


Table -16
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebral Cortex of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	46.00 ± 1.35	1.74 ± 0.05
Hx	16.00 ± 0.98*	2.12 ± 0.08*
Hx+G	62.00 ± 1.44 φφφ	1.65 ± 0.03 φφ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05 compared to Control
φφ p<0.01, φφφ p<0.001 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose treated – Hx+G

Figure –13
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebral Cortex of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

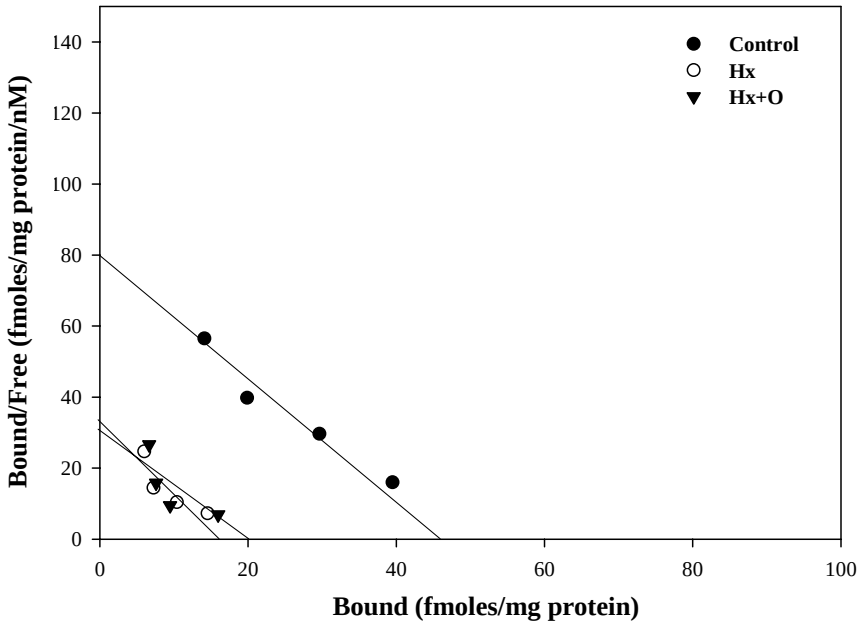


Table -17
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebral Cortex of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	46.00 ± 1.35	1.74 ± 0.05
Hx	16.00 ± 0.98 ***	2.12 ± 0.08*
Hx+O	20.00 ± 2.33 ***	1.60 ± 0.04 φφ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, ***p<0.001 compared to Control
φφ p<0.01 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats oxygen + glucose treated – Hx+O

Figure –14
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebral Cortex of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

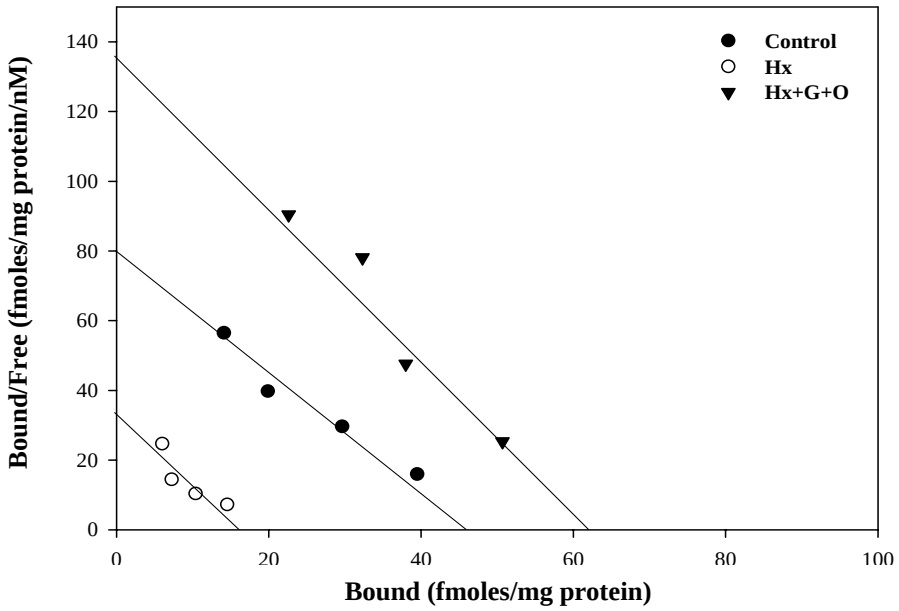


Table -18
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebral Cortex of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	46.00 ± 1.35	1.74 ± 0.05
Hx	16.00 ± 0.98***	2.12 ± 0.08*
Hx+G+O	63.00 ± 1.56 φφφ	1.55 ± 0.03φφ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, ***p<0.001 compared to Control
φφ p<0.01, φφφ p<0.001 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + oxygen treated – Hx+G+O

Figure –15
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebral Cortex of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

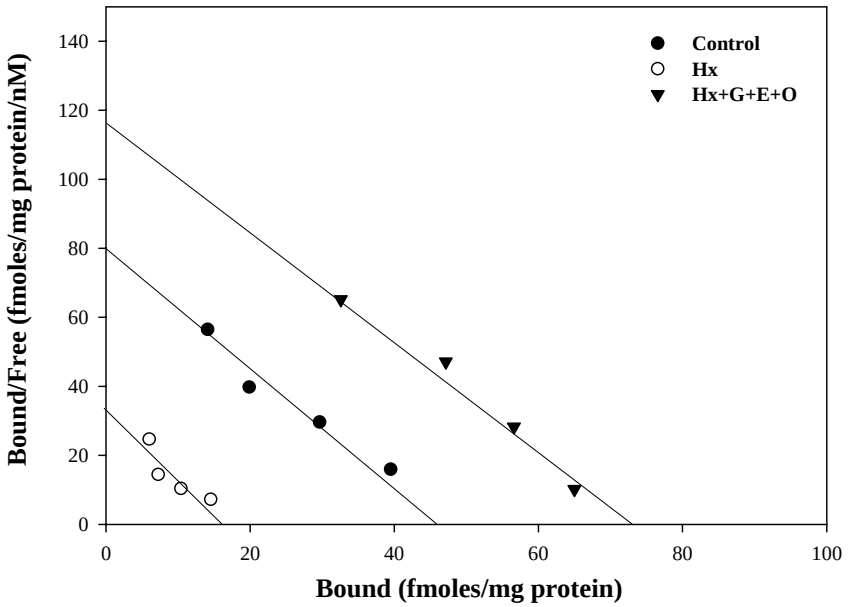
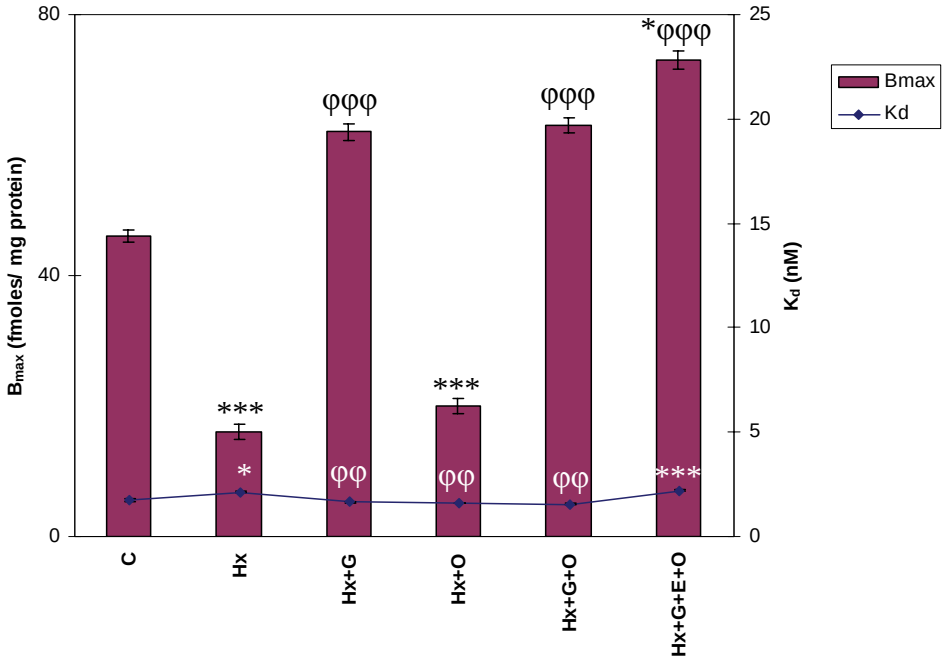


Table -19
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebral Cortex of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	46.00 ±1.35	1.74 ± 0.05
Hx	16.00 ± 0.98***	2.12 ± 0.08*
Hx+G+E+O	73.00 ±1.83 *φφφ	2.20 ± 0.04 ***

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, *** p<0.001 when compared to Control
φφφ p<0.001 when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + epinephrine + oxygen treated – Hx+G+E+O

Figure -16
Binding Parameters of [^{3}H]YM-09151-2 against Sulpiride in the Cerebral Cortex of Control and Experimental groups of Hypoxic Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, *** p<0.001 when compared to Control
φφ p<0.01, φφφ 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

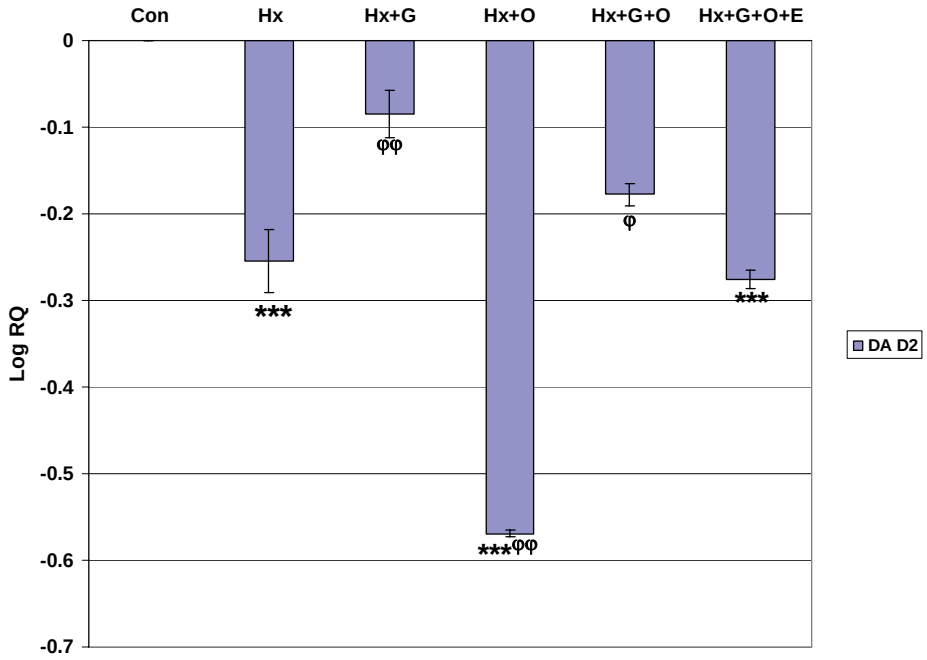
Table -20
Binding parameters of [^{3}H]YM-09151-2 against Sulpiride in the Cerebral Cortex of Control and Experimental Groups of Neonatal Rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
C	46.00 ± 1.35	1.74 ± 0.05
Hx	16.00 ± 0.98 ***	2.12 ± 0.08 *
Hx+G	62.00 ± 1.44 φφφ	1.65 ± 0.03 φφ
Hx+O	20.00 ± 2.33 ***	1.6 ± 0.04 φφ
Hx+G+O	63.00 ± 1.56 φφφ	1.55 ± 0.03 φφ
Hx+G+E+O	73.00 ±1.83 * φφφ	2.20 ± 0.04 ***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, *** p<0.001 when compared to Control
φφ p<0.01, φφφ 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 - 17
Real-Time PCR amplification of Dopamine D_2 receptor mRNA from the Cerebral cortex of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φ $p<0.05$, φφ $p<0.01$ when compared to Hx

Control rats - Con
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 – 21
Real-Time PCR amplification of Dopamine D_2 receptor mRNA from the Cerebral cortex of Control and Experimental Groups of Neonatal Rats

Animal Status	Log RQ
Con	0
Hx	-0.255 ± 0.036***
Hx+G	-0.085 ± 0.028φφ
Hx+O	-0.569 ± 0.004***φφ
Hx+G+O	-0.178 ± 0.012φ
Hx+G+E+O	-0.276 ± 0.010***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φ $p<0.05$, φφ $p<0.01$ when compared to Hx

Control rats - Con
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 -18
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Brainstem of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

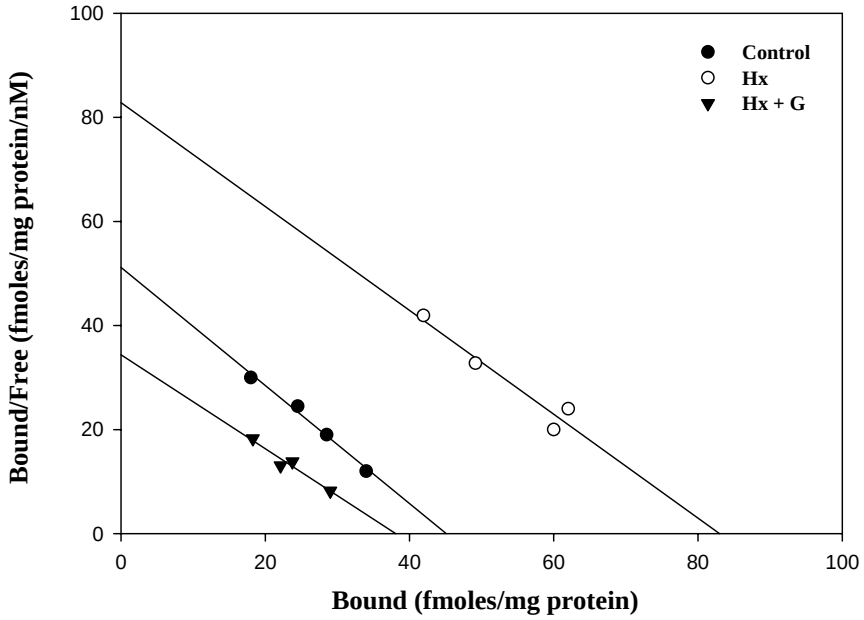


Table -22
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Brainstem of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	44.00 ±1.35	1.16 ± 0.05
Hx	83.00 ± 0.98 ***	0.96 ± 0.08
Hx+G	36.00 ± 1.44 φφφ	1.00 ± 0.03

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose treated – Hx+G

Figure –19
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Brainstem of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

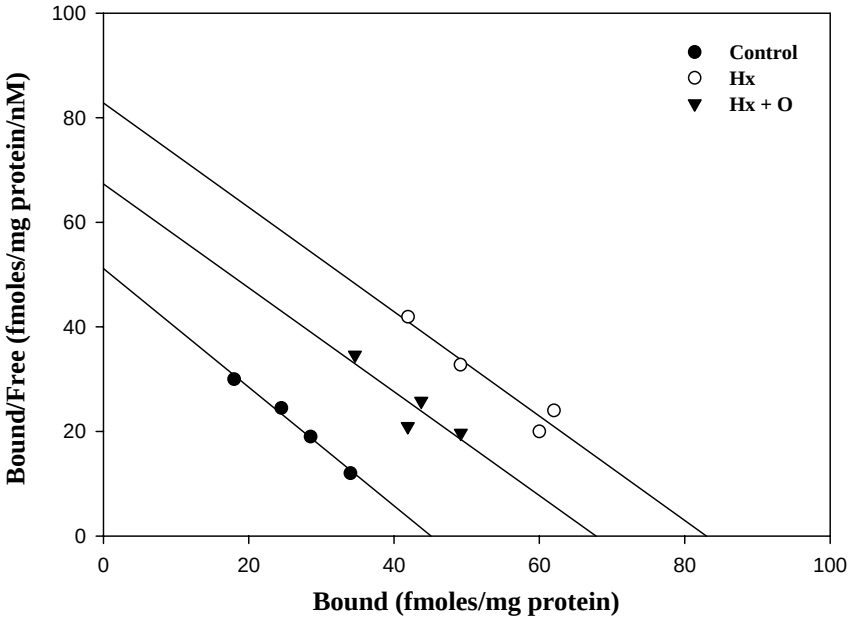


Table -23
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Brainstem of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	44.00 ± 1.35	1.16 ± 0.05
Hx	83.00 ± 0.98 ***	0.96 ± 0.08
Hx+O	65.00 ± 1.83 *φ	1.06 ± 0.04

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, *** p<0.001 when compared to Control
φ p<0.05 when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats oxygen treated – Hx+O

Figure –20
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Brainstem of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

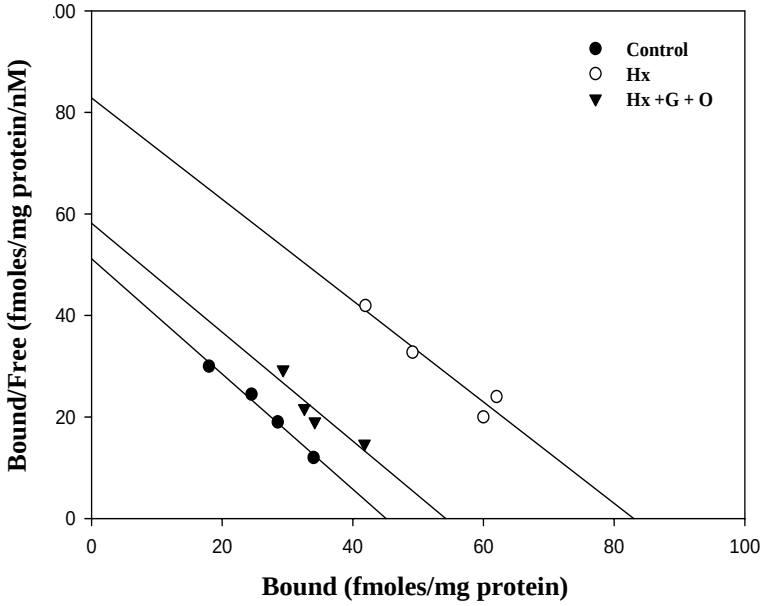


Table -24
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Brainstem of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	44.00 ± 1.35	1.16 ± 0.05
Hx	83.00 ± 0.98 ***	0.96 ± 0.08
Hx+G+O	54.00 ± 2.33 φφ	1.13 ± 0.03

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
***p<0.001 compared to Control
φφ p<0.01 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + oxygen treated – Hx+G+O

Figure -21
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Brainstem of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

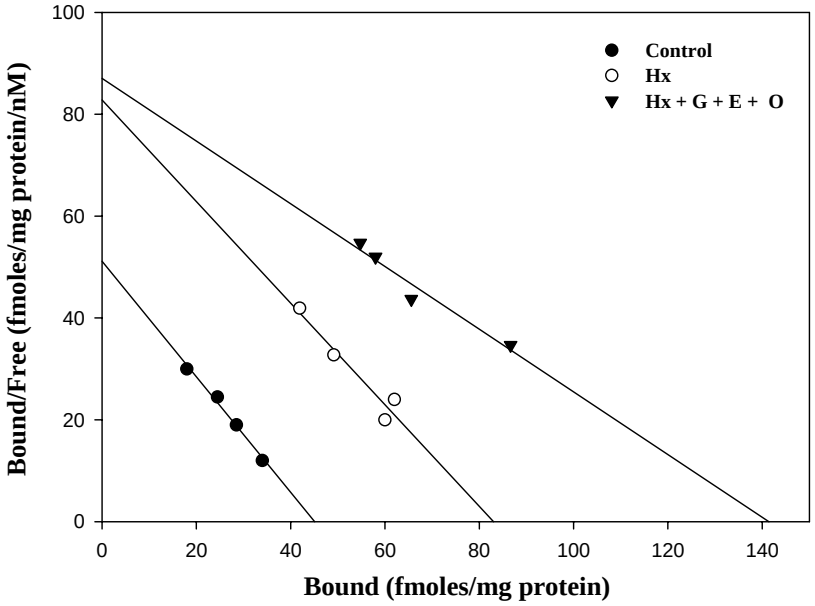
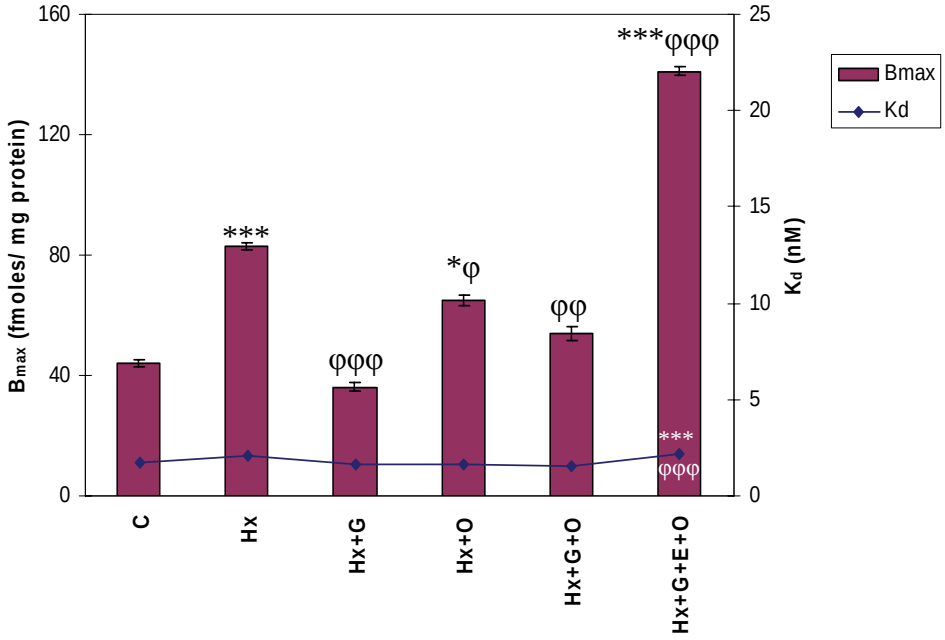


Table -25
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Brainstem of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	44.00 ± 1.35	1.16 ± 0.05
Hx	83.00 ± 0.98 ***	0.96 ± 0.08
Hx+G+E+O	141.00 ± 1.56 ***φφφ	0.63 ± 0.04***φφφ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
***p<0.001 compared to C
φφφ p<0.001 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + epinephrine + oxygen treated – Hx+G+E+O

Figure -22
Binding parameters of [^{3}H]Dopamine against Dopamine in the Brainstem of Control and Experimental groups of Hypoxic Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, *** p<0.001 when compared to Control
φ p<0.05,φφ p<0.01, φφφ 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 -26
Binding parameters of [^{3}H]Dopamine against Dopamine in the Brainstem of Control and Experimental Groups of Neonatal Rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
C	44.00 ± 1.35	1.16 ± 0.05
Hx	83.00 ± 0.98 ***	0.96 ± 0.08
Hx+G	36.00 ±1.44***φφφ	1.00 ± 0.03
Hx+O	65.00 ± 1.83 *φ	1.06 ± 0.04
Hx+G+O	54.00 ± 2.33 φφ	1.13 ± 0.03
Hx+G+E+O	141.00 ± 1.56 ***φφφ	0.63 ± 0.04***φφφ

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, *** p<0.001 when compared to Control
φ p<0.05, φφ p<0.01, φφφ 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 -23
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Brainstem of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

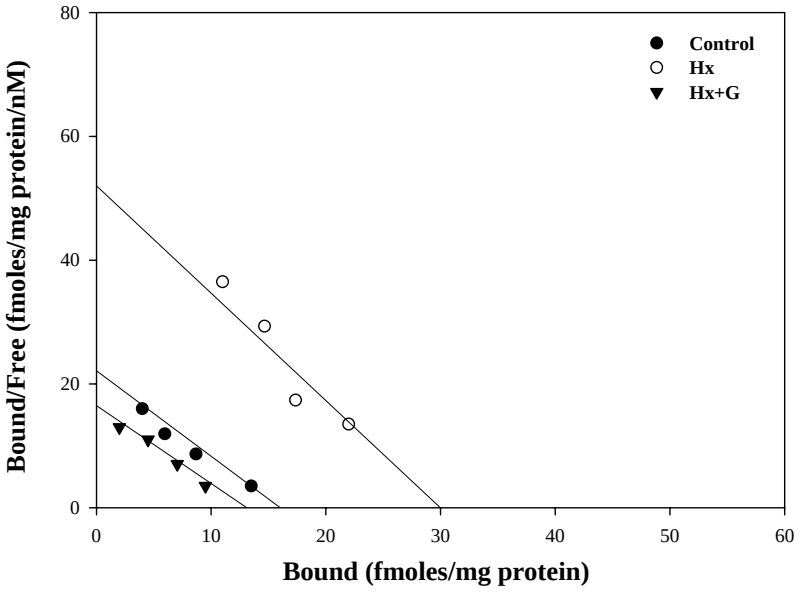


Table -27
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Brainstem of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	16.00 ± 1.02	1.38 ± 0.06
Hx	30.00 ± 0.89 **	1.73 ± 0.03**
Hx+G	13.00 ± 0.73 φφ	1.23 ± 0.06 φφ

Values are Mean±S.E.M of 4-6 separate experiments
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.01 compared to Control
φφ p<0.01 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose treated – Hx+G

Figure –24
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Brainstem of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

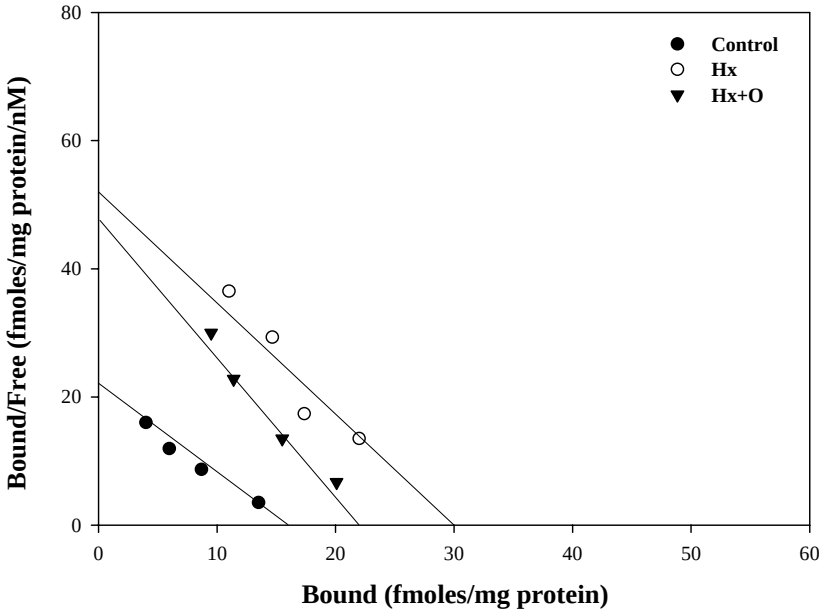


Table -28
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Brainstem of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg rotein)	K_d (nM)
C	16.00 ± 1.02	1.38 ± 0.06
Hx	30.00 ± 0.89 **	1.73 ± 0.03**
Hx+O	22.00 ± 0.65 φ	2.13 ± 0.04 **φ

Values are Mean±S.E.M of 4-6 separate experiments
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.01 compared to Control
φ p<0.05 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats oxygen treated – Hx+O

Figure –25
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Brainstem of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

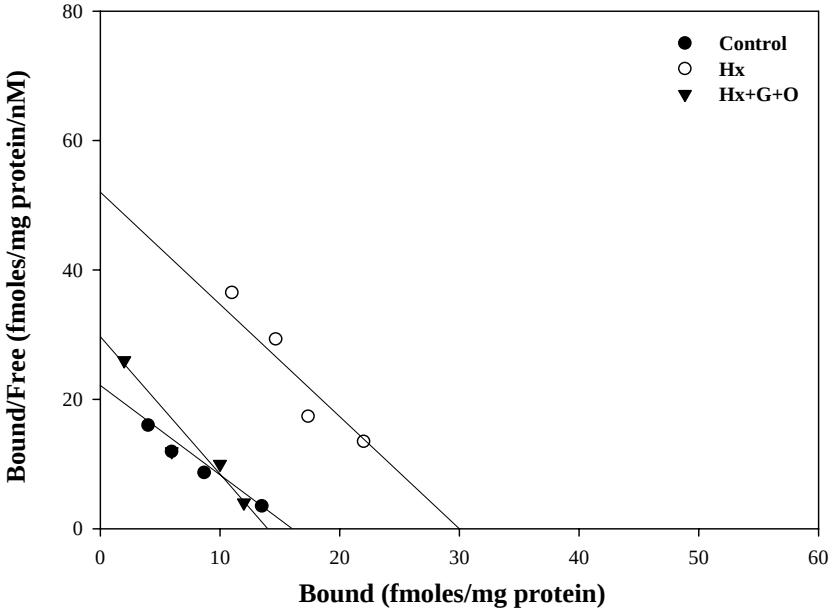


Table -29
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Brainstem of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	16.00 ± 1.02	1.38 ± 0.06
Hx	30.00 ± 0.89 **	1.73 ± 0.03**
Hx+G+O	14.00 ± 2.15 φφφ	2.14 ± 0.07 **φ

Values are Mean±S.E.M of 4-6 separate experiments
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.001 compared to Control
φ p<0.05, φφφ p<0.001 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + oxygen treated – Hx+G+O

Figure -26
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Brainstem of Control, Hypoxic and Glucose +Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

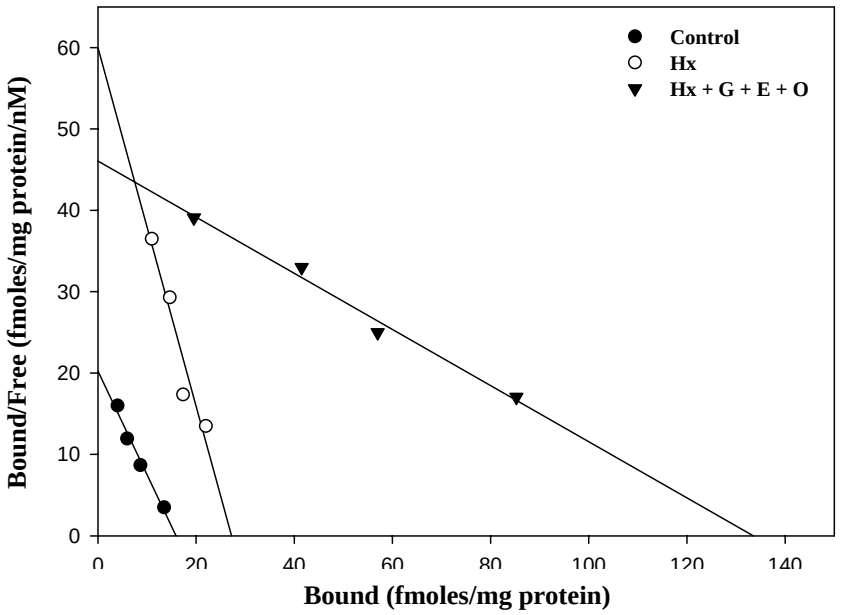
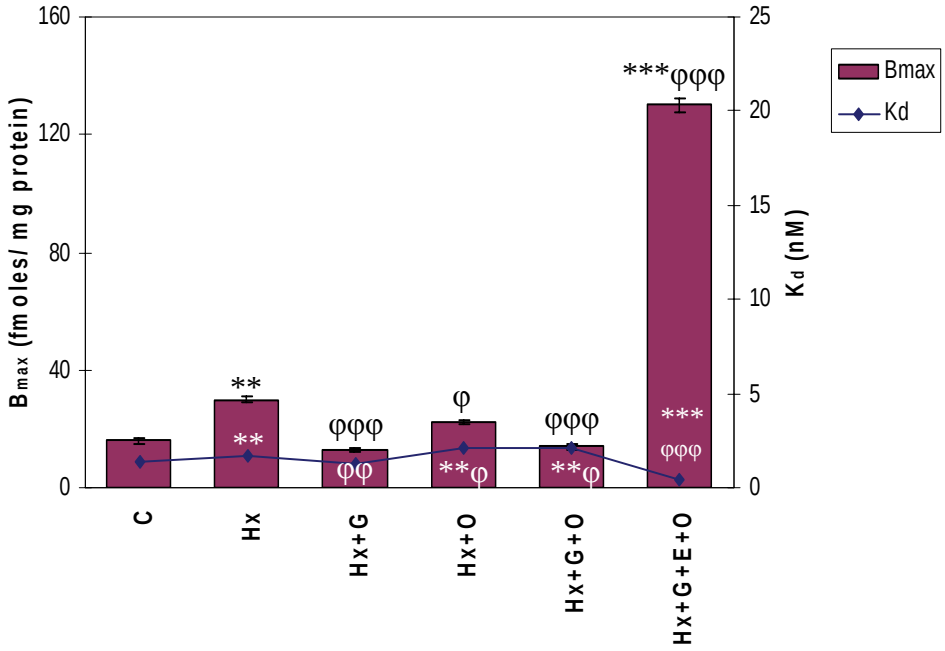


Table -30
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Brainstem of Control, Hypoxic and Glucose +Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	16.00 ± 1.02	1.38 ± 0.06
Hx	30.00 ± 0.89**	1.73 ± 0.03**
Hx+G+E+O	130.00 ± 2.15 ***φφφ	0.37 ± 0.05 **φφφ

Values are Mean±S.E.M of 4-6 separate experiments
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.01, ***p<0.001 compared to Control
φφφ p<0.001 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + epinephrine + oxyegn treated – Hx+G+E+O

Figure -27
Binding parameters of [^{3}H]SCH 23390 against SCH 23390 in the Brainstem of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
**p<0.01, *** p<0.001 when compared to Control
φ p<0.05,φφ p<0.01, φφφ 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

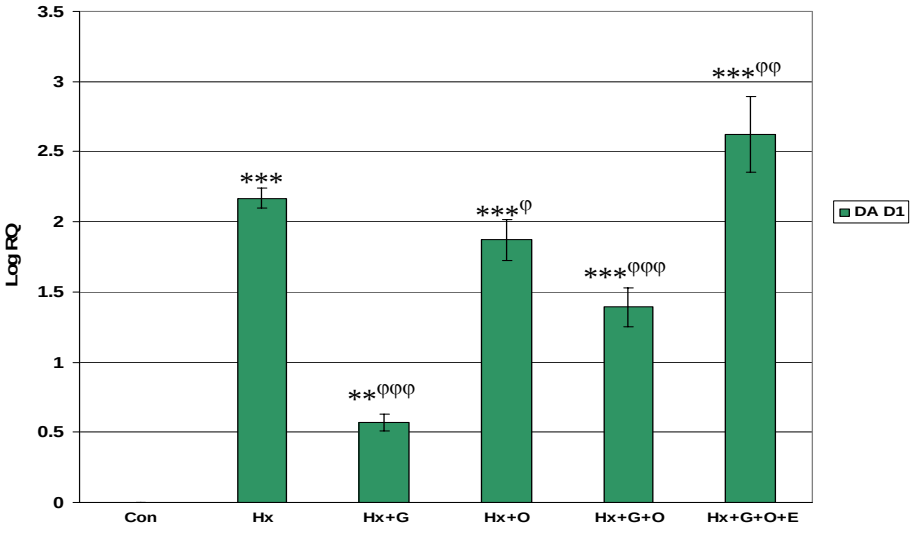
Table -31
Binding parameters of [^{3}H]SCH 23390 against SCH 23390 in the Brainstem of Control and Experimental Groups of Neonatal Rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
C	16.00 ± 1.02	1.38 ± 0.06
Hx	30.00 ± 0.89**	1.73 ± 0.03**
Hx+G	13.00 ± 0.73 φφφ	1.23 ± 0.06 φφ
Hx+O	22.00 ± 0.65 φ	2.13 ± 0.04 **φ
Hx+G+O	14.00 ± 1.10 φφφ	2.14 ± 0.07 **φ
Hx+G+E+O	130.00 ± 2.15 ***φφφ	0.37 ± 0.05 ***φφφ

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
**p<0.01, *** p<0.001 when compared to Control
φ p<0.05, φφ p<0.01, φφφ p<0.001 when compared to Hx

Control rats - Con
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 -28
Real-Time PCR amplification of Dopamine D_1 receptor mRNA from the Brainstem of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
**p<0.05, *** p<0.001 when compared to Control
φ p<0.05, φφ p<0.01, φφφ p<0.001 when compared to Hx

Control rats - Con
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 -32
Real-Time PCR amplification of Dopamine D_1 receptor mRNA from the Brainstem of Control and Experimental Groups of Neonatal Rats

Animal Status	Log RQ
C	0
Hx	2.169 ± 0.071***
Hx+G	0.569 ± 0.061**φφφ
Hx+O	1.871 ± 0.145***φ
Hx+G+O	0.392 ± 0.139***φφφ
Hx+G+E+O	2.622 ± 0.268***φφ

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
**p<0.05, *** p<0.001 when compared to Control
φ p<0.05, φφ p<0.01, φφφ p<0.001 when compared to Hx

Control rats - Con
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 -29
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Brainstem of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

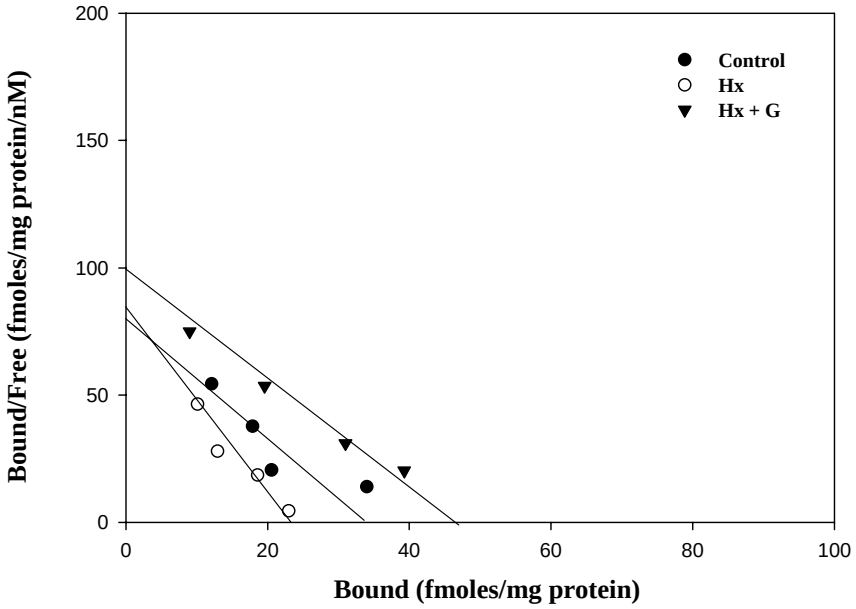


Table - 33
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Brainstem of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	34.00 ± 0.86	2.35 ± 0.04
Hx	23.00 ± 1.02**	3.69 ± 0.03***
Hx+G	46.00 ± 0.93φφφ	2.15 ± 0.06 φφ

Values are Mean±S.E.M of 4-6 separate experiments
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.01, ***p<0.001 when compared to Control
φφ p<0.01, φφφ p<0.001 when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose treated – Hx+G

Figure – 30
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Brainstem of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

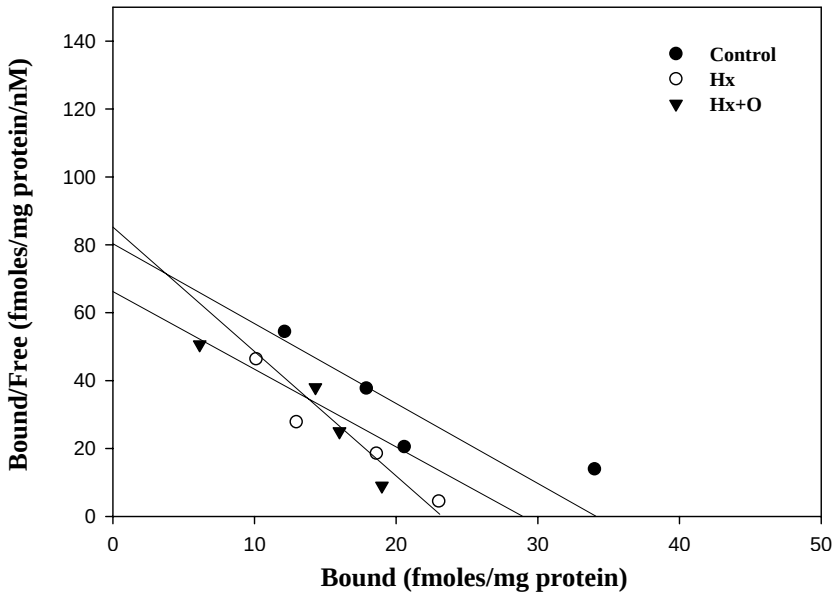


Table - 34
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Brainstem of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	34.00 ± 0.86	2.35 ± 0.04
Hx	23.00 ± 1.02**	3.69 ± 0.03***
Hx+O	29.00 ± 1.83*	2.29 ± 0.03φφφ

Values are Mean±S.E.M of 4-6 separate experiments
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, **p<0.01, ***p<0.001 when compared to Control
φφφ p<0.001 when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats oxygen treated – Hx+O

Figure -31
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Brainstem of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

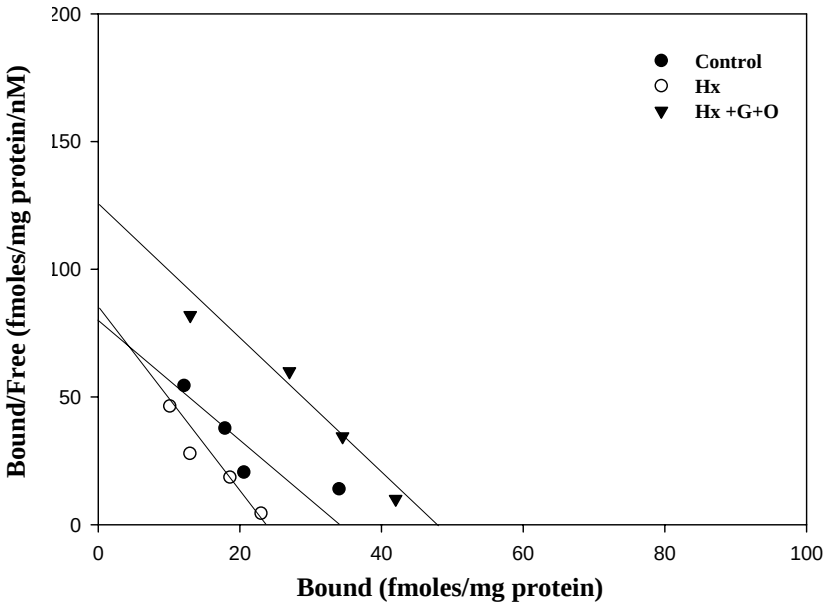


Table -35
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Brainstem of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	34.00 ± 0.86	2.35 ± 0.04
Hx	23.00 ± 1.02**	3.69 ± 0.03***
Hx+G+O	48.00 ± 2.33 *φφφ	2.61 ± 0.04φφ

Values are Mean±S.E.M of 4-6 separate experiments
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, **p<0.01, ***p<0.001 when compared to Control
φφ p<0.01, φφφ p<0.001 when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + oxygen treated – Hx+G+O

Figure -32
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Brainstem of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

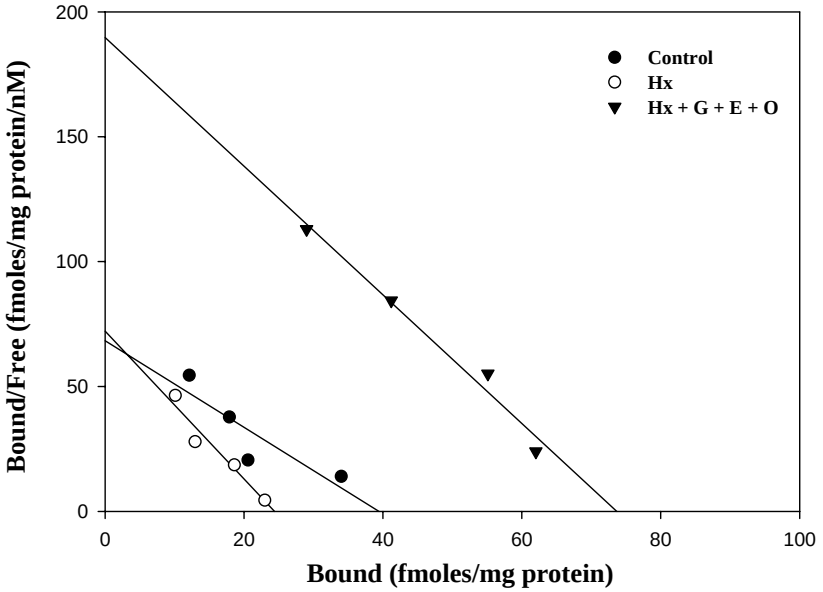
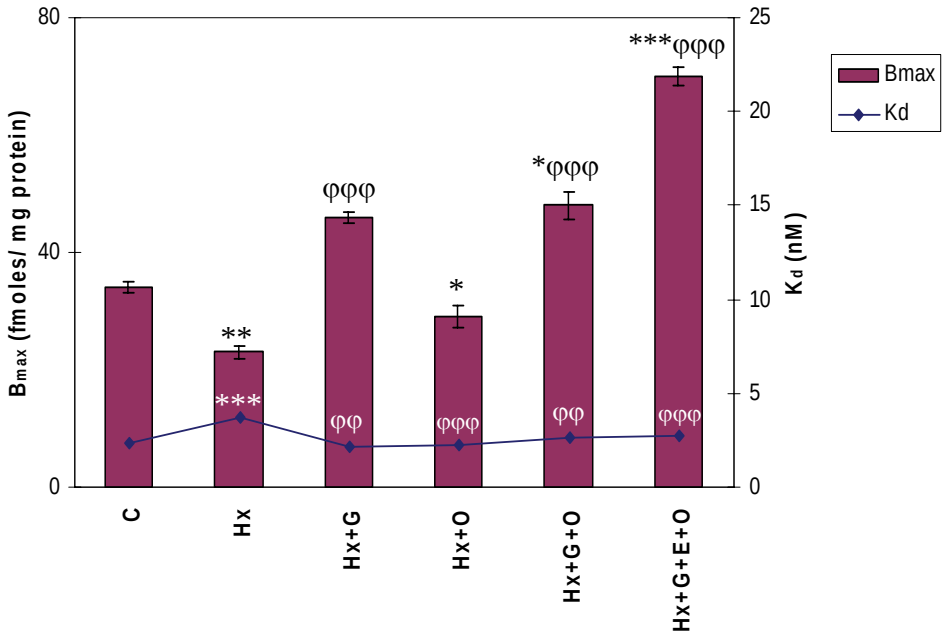


Table -36
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Brainstem of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	34.00 ± 0.86	2.35 ± 0.04
Hx	23.00 ± 1.02**	3.69 ± 0.03***
Hx+G+E+O	70.00 ± 1.56 ***φφφ	2.71 ± 0.03φφφ

Values are Mean±S.E.M of 4-6 separate experiments
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.01, ***p<0.001 when compared to Control
φφφ p<0.001 when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + epinephrine + oxygen treated – Hx+G+E+O

Figure -33
Binding Parameters of [^{3}H]YM-09151-2 Binding against Sulpiride in the Brainstem of Control and Experimental groups of Hypoxic Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, **p<0.01, *** p<0.001 when compared to Control
φφ p<0.01, φφφ 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

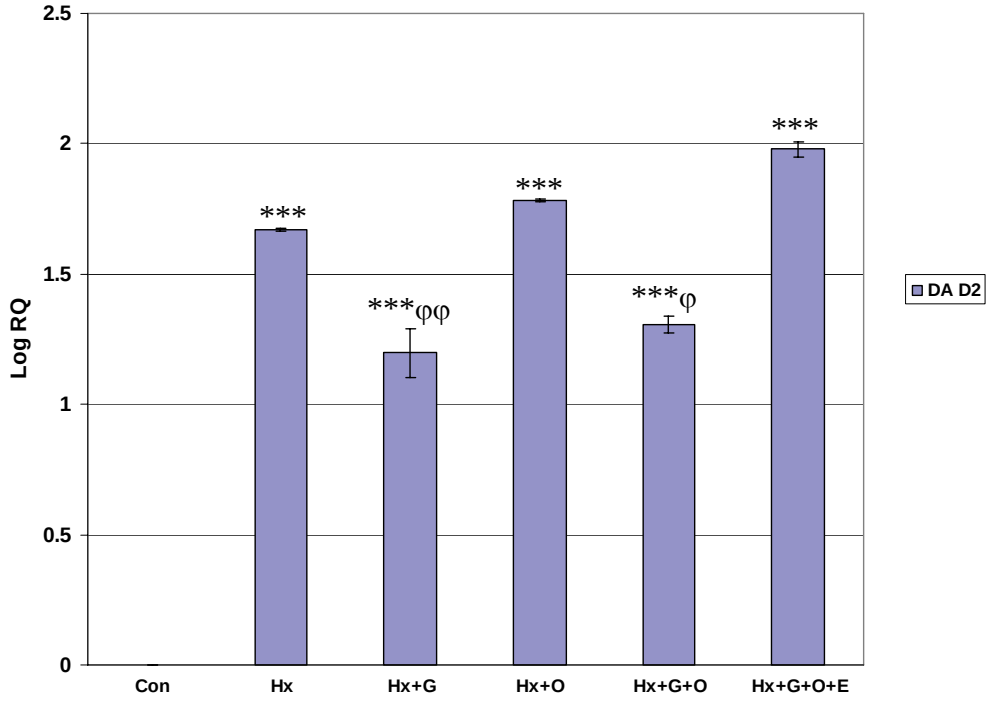
Table -37
Binding Parameters of [^{3}H]YM-09151-2 Binding against Sulpiride in the Brainstem of Control and Experimental Groups of Neonatal Rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
C	34.00 ± 0.86	2.35 ± 0.04
Hx	23.00 ± 1.02**	3.69 ± 0.03***
Hx+G	46.00 ± 0.93φφφ	2.15 ± 0.06 φφ
Hx+O	29.00 ± 1.83*	2.29 ± 0.03φφφ
Hx+G+O	48.00 ± 2.33 *φφφ	2.61 ± 0.04φφ
Hx+G+E+O	70.00 ± 1.56 ***φφφ	2.71 ± 0.03φφφ

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, **p<0.01, *** p<0.001 when compared to Control
φφ p<0.01, φφφ 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 -34
Real-Time PCR amplification of Dopamine D_2 receptor mRNA from the Brainstem of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φ $p<0.05$, φφ $p<0.01$when compared to Hx

Control rats - Con
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 -38
Real-Time PCR amplification of Dopamine D_2 receptor mRNA from the Brainstem of Control and Experimental Groups of Neonatal Rats

Animal Status	Log RQ
Con	0
Hx	1.672 ± 0.005***
Hx+G	1.197 ± 0.093***φφ
Hx+O	1.782 ± 0.007***
Hx+G+O	1.306 ± 0.034***φ
Hx+G+E+O	1.979 ± 0.030***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φ $p<0.05$, φφ $p<0.01$when compared to Hx

Control rats - Con
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 -35
Scatchard Analysis of [^{3}H]Dopamine Binding Against Dopamine in the Cerebellum of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

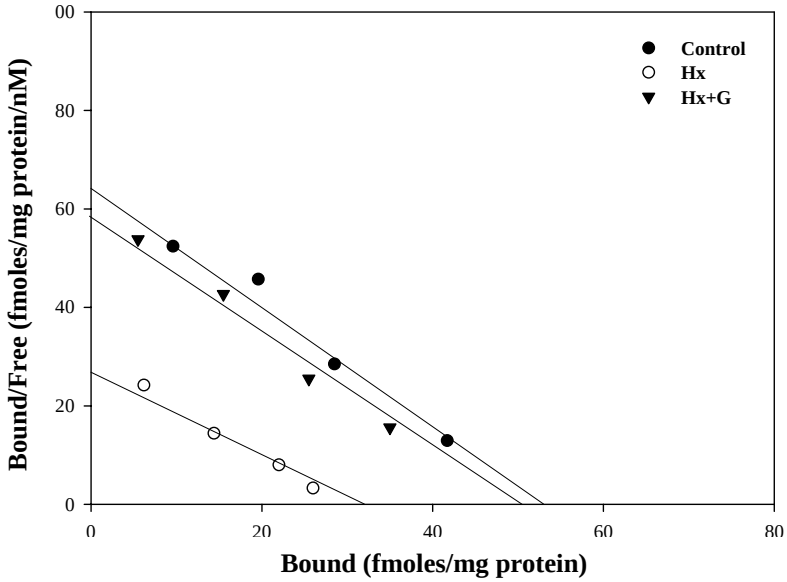


Table -39
Scatchard Analysis of [^{3}H]Dopamine Binding Against Dopamine in the Cerebellum of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	53.00 ± 0.74	1.21 ± 0.02
Hx	28.00 ± 1.22 **	0.85 ± 0.04 **
Hx+G	50.00 ± 0.86 φφ	1.18 ± 0.08 φφ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.01 when compared to Control
φφ p<0.01 when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose treated – Hx+G

Figure –36
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebellum of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

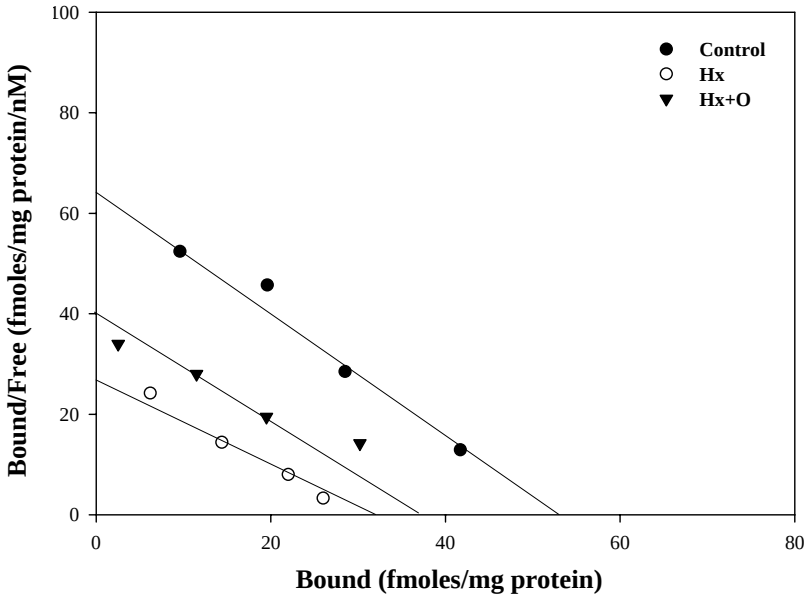


Table -40
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebellum of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	53.00 ± 0.74	1.21 ± 0.02
Hx	28.00 ± 1.22 **	0.85 ± 0.04 **
Hx+O	37.00 ± 1.31 *φ	1.08 ± 0.02 *φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
* $p<0.05$, **$p<0.01$ when compared to C
φ $p<0.05$ when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats oxygen treated – Hx+O

Figure –37
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebellum of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

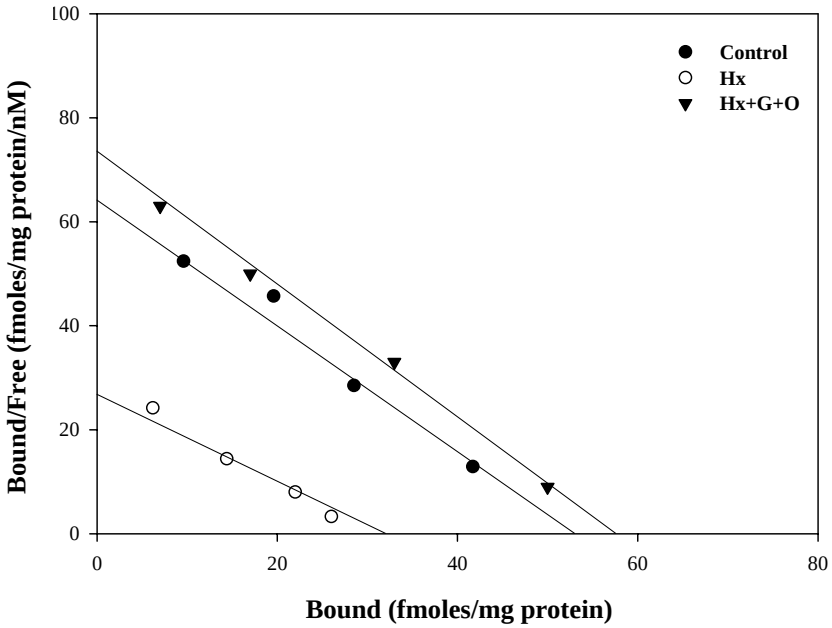


Table -41
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebellum of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	53.00 ± 0.74	1.21 ± 0.02
Hx	28.00 ± 1.22 **	0.85 ± 0.04**
Hx+G+O	57.00 ± 1.53 φφφ	1.28 ± 0.06 φφ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.01 when compared to C
φφ p<0.01, φφφ p<0.001when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + oxygen treated – Hx+G+O

Figure -38
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebellum of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

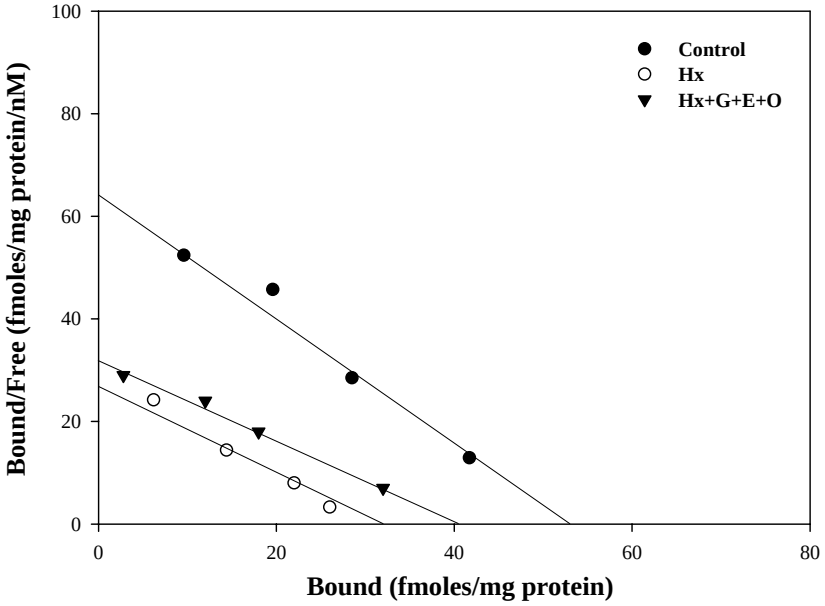
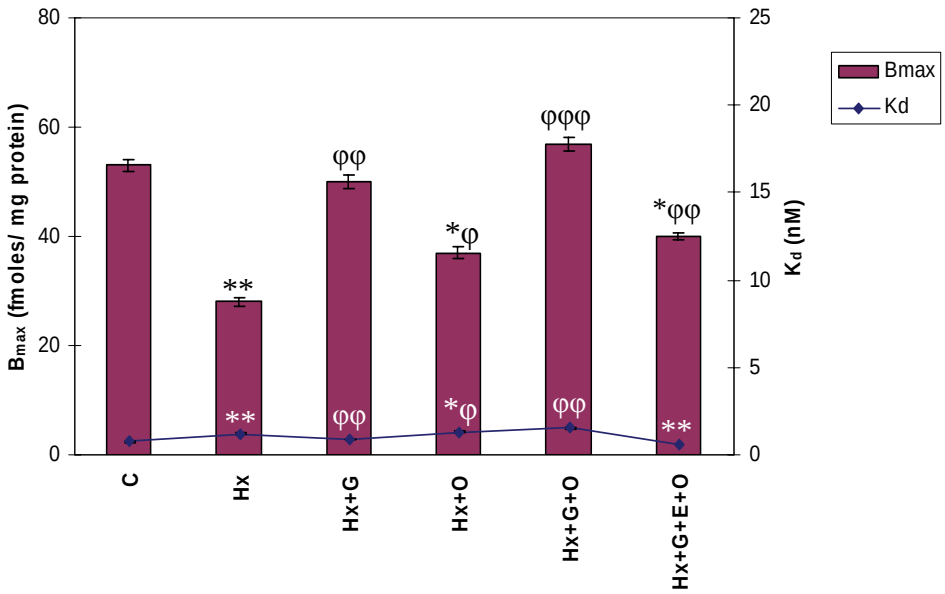


Table -42
Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebellum of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	53.00 ± 0.74	1.21 ± 0.02
Hx	28.00 ± 1.22 **	0.85 ± 0.04
Hx+G+E+O	40.00 ± 0.63 *φφ	0.78 ± 0.03**

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, **p<0.01when compared to C
φφ p<0.01 when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + epinephrine + oxygen treated – Hx+G+E+O

Figure -39
Binding parameters of [^{3}H]Dopamine against Dopamine in the Cerebellum of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, ** p<0.01 when compared to Control
φφ p<0.01, φφφ 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 -43
Binding parameters of [^{3}H]Dopamine against Dopamine in the Cerebellum of Control and Experimental and Control Groups of Neonatal Rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
C	53.00 ± 0.74	1.21 ± 0.02
Hx	28.00 ± 1.22 **	0.85 ± 0.04**
Hx+G	50.00 ± 0.86 φφ	1.18 ± 0.08 φφ
Hx+O	37.00 ± 1.31 *φ	1.08 ± 0.02 *φ
Hx+G+O	57.00 ± 1.53 φφφ	1.28 ± 0.06 φφ
Hx+G+E+O	40.00 ± 0.63 *φφ	0.78 ± 0.03**

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*$p<0.05$, ** $p<0.01$ when compared to Control
φ $p<0.05$, φφ $p<0.01$, φφφ $p<0.001$ 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 -40
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Cerebellum of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

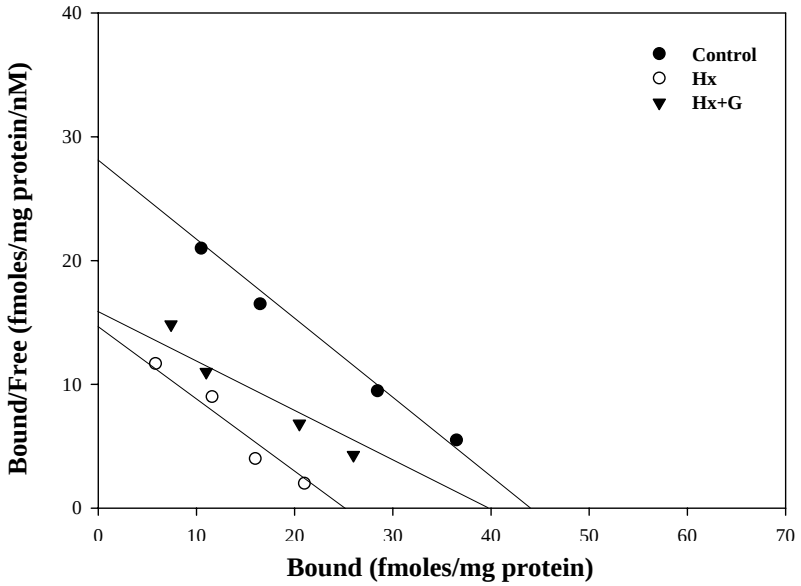


Table -44
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Cerebellum of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	44.00 ± 1.02	0.64 ± 0.03
Hx	24.00 ± 0.56 **	0.59 ± 0.06
Hx+G	40.00 ± 0.73 φφ	0.38 ± 0.04**φφ

Values are Mean±S.E.M of 4-6 separate experiments
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.01 when compared to Control
φφ p<0.01 when compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose treated – Hx+G

Figure –41
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Cerebellum of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

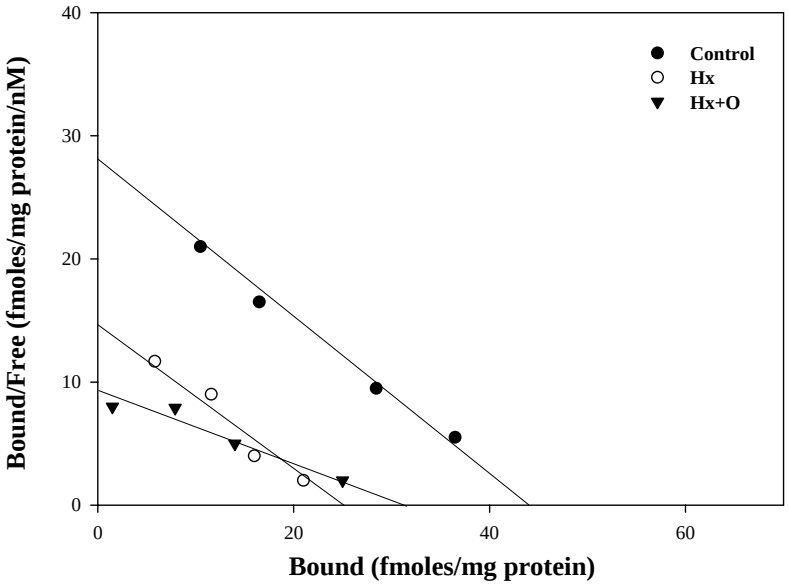


Table -45
Scatchard Analysis of [^{3}H]SCH 23390 Binding Against SCH 23390 in the Cerebellum of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	44.00 ± 1.02	0.64 ± 0.03
Hx	24.00 ± 0.56 **	0.59 ± 0.06
Hx+O	32.00 ± 0.89 *φ	0.28 ± 0.08 ***φφφ

Values are Mean±S.E.M of 4-6 separate experiments
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, **p<0.01, ***p<0.001 compared to Control
φ p<0.05, φφφ p<0.001 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats oxygen treated – Hx+O

Figure –42
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Cerebellum of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

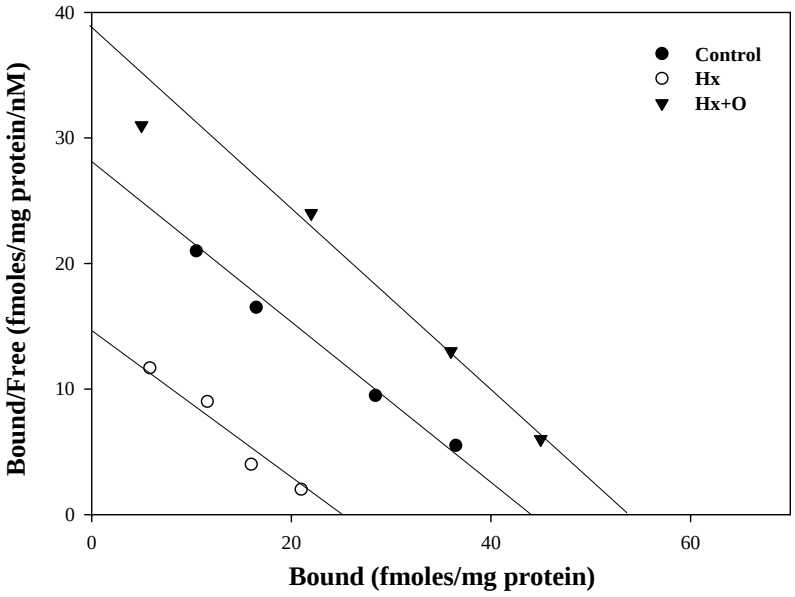


Table -46
Scatchard Analysis of [^{3}H]SCH 23390 Binding Against SCH 23390 in the Cerebellum of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	44.00 ± 1.02	0.64 ± 0.03
Hx	24.00 ± 0.56 **	0.59 ± 0.06
Hx+G+O	53.00 ± 1.22 *φφφ	0.71 ± 0.05 **φφ

Values are Mean±S.E.M of 4-6 separate experiments
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.01, *p<0.01 compared to Control
φφ p<0.01, φφφ p<0.001 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + oxygen treated – Hx+G+O

Figure -43
Scatchard Analysis of [^{3}H]SCH 23390 Binding against SCH 23390 in the Cerebellum of Control, Hypoxic and Glucose +Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

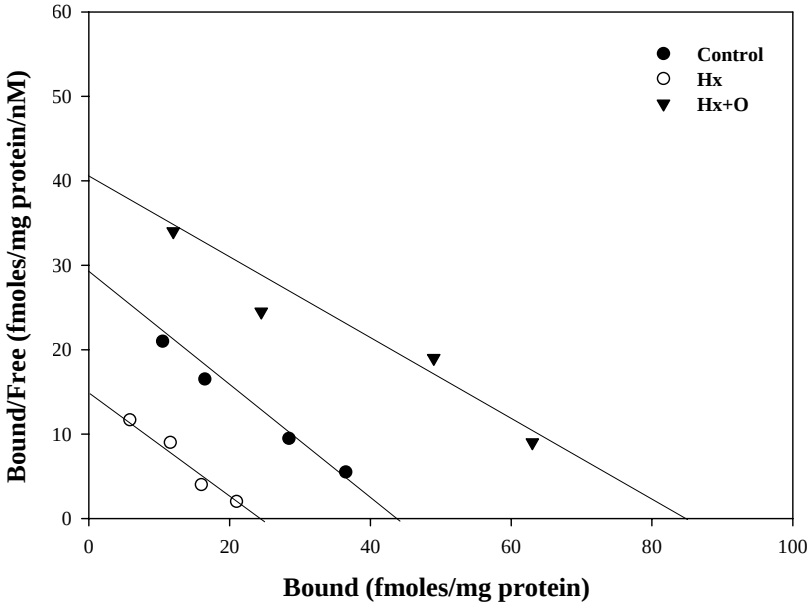
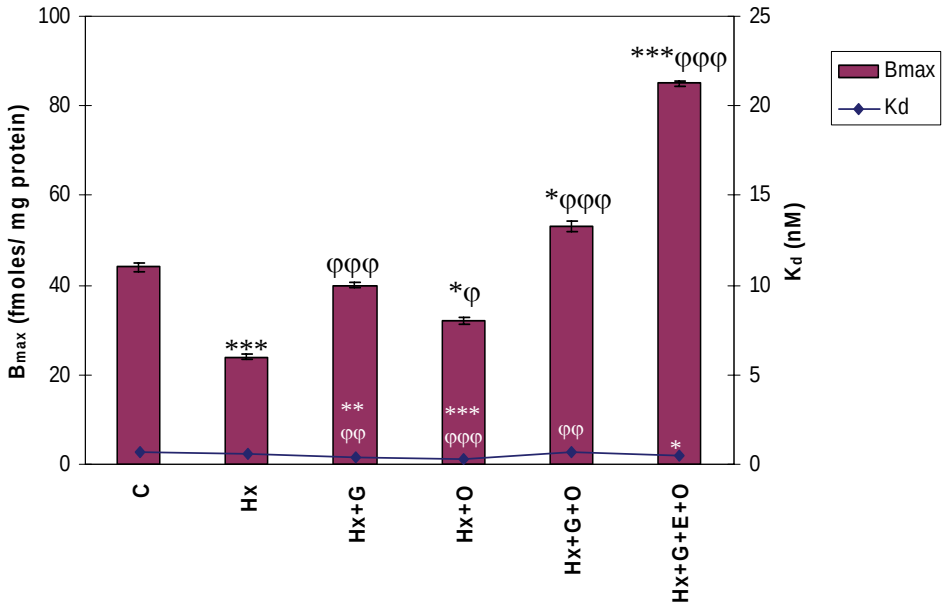


Table -47
Scatchard Analysis of [^{3}H]SCH 23390 Binding Against SCH 23390 in the Cerebellum of Control, Hypoxic and Glucose +Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	44.00 ± 1.02	0.64 ± 0.03
Hx	24.00 ± 0.56 **	0.59 ± 0.06
Hx+G+E+O	85.00 ± 0.73 ***φφφ	0.48 ± 0.07 *

Values are Mean±S.E.M of 4-6 separate experiments
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, **p<0.01,***p<0.001 compared to Control
φφφ p<0.001 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + epinephrine + oxygen treated – Hx+G+E+O

Figure -44
Binding parameters of [^{3}H]SCH 23390 against SCH 23390 in the Cerebellum of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, **p<0.01, *** p<0.001 when compared to Control
φ p<0.05,φφ p<0.01, φφφ 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

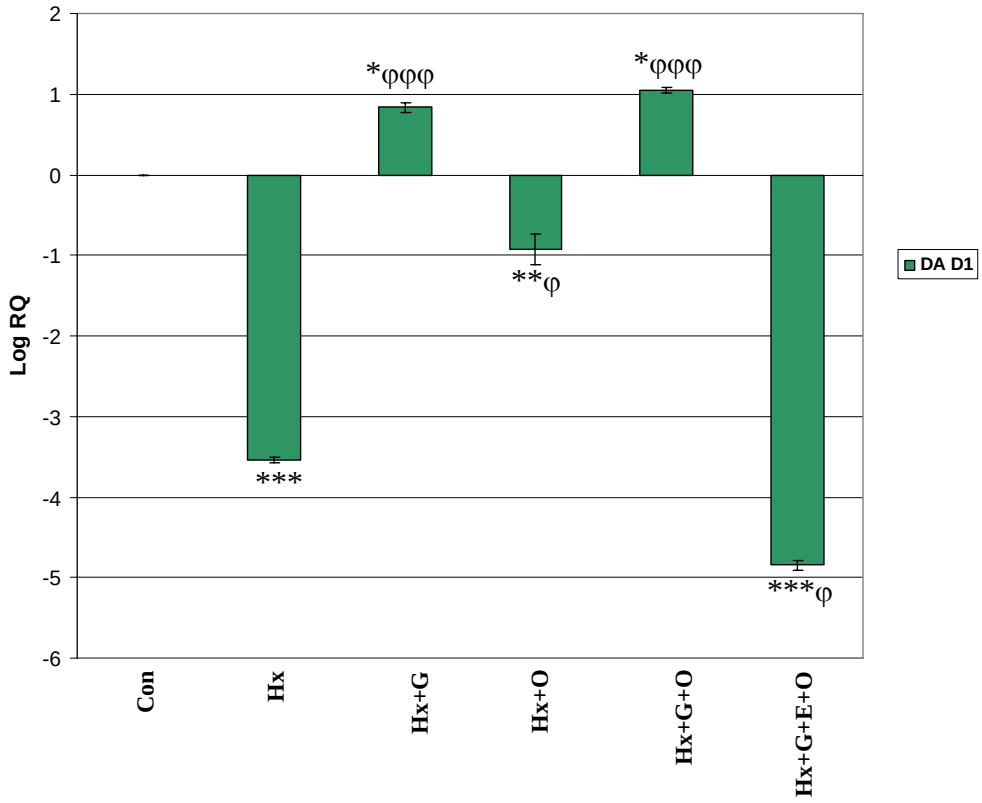
Table -48
Binding parameters of [^{3}H]SCH 23390 against SCH 23390 in the Cerebellum of Control and Experimental Groups of Neonatal Rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
C	44.00 ± 1.02	0.64 ± 0.03
Hx	24.00 ± 0.56 ***	0.59 ± 0.06
Hx+G	40.00 ± 0.73 φφφ	0.38 ± 0.04 **φφ
Hx+O	32.00 ± 0.89 *φ	0.28 ± 0.08 ***φφφ
Hx+G+O	53.00 ± 1.22 *φφφ	0. 71 ± 0.05 φφ
Hx+G+E+O	85.00 ± 0.73 ***φφφ	0.48 ± 0.07 *

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared to Control
φ $p<0.05$, φφ $p<0.01$, φφφ $p<0.001$ 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 -45
Real-Time PCR amplification of Dopamine D_1 receptor mRNA from the Cerebellum of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
* p<0.05, ** p<0.01, *** p<0.001 when compared to Control
φ p<0.05, φφφ p<0.001 when compared to Hx

Control rats - Con
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 -49
Real-Time PCR amplification of Dopamine D_1 receptor mRNA from the Cerebellum of Control and Experimental Groups of Neonatal Rats

Animal Status	Log RQ
Con	0
Hx	-3.545 ± 0.032***
Hx+G	0.834 ± 0.066*$^{\varphi\varphi\varphi}$
Hx+O	-0.923 ± 0.186**$^{\varphi}$
Hx+G+O	1.045 ± 0.035*$^{\varphi\varphi\varphi}$
Hx+G+E+O	-4.847 ± 0.064***$^{\varphi}$

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared to Control
φ $p<0.05$, φφφ $p<0.001$ when compared to Hx

Control rats - Con
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 -46
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebellum of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

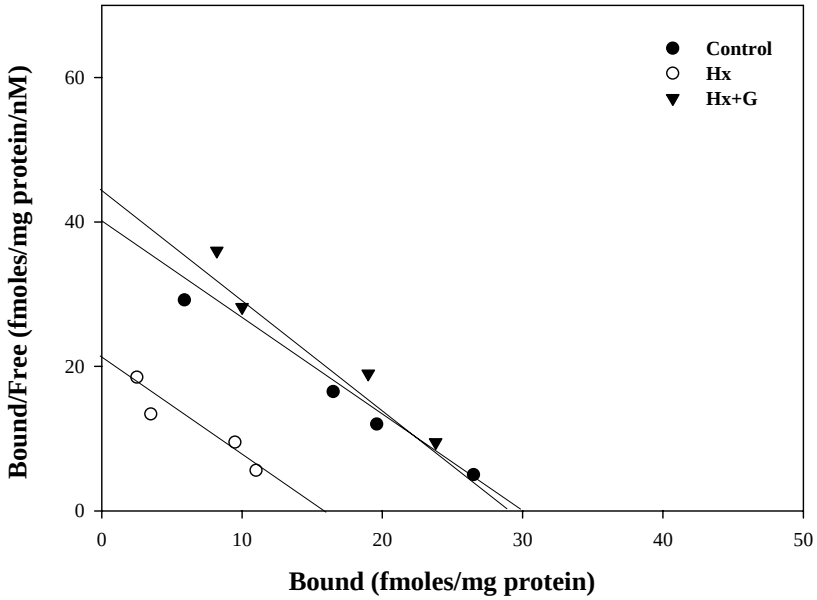


Table -50
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebellum of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	28.00 ± 0.58	1.45 ± 0.06
Hx	16.00 ± 1.02**	1.36 ± 0.04
Hx+G	30.00 ± 0.47φφ	1.48 ± 0.08

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.01 compared to Control
φφ p<0.01 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose treated – Hx+G

Figure – 47
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebellum of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

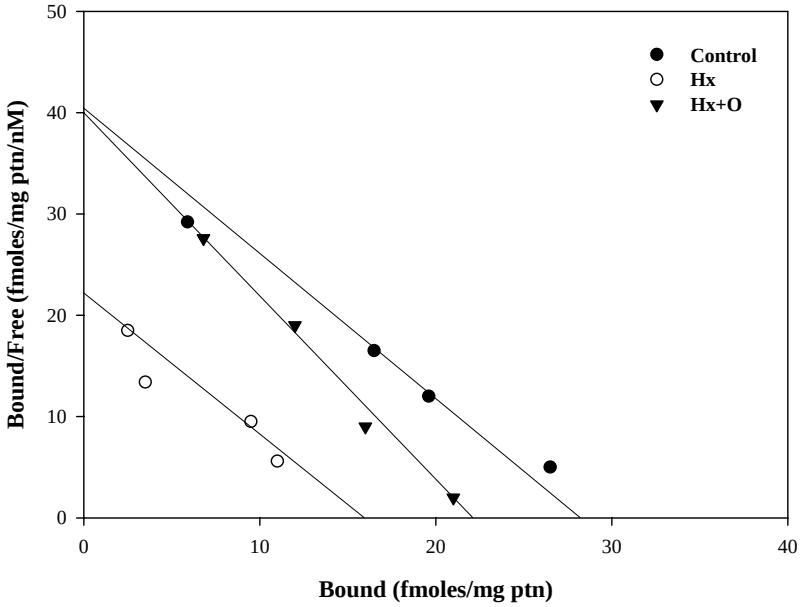


Table - 51
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebellum of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	28.00 ± 0.58	1.45 ± 0.06
Hx	16.00 ± 1.02 **	1.36 ± 0.04
Hx+O	22.00 ± 0.62 *φ	1.32 ± 0.05

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, **p<0.01 compared to Control
φp<0.05 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats oxygen treated – Hx+O

Figure -48
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebellum of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

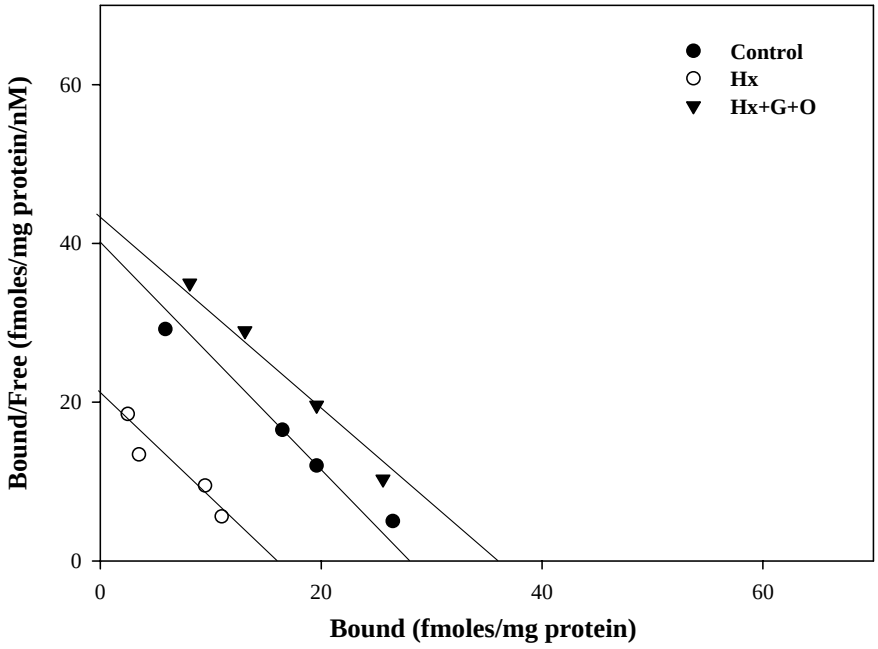


Table -52
Scatchard Analysis of [^{3}H]YM-09151-2 Binding Against Sulpiride in the Cerebellum of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	28.00 ± 0.58	1.45 ± 0.06
Hx	16.00 ± 1.02 **	1.36 ± 0.04
Hx+G+O	36.00 ± 1.22 *φφφ	1.22 ± 0.05φφ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, **p<0.01 compared to Control
φφ p<0.01, φφφ p<0.001 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + oxygen treated – Hx+G+O

Figure -49
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebellum of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

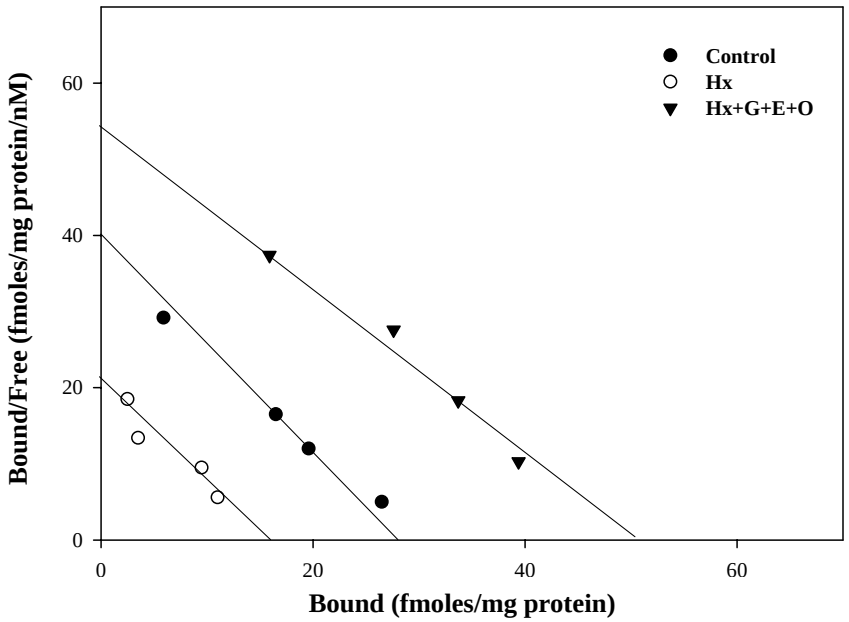
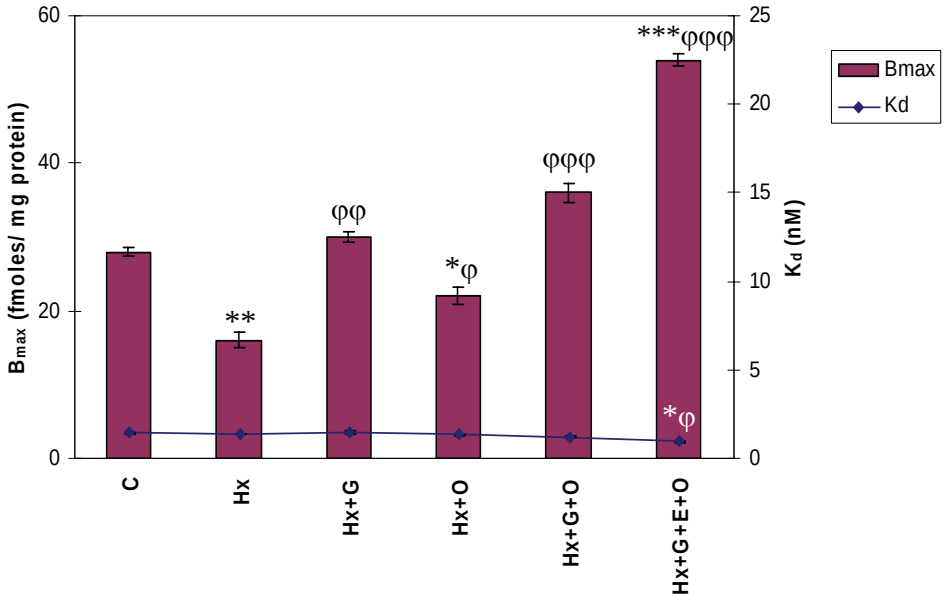


Table -53
Scatchard Analysis of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebellum of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	28.00 ± 0.58	1.45 ± 0.06
Hx	16.00 ± 1.02**	1.36 ± 0.04
Hx+G+E+O	54.00 ± 1.27 ***φφ	0.93 ± 0.04 *φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.01, ***p<0.001 compared to Control
φ p<0.05, φφ p<0.01 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose + epinephrine + oxygen treated – Hx+G+E+O

Figure -50
Binding parameters of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebellum of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, **p<0.01, *** p<0.001 when compared to Control
φ p<0.05, φφ p<0.01, φφφ 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

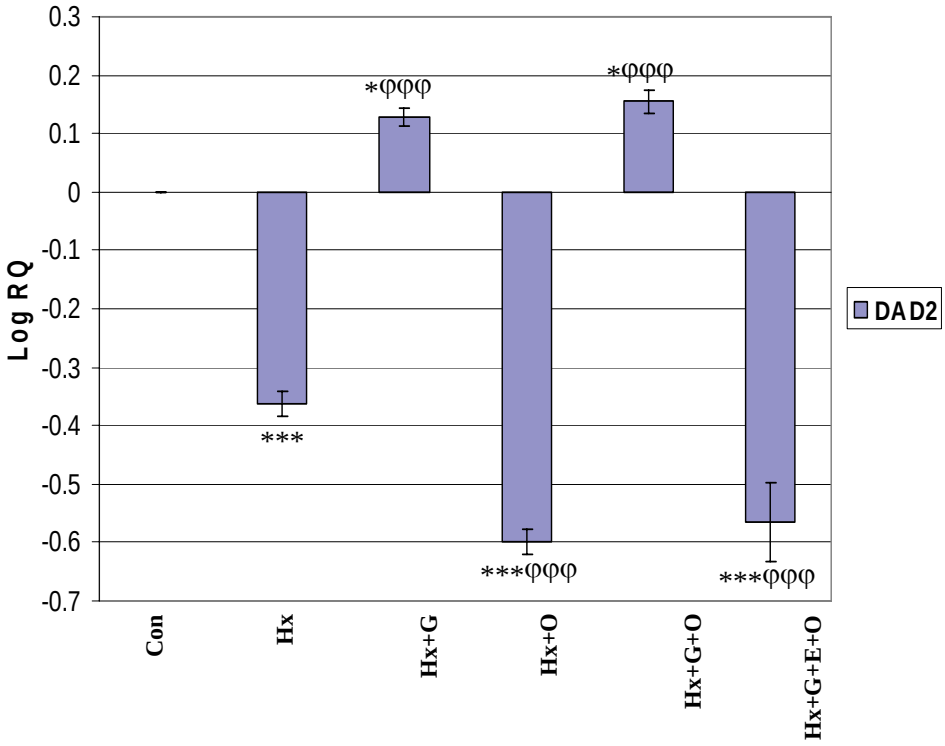
Table -54
Binding parameters of [^{3}H]YM-09151-2 Binding against Sulpiride in the Cerebellum of Control and Experimental Groups of Neonatal Rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
C	28.00 ± 0.58	1.45 ± 0.06
Hx	16.00 ± 1.02 **	1.36 ± 0.04
Hx+G	30.00 ± 0.47 φφ	1.48 ± 0.08
Hx+O	22.00 ± 0.62 *φ	1.32 ± 0.05
Hx+G+O	36.00 ± 1.22 φφφ	1.22 ± 0.05
Hx+G+E+O	54.00 ± 1.27 ***φφφ	0.93 ± 0.04 *φ

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, **p<0.01, *** p<0.001 when compared to Control
φ p<0.05, φφ p<0.01, φφφ 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 -51
Real-Time PCR amplification of Dopamine D_2 receptor mRNA from the Brainstem of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*p<0.05, *** p<0.001 when compared to Control
φφφ p<0.001when compared to Hx

Control rats - Con
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 -55
Real-Time PCR amplification of Dopamine D_2 receptor mRNA from the Brainstem of Control and Experimental Groups of Neonatal Rats

Animal Status	Log RQ
Con	0
Hx	-0.362 ± 0.022***
Hx+G	0.128 ± 0.015*φφφ
Hx+O	-0.599 ± 0.022***φφφ
Hx+G+O	0.155 ± 0.019*φφφ
Hx+G+E+O	-0.564 ± 0.067***φφφ

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*p<0.05, *** p<0.001 when compared to Control
φφφ p<0.001when compared to Hx

Control rats - Con
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 -52
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebral Cortex of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

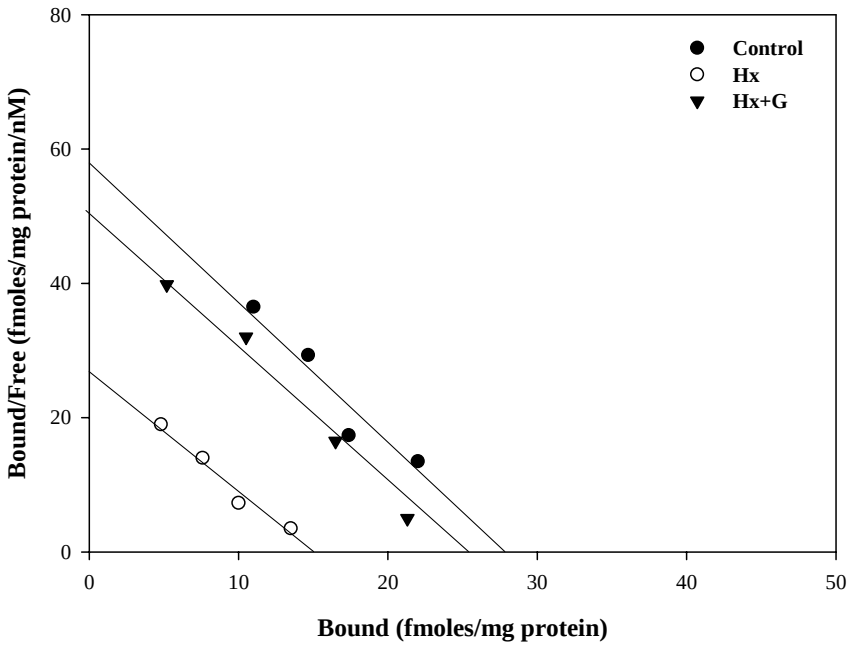


Table -56
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebral Cortex of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	27.82 ± 3.24	2.08 ± 0.03
Hx	15.02 ± 2.27 **	1.79 ± 0.02 *
Hx+G	25.49 ± 3.32 φφ	1.98 ± 0.01 φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
* $p<0.05$, ** $p<0.01$ compared to Control
φ $p<0.05$, φφ $p<0.01$ compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose treated - Hx+G

Figure -53
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebral Cortex of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

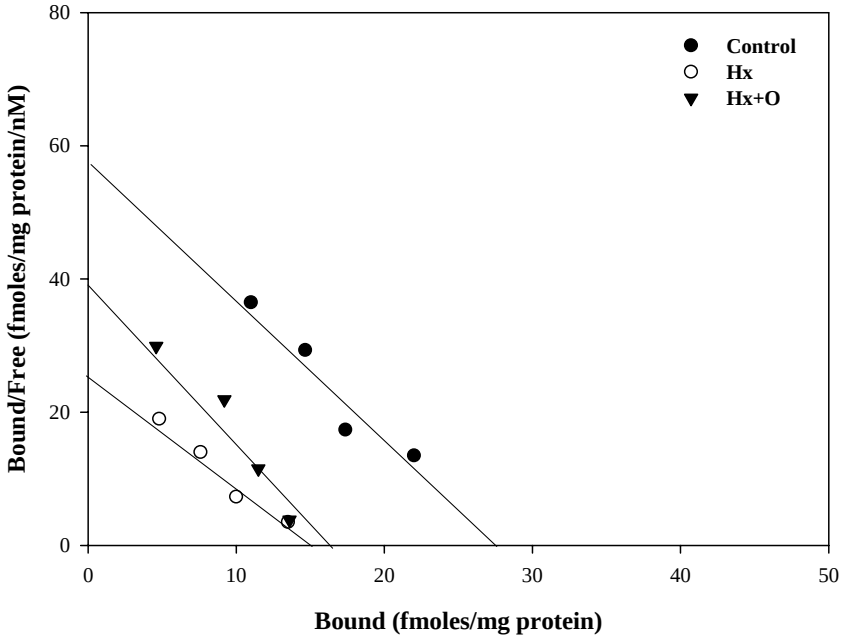


Table -57
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebral Cortex of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	27.82 ± 3.24	2.08 ± 0.03
Hx	15.02 ± 2.27 **	1.79 ± 0.02 *
Hx+O	16.39 ± 4.21 **	2.38 ± 0.07 *φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, **p<0.01compared to Control
φ p<0.05 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats oxygen treated - Hx+O

Figure -54
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebral Cortex of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

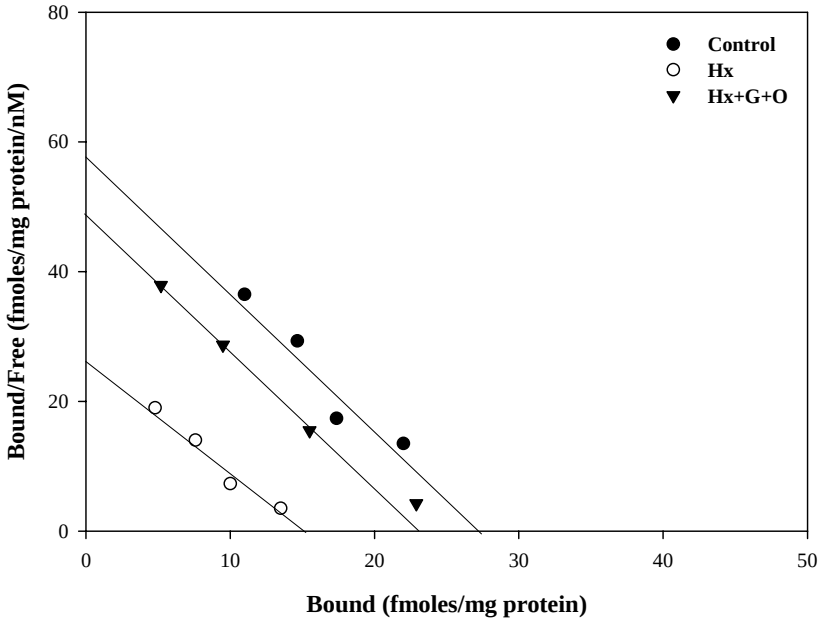


Table -58
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebral Cortex of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	27.82 ± 3.24	2.08 ± 0.03
Hx	15.02 ± 2.27 **	1.79 ± 0.02 *
Hx+G+O	23.06 ± 2.11 φφ	2.07 ± 0.05 φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, **p<0.01compared to Control
φ p<0.05, φφ p<0.01compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure -55
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebral Cortex of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

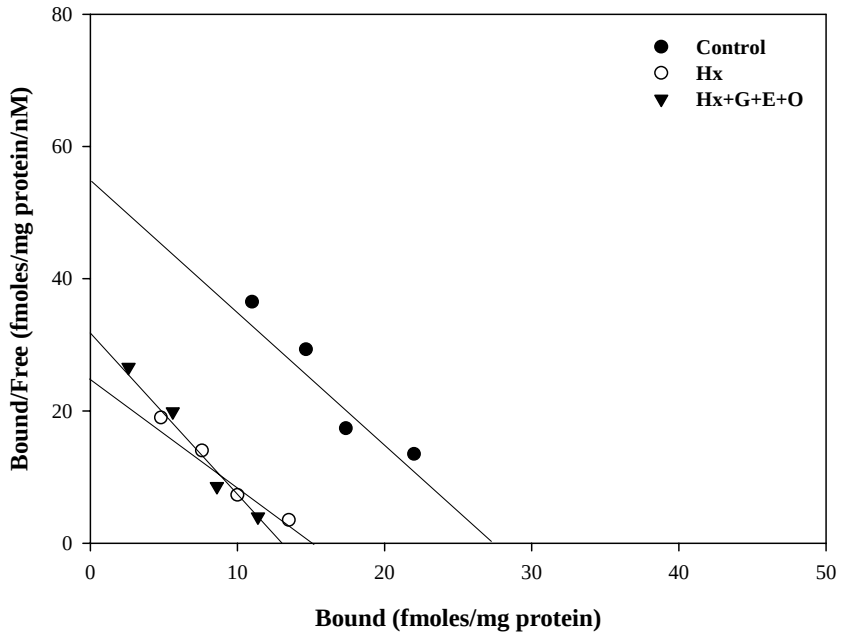
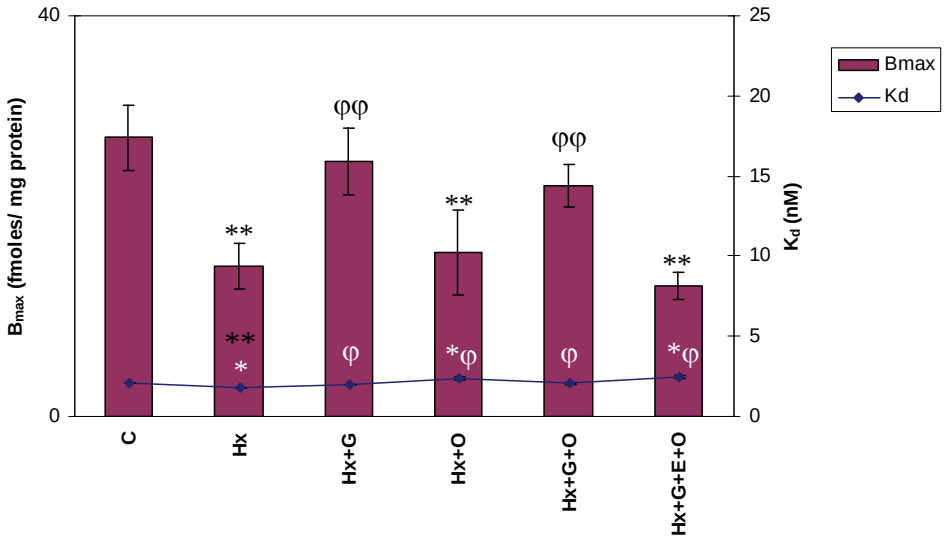


Table -59
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebral Cortex of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	27.82 ± 3.24	2.08 ± 0.03
Hx	15.02 ± 2.27 **	1.79 ± 0.02 *
Hx+G+E+O	13.01 ± 1.35 **	2.45 ± 0.06 *φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, **p<0.01compared to Control
φ p<0.05 compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure -56
Binding parameters of [^{3}H]MK-801 Binding Against MK-801 in the Cerebral Cortex of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, **p<0.01 when compared to Control
φ p<0.05, φφ 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

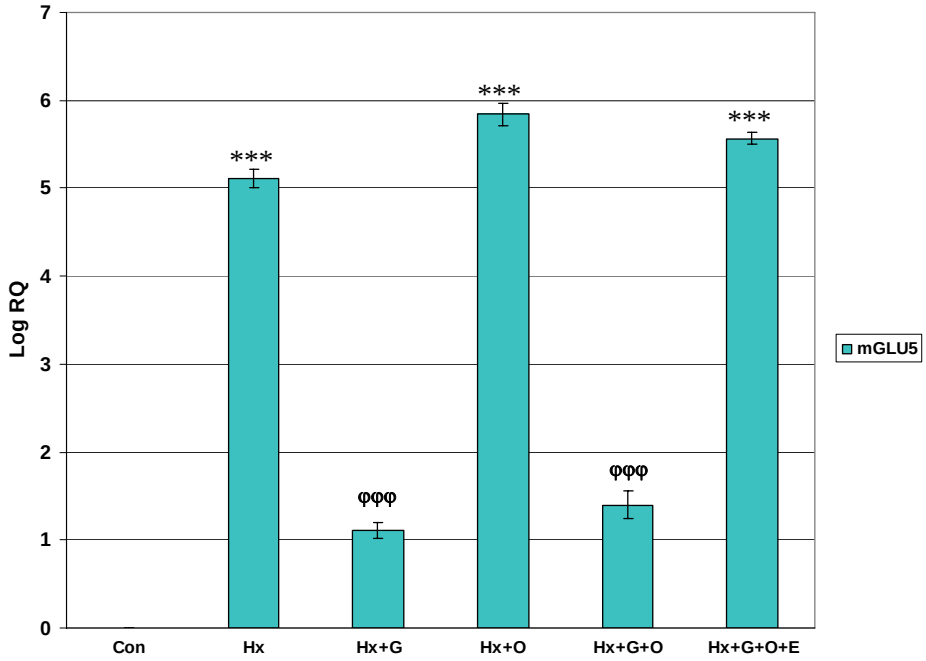
Table -60
Binding parameters of [^{3}H]MK-801 Binding Against MK-801 in the Cerebral Cortex of Control and Experimental Groups of Neonatal Rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
C	27.82 ± 3.24	2.08 ± 0.03
Hx	15.02 ± 2.27 **	1.79 ± 0.02 *
Hx+G	25.49 ± 3.32 φφ	1.98 ± 0.01 φ
Hx+O	16.39 ± 4.21 **	2.38 ± 0.07 *φ
Hx+G+O	23.06 ± 2.11 φφ	2.07 ± 0.05 φ
Hx+G+E+O	13.01 ± 1.35 **	2.45 ± 0.06 *φ

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*$p<0.05$, **$p<0.01$ when compared to Control
φ $p<0.05$, φφ $p<0.01$ 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 –57
Real-time PCR amplification of Metabotropic Glutamate 5 (mGLU5) receptor mRNA from the cerebral cortex of control and experimental neonatal rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ when compared to Hx

Control rats - Con
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 –61

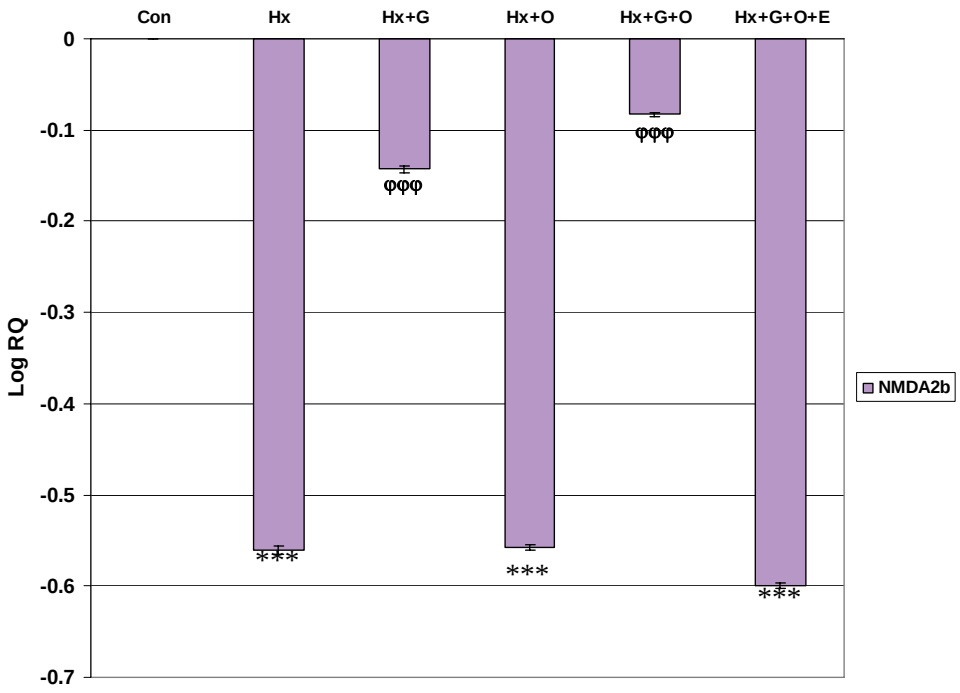
Real-time PCR amplification of Metabotropic Glutamate 5 (mGLU5) receptor mRNA from the cerebral cortex of control and experimental neonatal rats

Animal Status	Log RQ
Con	0
Hx	5.109 ± 0.108***
Hx+G	1.105 ± 0.085φφφ
Hx+O	5.843 ± 0.127***
Hx+G+O	1.400 ± 0.152φφφ
Hx+G+E+O	5.565 ± 0.065***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ when compared to Hx

Control rats - Con
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 –58
Real-time PCR amplification of NMDA 2b receptor mRNA from the cerebral cortex of control and experimental neonatal rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** p<0.001 when compared to Control
φφφ p<0.001 when compared to Hx

Control rats - Con
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 –62

Real-time PCR amplification of NMDA 2b receptor mRNA from the cerebral cortex of control and experimental neonatal rats

Animal Status	Log RQ
Con	0
Hx	-0.560 ± 0.004 ***
Hx+G	-0.143 ± 0.005 φφφ
Hx+O	-0.558 ± 0.003 ***
Hx+G+O	-0.083 ± 0.002 φφφ
Hx+G+E+O	-0.683 ± 0.003 ***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ when compared to Hx

Control rats - Con
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 -59
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Brainstem of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

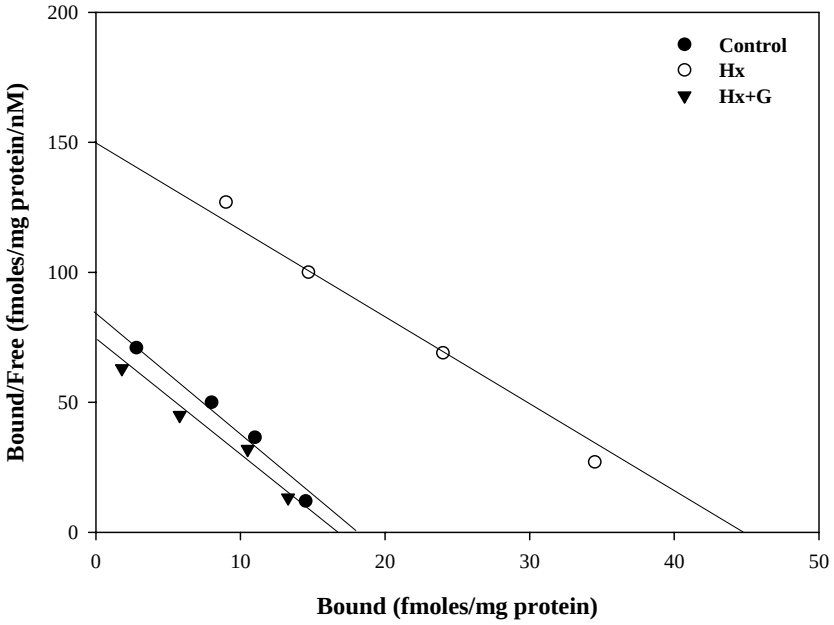


Table -63
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Brainstem of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	18.18 ± 2.73	4.64 ± 0.04
Hx	44.79 ± 5.22 ***	3.35 ± 0.02 *
Hx+G	16.81 ± 4.16 φφφ	4.38 ± 0.03 φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, ***p<0.001compared to Control
φ p<0.05, φφφ p<0.001compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose treated - Hx+G

Figure -60
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Brainstem of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

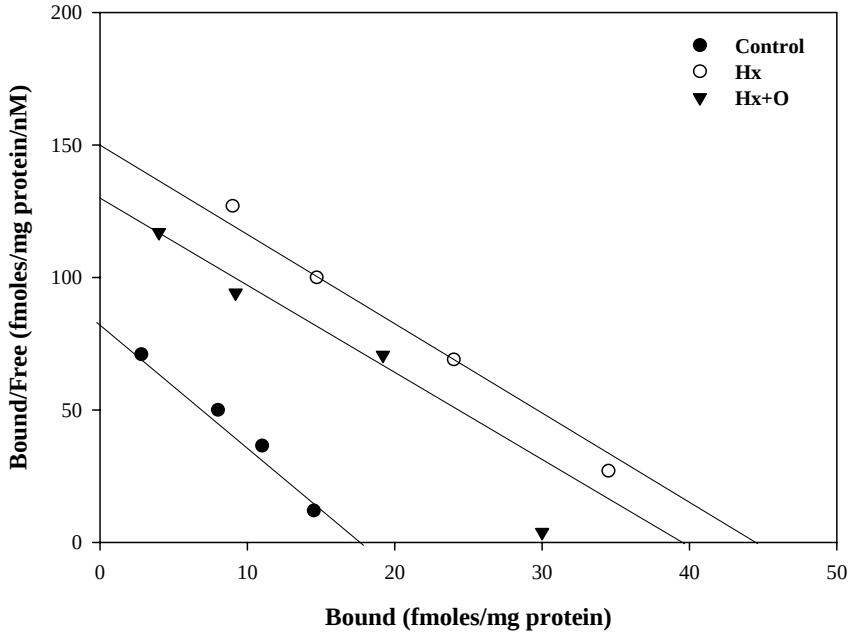


Table -64
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Brainstem of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	18.18 ± 2.73	4.64 ± 0.04
Hx	44.79 ± 5.22 ***	3.35 ± 0.02 *
Hx+O	39.67 ± 3.64 ***	3.28 ± 0.05 *

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
* $p<0.05$, *** $p<0.001$ compared to Control
Control rats – C; Hypoxic rats – Hx; Hypoxic rats oxygen treated - Hx+O

Figure -61
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Brainstem of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

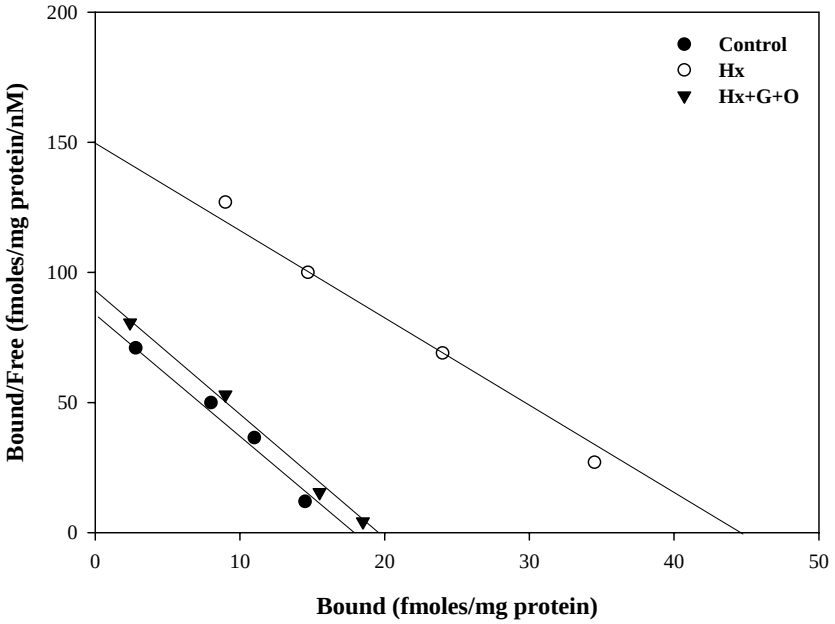


Table -65
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Brainstem of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	18.18 ± 2.73	4.64 ± 0.04
Hx	44.79 ± 5.22 ***	3.35 ± 0.02 *
Hx+G+O	19.68 ± 3.58 φφφ	4.70 ± 0.04 φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, ***p<0.001compared to Control
φφφ p<0.001compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure -62
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Brainstem of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

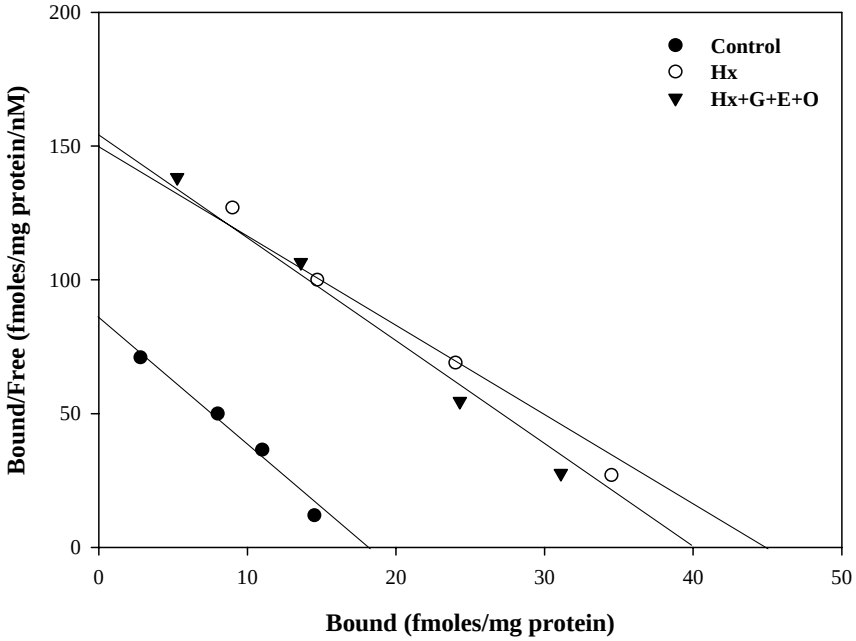
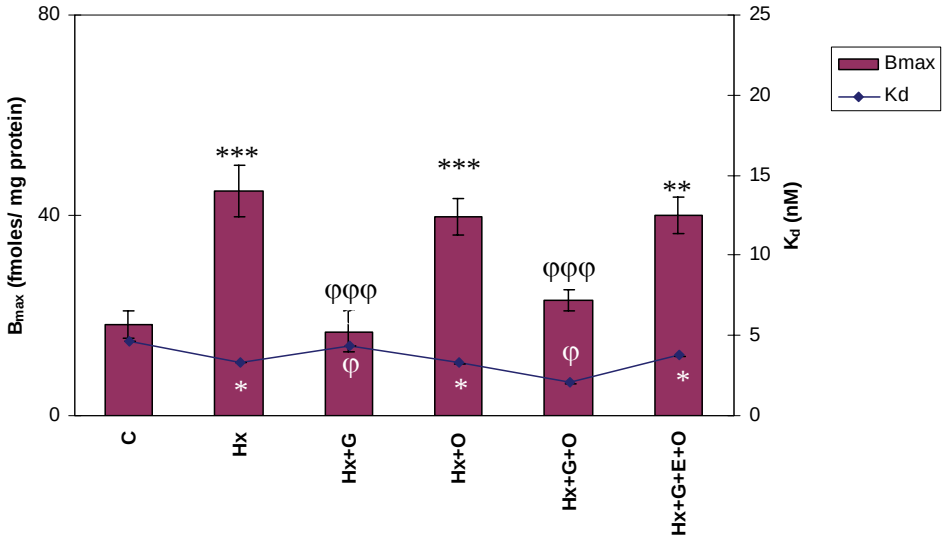


Table -66
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Brainstem of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	18.18 ± 2.73	4.64 ± 0.04
Hx	44.79 ± 5.22 ***	3.35 ± 0.02 *
Hx+G+E+O	39.89 ± 3.61 ***	3.76 ± 0.03 *

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
*p<0.05, ***p<0.001compared to Control
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure -63
Binding parameters of [^{3}H]MK-801 Binding Against MK-801 in the Brainstem of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, **p<0.01, ***p<0.001 when compared to Control
φ p<0.05, φφ p<0.01, φφφ p<0.001when 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

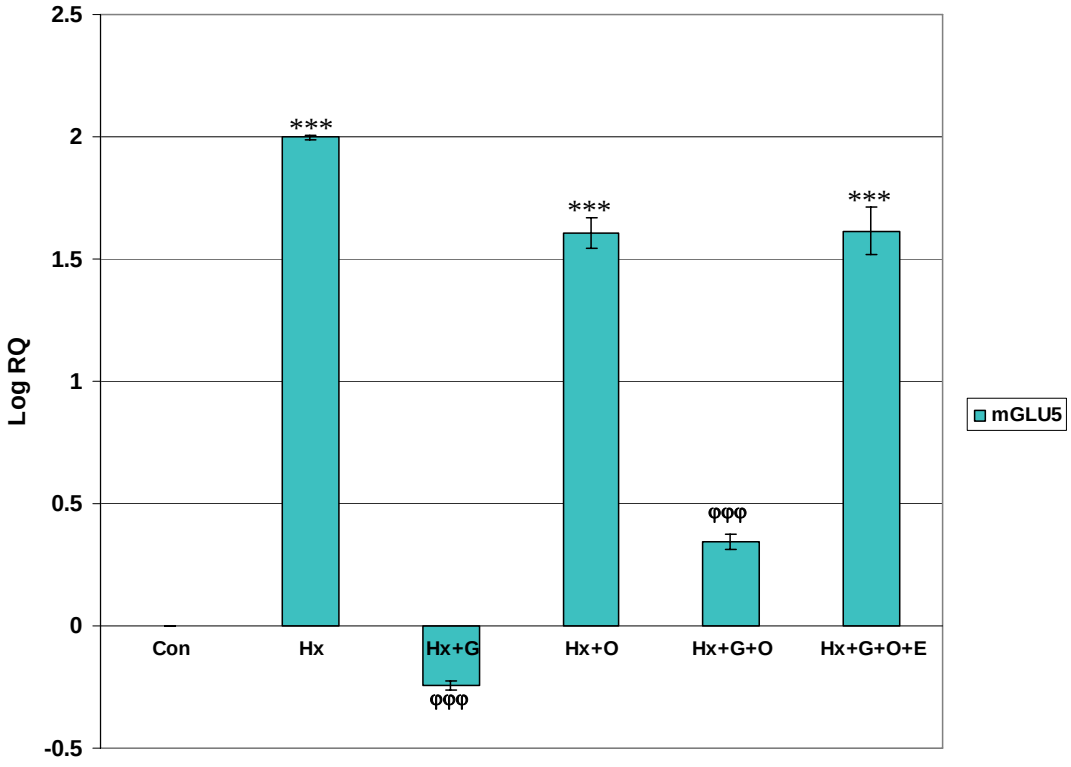
Table -67
Binding parameters of [[^{3}H]MK-801 Binding Against MK-801 in the Brainstem of Control and Experimental Groups of Neonatal Rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
C	18.18 ± 2.73	4.64 ± 0.04
Hx	44.79 ± 5.22 ***	3.35 ± 0.02 *
Hx+G	16.81 ± 4.16 φφφ	4.38 ± 0.03 φ
Hx+O	39.67 ± 3.64 ***	3.28 ± 0.05 *
Hx+G+O	19.68 ± 3.58 φφφ	4.70 ± 0.04 φ
Hx+G+E+O	39.89 ± 3.61 ***	3.76 ± 0.03 *

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
*p<0.05, **p<0.05, ***p<0.001 when compared to Control
φ p<0.05, φφ p<0.01, φφφ p<0.001when 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 –64
Real-time PCR amplification of Metabotropic Glutamate 5 (mGLU5) receptor mRNA from the brainstem of control and experimental neonatal rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ when compared to Hx

Control rats - Con
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 –68

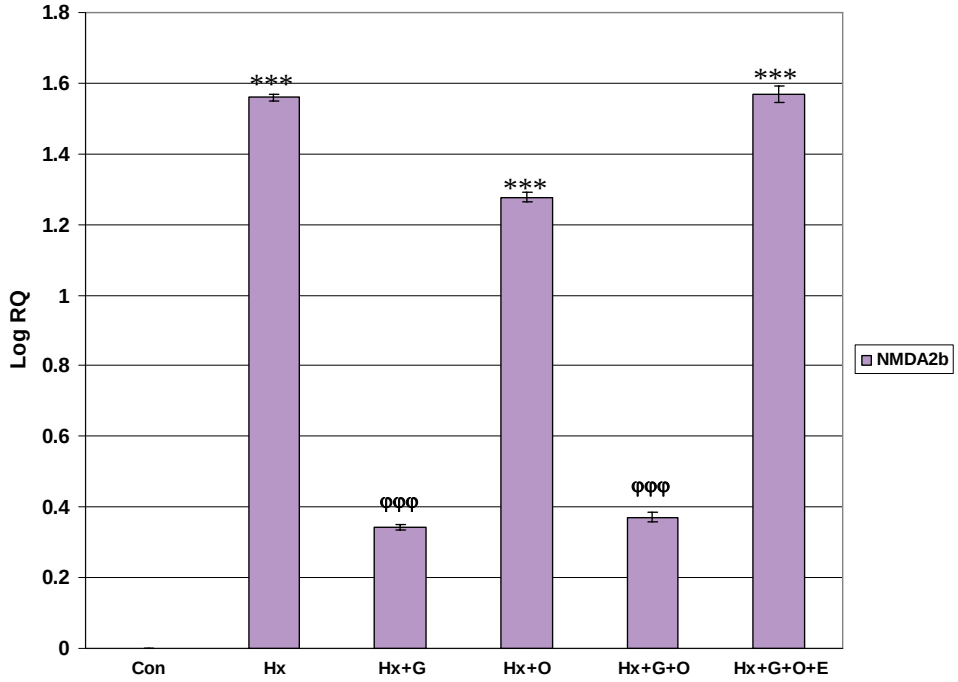
Real-time PCR amplification of Metabotropic Glutamate 5 (mGLU5) receptor mRNA from the brainstem of control and experimental neonatal rats

Animal Status	Log RQ
Con	0
Hx	1.997 ± 0.103***
Hx+G	-0.243 ± 0.017 φφφ
Hx+O	1.608 ± 0.062 ***
Hx+G+O	0.343 ± 0.029 φφφ
Hx+G+E+O	1.615 ± 0.098 ***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ when compared to Hx

Control rats - Con
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 –65
Real-time PCR amplification of NMDA 2b receptor mRNA from the brainstem of control and experimental neonatal rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ when compared to Hx

Control rats - Con
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 –69

Real-time PCR amplification of NMDA 2b receptor mRNA from the brainstem of control and experimental neonatal rats

Animal Status	Log RQ
Con	0
Hx	1.527 ± 0.008 ***
Hx+G	0.342 ± 0.006 φφφ
Hx+O	1.277 ± 0.013 ***
Hx+G+O	0.372 ± 0.014 φφφ
Hx+G+E+O	1.568 ± 0.023 ***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ when compared to Hx

Control rats - Con
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 -66
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebellum of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

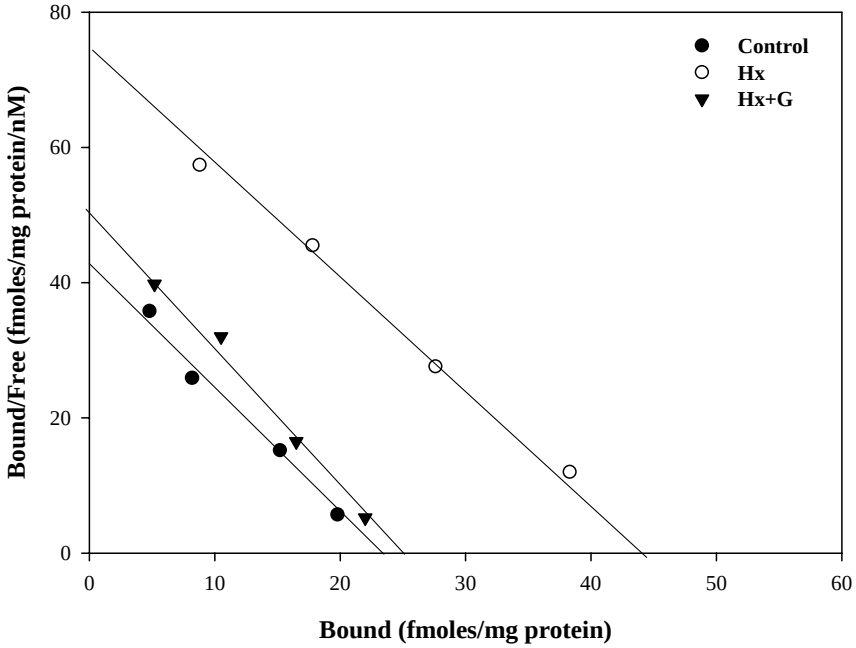


Table -70
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebellum of Control, Hypoxic and Glucose Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	23.48 ± 1.18	1.83 ± 0.03
Hx	44.18 ± 3.17 ***	1.69 ± 0.07
Hx+G	25.02 ± 2.28 φφφ	1.92 ± 0.05 φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
***p<0.001compared to Control
φ p<0.05, φφφ p<0.001compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose treated - Hx+G

Figure -67
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebellum of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

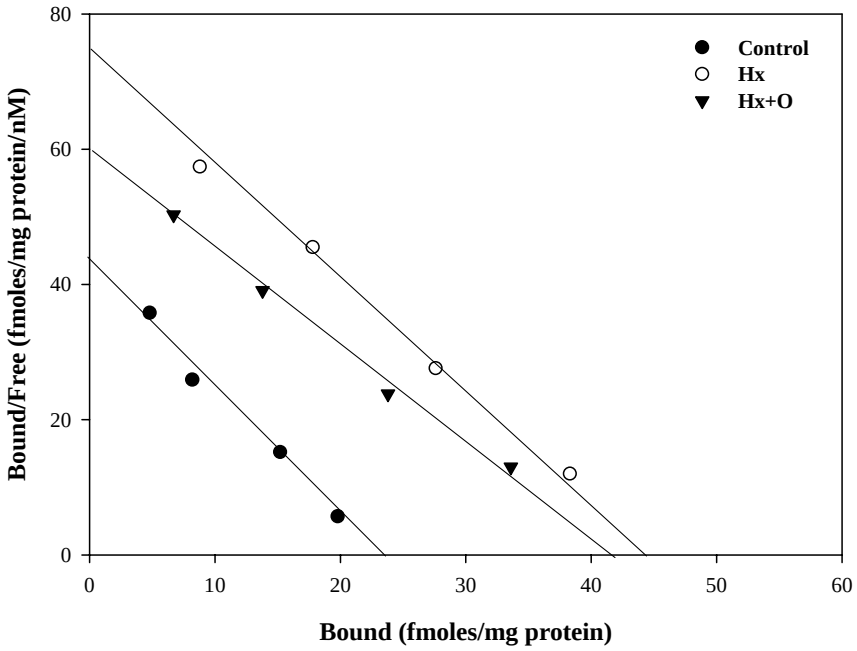


Table -71
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebellum of Control, Hypoxic and Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	23.48 ± 1.18	1.83 ± 0.03
Hx	44.18 ± 3.17 ***	1.69 ± 0.07
Hx+O	41.89 ± 5.72 ***	1.43 ± 0.02 *

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
* $p<0.05$, *** $p<0.001$ compared to Control
Control rats – C; Hypoxic rats – Hx; Hypoxic rats oxygen treated - Hx+O

Figure -68
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebellum of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

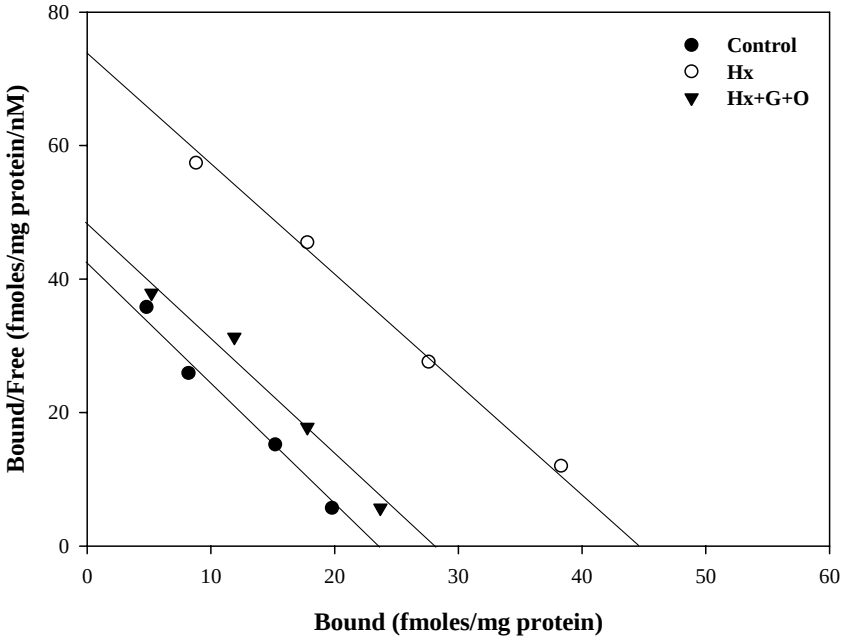


Table -72
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebellum of Control, Hypoxic and Glucose + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	23.48 ± 1.18	1.83 ± 0.03
Hx	44.18 ± 3.17 ***	1.69 ± 0.07
Hx+G+O	25.63 ± 4.61 φφφ	1.88 ± 0.03 φ

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
***p<0.001compared to Control
φ p<0.05, φφφ p<0.001compared to Hx
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose and oxygen treated - Hx+G+O

Figure -69
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebellum of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

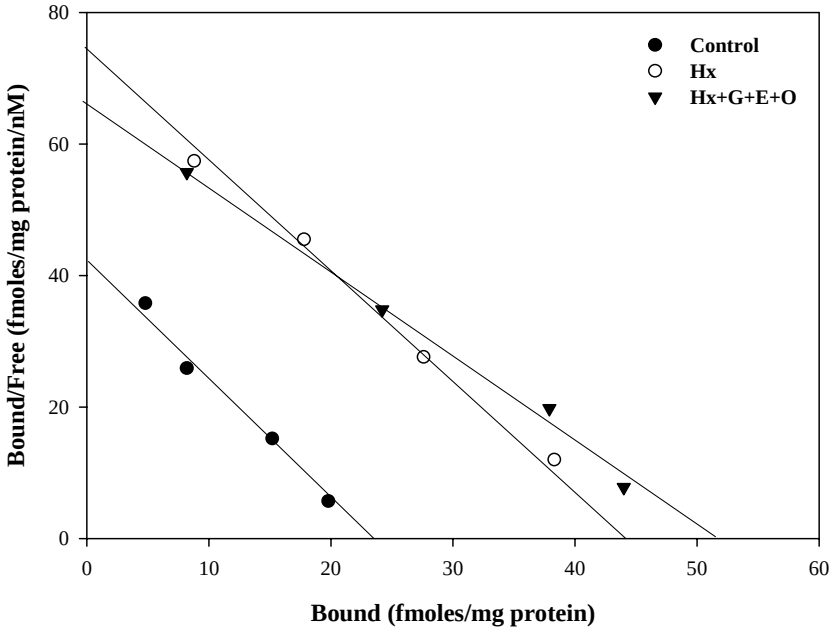
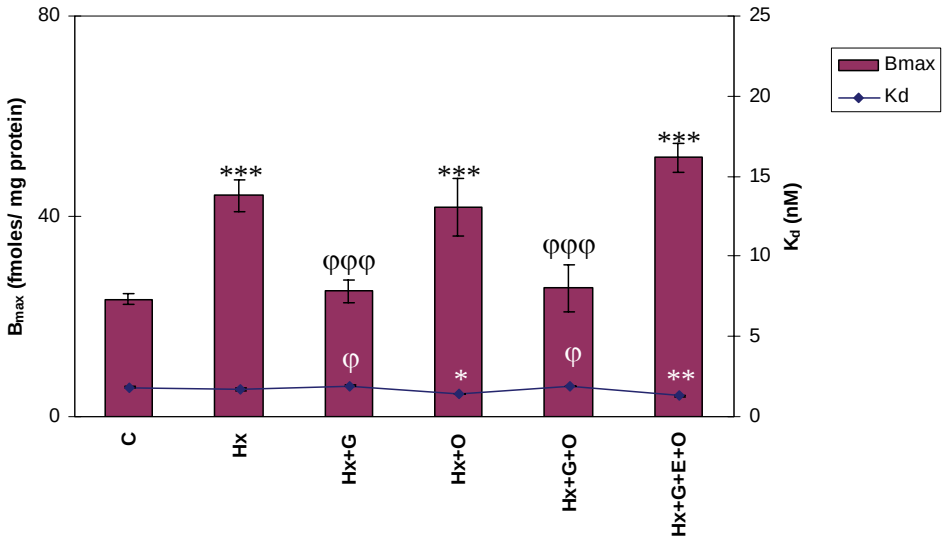


Table -73
Scatchard Analysis of [^{3}H]MK-801 Binding Against MK-801 in the Cerebellum of Control, Hypoxic and Glucose + Epinephrine + Oxygen Treated Hypoxic Neonatal Rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
C	23.48 ± 1.18	1.83 ± 0.03
Hx	44.18 ± 3.17 ***	1.69 ± 0.07
Hx+G+E+O	51.68 ± 2.84 ***	1.28 ± 0.06 **

Values are Mean±S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} – Maximal binding; K_d – Dissociation constant
**p<0.01, ***p<0.001compared to Control
Control rats – C; Hypoxic rats – Hx; Hypoxic rats glucose, epinephrine and oxygen treated - Hx+G+E+O

Figure -70
Binding parameters of [^{3}H]MK-801 Binding Against MK-801 in the Cerebellum of Control and Experimental Groups of Neonatal Rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared to Control
φ $p<0.05$, φφφ $p<0.001$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

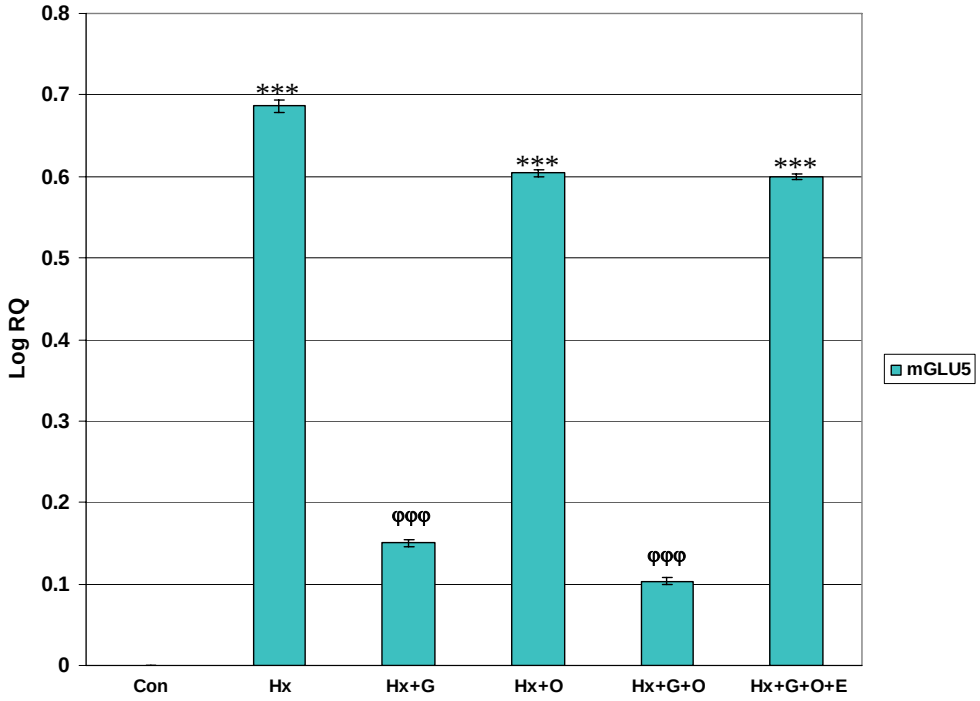
Table -74
Binding parameters of [[^{3}H]MK-801 Binding Against MK-801 in the Cerebellum of Control and Experimental Groups of Neonatal Rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
C	23.48 ± 1.18	1.83 ± 0.03
Hx	44.18 ± 3.17 ***	1.69 ± 0.07
Hx+G	25.02 ± 2.28 φφφ	1.92 ± 0.05 φ
Hx+O	41.89 ± 5.72 ***	1.43 ± 0.02 *
Hx+G+O	25.63 ± 4.61 φφφ	1.88 ± 0.03 φ
Hx+G+E+O	51.68 ± 2.84 ***	1.28 ± 0.06 **

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
B_{max} - Maximal binding; K_d - Dissociation constant
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared to Control
φ $p<0.05$, φφφ $p<0.001$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 –71
Real-time PCR amplification of Metabotropic Glutamate 5 (mGLU5) receptor mRNA from the cerebellum of control and experimental neonatal rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ when compared to Hx

Control rats - Con
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 –75

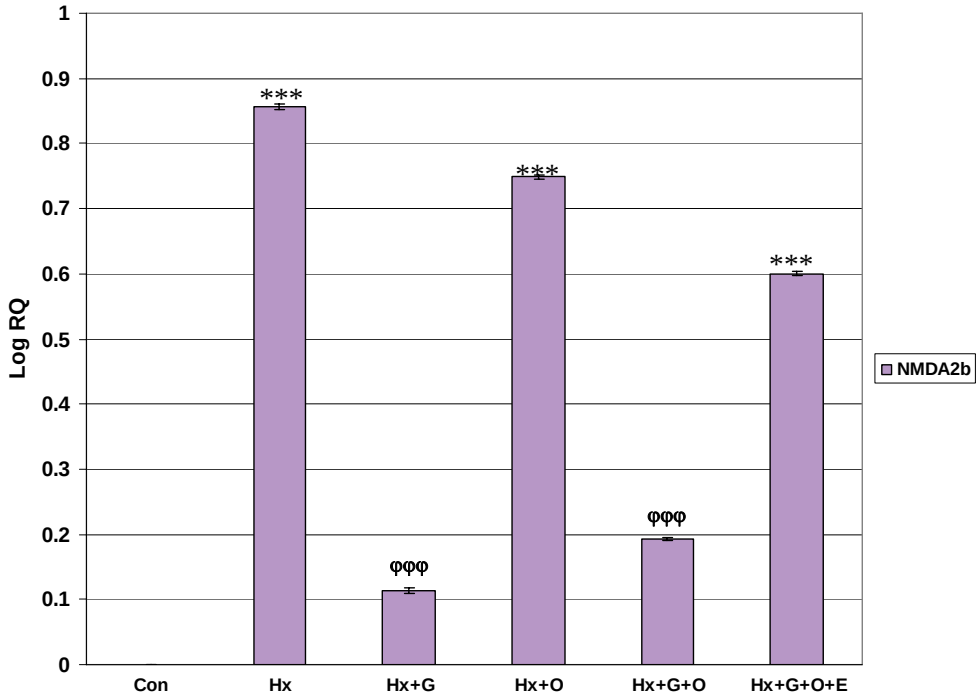
Real-time PCR amplification of Metabotropic Glutamate 5 (mGLU5) receptor mRNA from the cerebellum of control and experimental neonatal rats

Animal Status	Log RQ
Con	0
Hx	0.686 ± 0.008***
Hx+G	0.151 ± 0.004 φφφ
Hx+O	0.604 ± 0.004 ***
Hx+G+O	0.103 ± 0.004 φφφ
Hx+G+E+O	0.596 ± 0.003 ***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ when compared to Hx

Control rats - Con
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 –72
Real-time PCR amplification of NMDA 2b receptor mRNA from the cerebellum of control and experimental neonatal rats



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ when compared to Hx

Control rats - Con
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 –76

Real-time PCR amplification of NMDA 2b receptor mRNA from the cerebellum of control and experimental neonatal rats

Animal Status	Log RQ
Con	0
Hx	0.856 ± 0.004 ***
Hx+G	0.114 ± 0.006 φφφ
Hx+O	0.749 ± 0.003 ***
Hx+G+O	0.193 ± 0.002 φφφ
Hx+G+E+O	0.611 ± 0.008 ***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ when compared to Hx

Control rats - Con
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-73
cGMP Content in the Cerebral Cortex of Control and Experimental Groups of Neonatal Rats

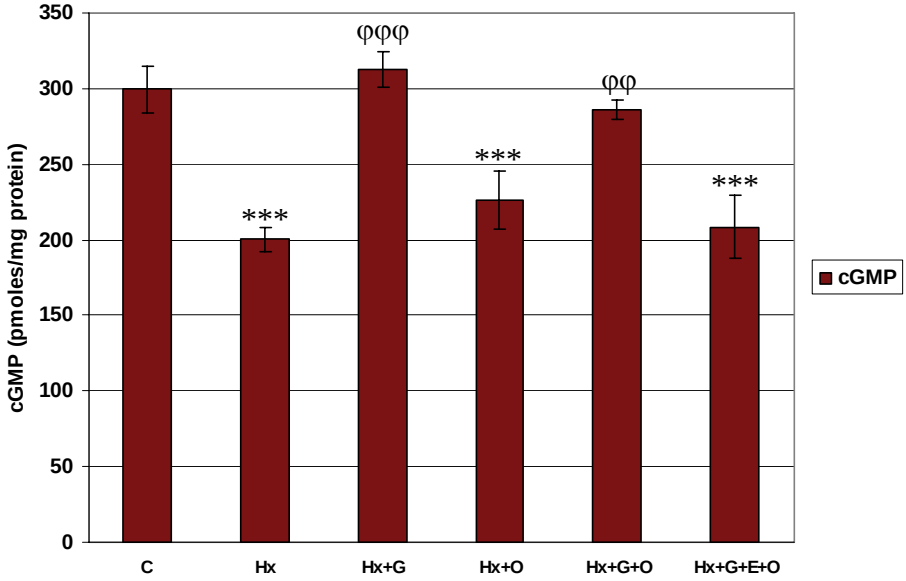


Table-77
cGMP Content in the Cerebral Cortex of Control and Experimental Groups of Neonatal Rats

Animal status	cGMP (pmoles/mg protein)
C	299.46 ± 15.62
Hx	200.08 ± 8.65***
Hx+G	312.89 ± 11.56φφφ
Hx+O	226.49 ± 19.33***
Hx+G+O	286.17 ± 6.57φφ
Hx+G+E+O	208.14 ± 20.86***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφ $p<0.01$, φφφ $p<0.001$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-74
cGMP Content in the Brainstem of Control and Experimental Groups of Neonatal Rats

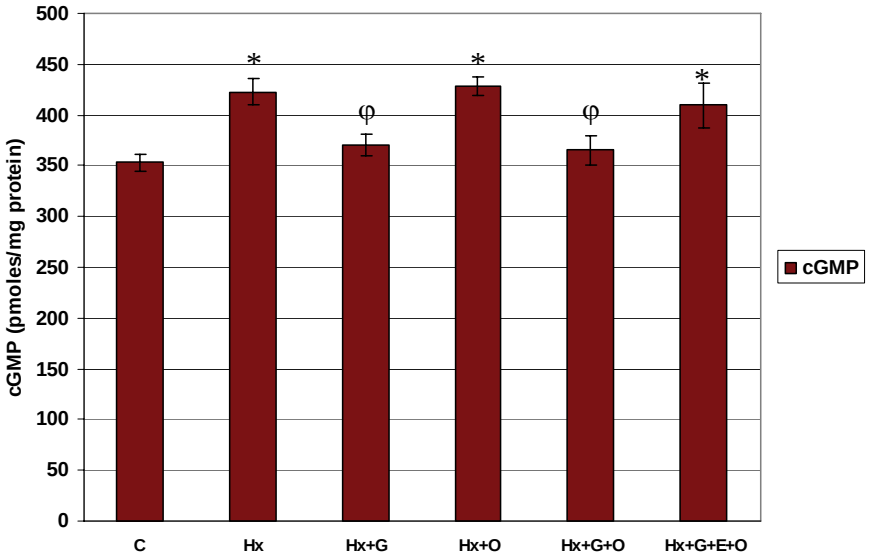


Table-78
cGMP Content in the Brainstem of Control and Experimental Groups of Neonatal Rats

Animal status	cGMP (pmoles/mg protein)
C	353.17 ± 8.26
Hx	423.00 ± 12.35 *
Hx+G	370.34 ± 11.24 φ
Hx+O	428.37 ± 9.55 *
Hx+G+O	365.34 ± 14.68 φ
Hx+G+E+O	409.57 ± 22.24 *

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*$p<0.05$ when compared to Control
φ $p<0.05$ 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-75
cGMP Content in the Cerebellum of Control and Experimental Groups of Neonatal Rats

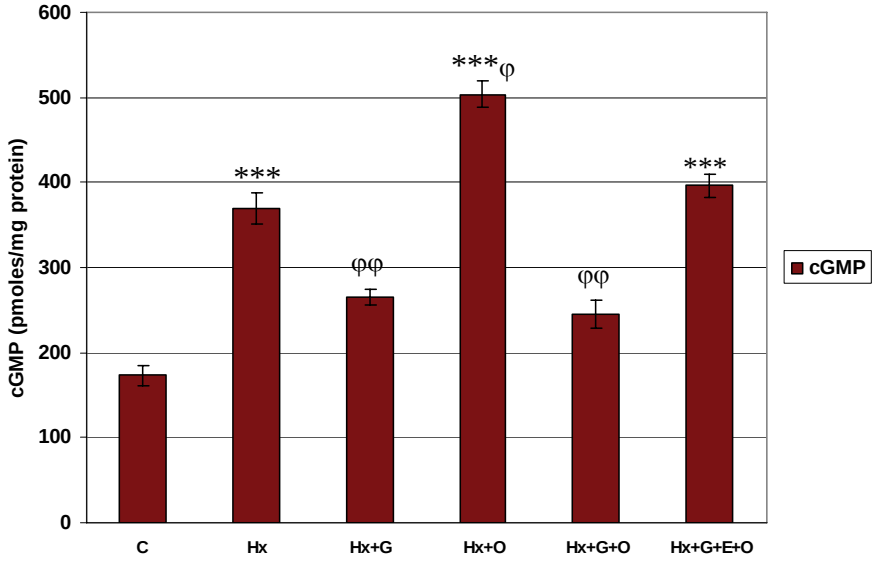


Table-79
cGMP Content in the Cerebellum of Control and Experimental Groups of Neonatal Rats

Animal status	cGMP (pmoles/mg protein)
C	173.22 ± 11.35
Hx	369.28 ± 18.65 ***
Hx+G	265.54 ± 9.65 φφ
Hx+O	503.57 ± 15.54 ***φ
Hx+G+O	245.74 ± 16.45 φφ
Hx+G+E+O	396.14 ± 13.22 ***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φ $p<0.05$, φφ $p<0.01$ 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-76
cAMP content in the Brainstem of Control and Experimental Groups of Neonatal Rats

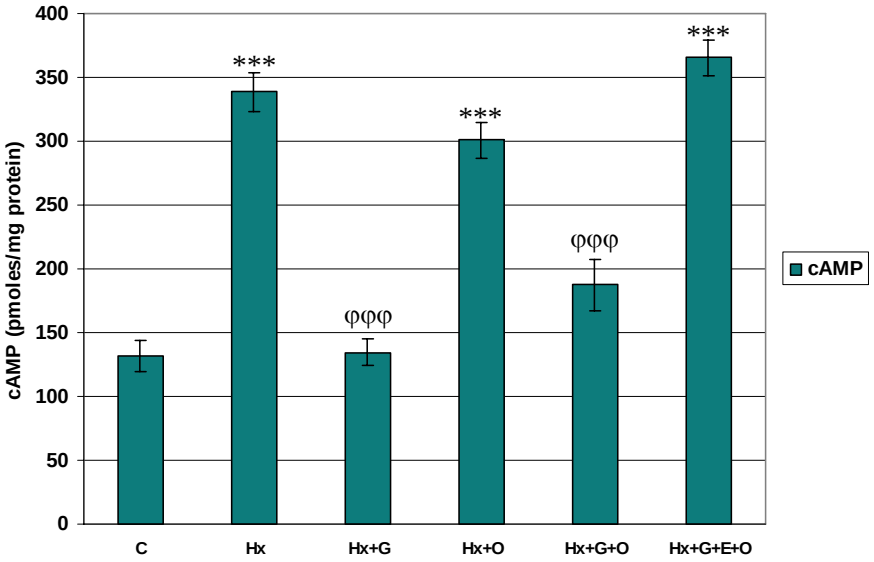


Table-80
cAMP Content in the Brainstem of Control and Experimental Groups of Neonatal Rats

Animal status	cAMP (pmoles/mg protein)
C	131.62 ± 12.55
Hx	338.43 ± 15.24 ***
Hx+G	134.28 ± 10.35 φφφ
Hx+O	300.81 ± 14.17 ***
Hx+G+O	187.37 ± 19.87 φφφ
Hx+G+E+O	365.25 ± 13.75 ***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$ when compared to Control
φφφ $p<0.001$ 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-77
cAMP Content in the Cerebellum of Control and Experimental Groups of Neonatal Rats

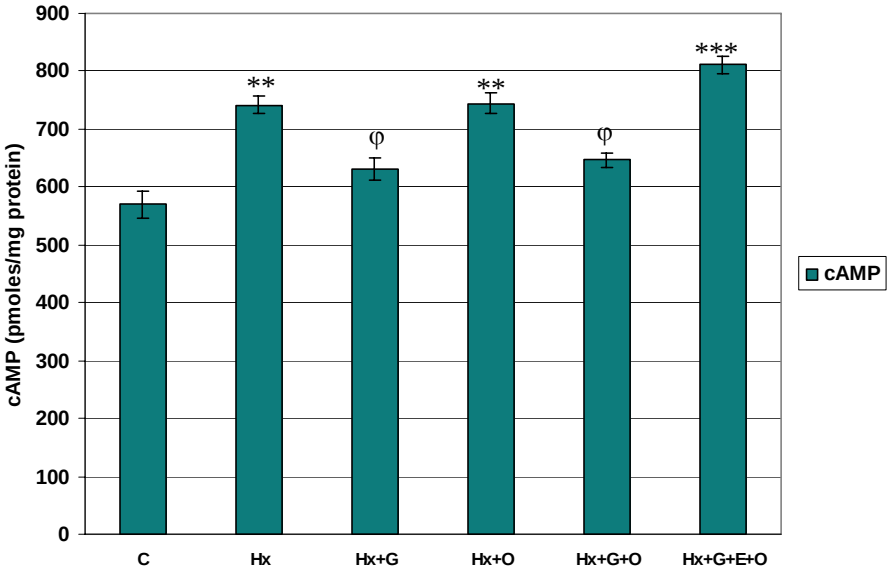


Table-81
cAMP Content in the Cerebellum of Control and Experimental Groups of Neonatal Rats

Animal status	cAMP (pmoles/mg protein)
C	569.37 ± 22.45
Hx	741.25 ± 15.28 **
Hx+G	631.14 ± 20.52 φ
Hx+O	743.94 ± 17.77 **
Hx+G+O	647.25 ± 12.43 φ
Hx+G+E+O	811.08 ± 15.26 ***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
** $p<0.01$, *** $p<0.001$when compared to Control
φ $p<0.05$ 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-78
IP3 Content in the Cerebral Cortex of Control and Experimental Groups of Neonatal Rats

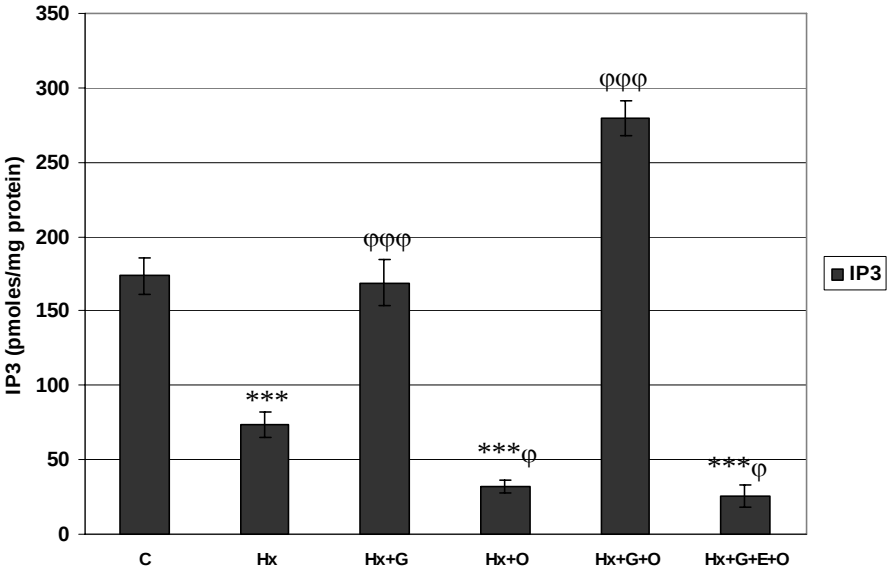


Table-82
IP3 Content in the Cerebral Cortex of Control and Experimental Groups of Neonatal Rats

Animal status	cAMP (pmoles/mg protein)
C	173.75 ± 12.16
Hx	73.75 ± 8.26 ***
Hx+G	168.75 ± 15.37 φφφ
Hx+O	32.08 ± 4.32 ***φ
Hx+G+O	280.00 ± 11.65 φφφ
Hx+G+E+O	25.42 ± 7.22 ***φ

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
*** $p<0.001$when compared to Control
φ $p<0.05$, φφφ $p<0.001$ 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-79
IP3 Content in the Brainstem of Control and Experimental Groups of Neonatal Rats

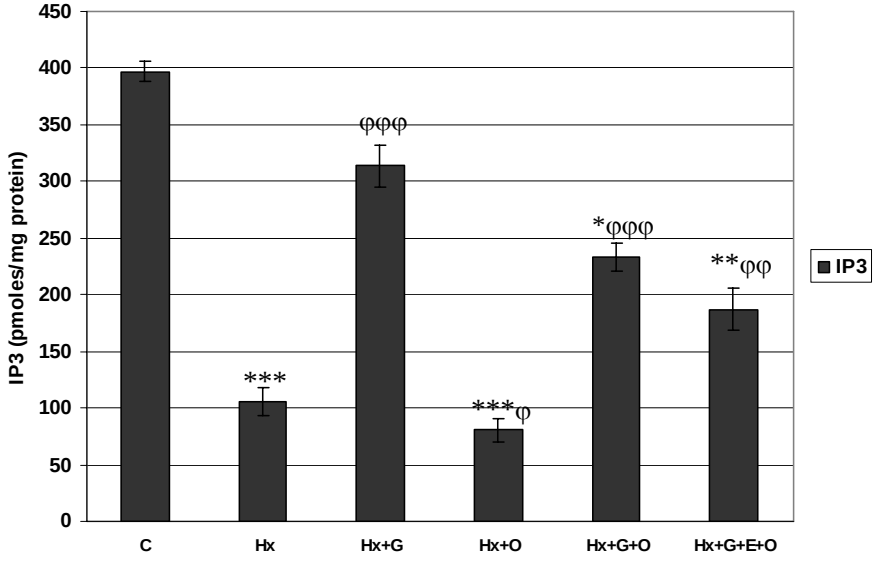


Table-83
IP3 Content in the Brainstem of Control and Experimental Groups of Neonatal Rats

Animal status	cAMP (pmoles/mg protein)
C	397.08 ± 8.37
Hx	105.41 ± 12.28 ***
Hx+G	313.75 ± 18.13 φφφ
Hx+O	80.41 ± 9.96 ***φ
Hx+G+O	280.00 ± 11.65 *φφφ
Hx+G+E+O	233.75 ± 12.25 **φφ

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
* p<0.05,** p<0.01, *** p<0.001when compared to Control
φ p<0.05, φφ p<0.01, φφφ p<0.001when 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-80
IP3 Content in the Cerebellum of Control and Experimental Groups of Neonatal Rats

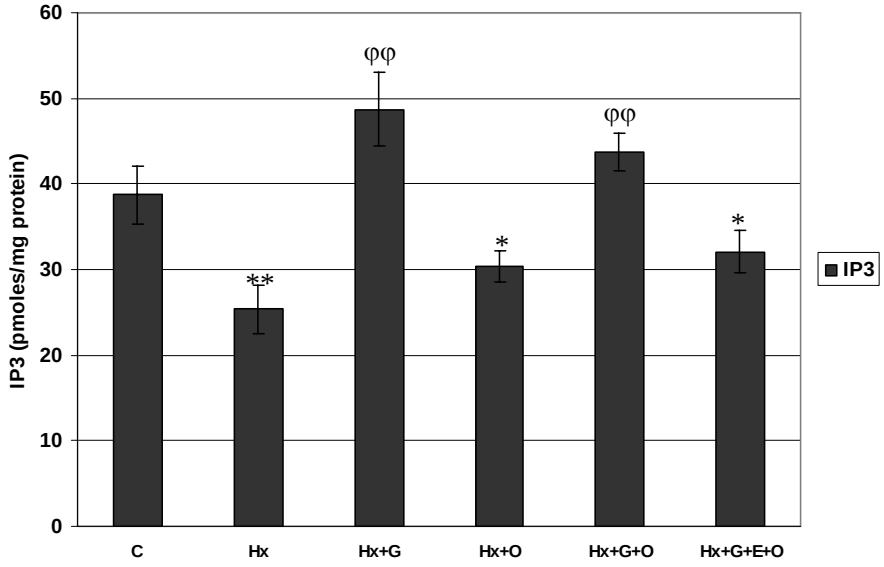


Table-84
IP3 Content in the Cerebellum of Control and Experimental Groups of Neonatal Rats

Animal status	cAMP (pmoles/mg protein)
C	38.75 ± 3.37
Hx	25.40 ± 2.81 **
Hx+G	48.75 ± 4.27 φφ
Hx+O	30.41 ± 1.83 *
Hx+G+O	43.75 ± 2.15 φφ
Hx+G+E+O	32.08 ± 2.52 *

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
* $p<0.05$,** $p<0.01$when compared to Control
φφ $p<0.01$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

Table-85
Body Weight of Experimental Animals (1 month) used for Behavioural Study

Animal status	Body weight (g)
C	75.32 ± 0.35
Hx	65.53 ± 0.74 **
Hx+G	72.35 ± 0.57 φφ
Hx+O	68.22 ± 0.25 **
Hx+G+O	73.61 ± 0.94 φφ
Hx+G+E+O	60.24 ± 0.82 ***φ

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
**p<0.01, *** p<0.001 when compared to Control
φ p<0.05, φφ p<0.01when 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 - 81
Rotarod Performance of Control and Experimental Groups of Rats

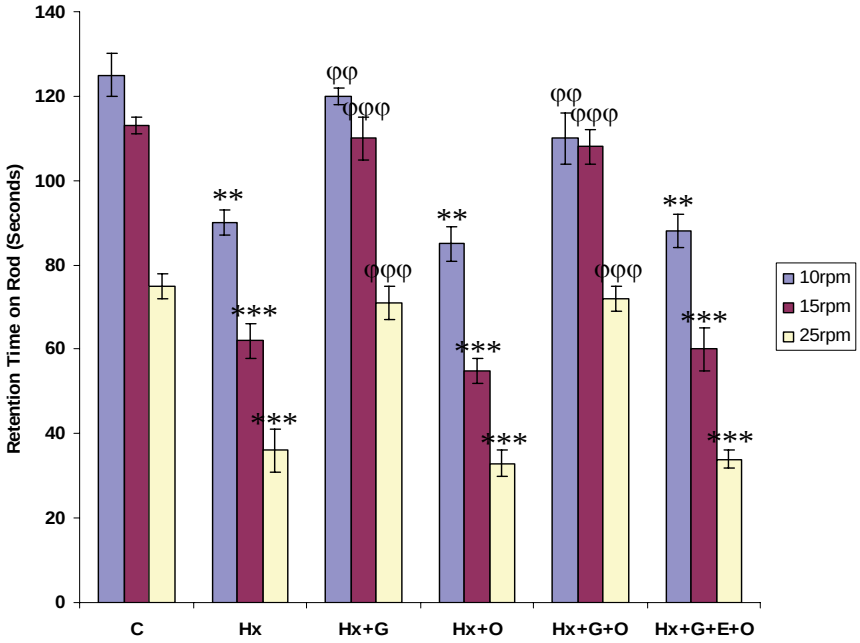
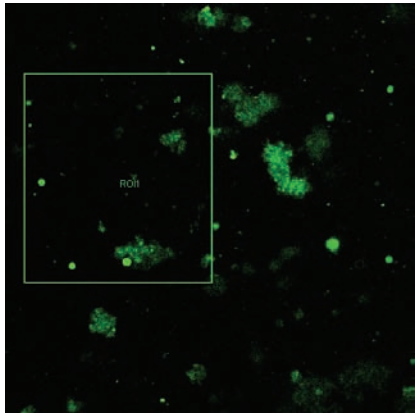


Table-86
Rotarod Performance of Control and Experimental Groups of Rats

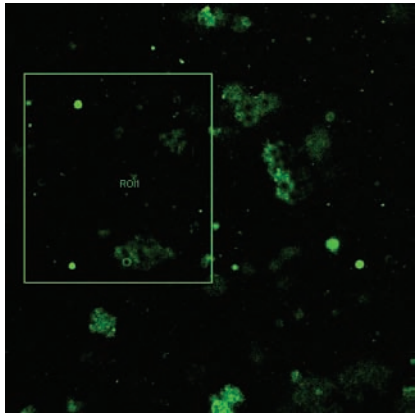
Experimental Groups	Retention Time on the Rod (in seconds)		
	10 rpm	15 rpm	25 rpm
C	125 ± 5	113±2	75±3
Hx	90 ± 3 **	62 ± 4 ***	36 ± 5 ***
Hx+G	120 ± 2 φφ	110 ± 5 φφφ	71 ± 4 φφφ
Hx+O	85 ± 4 **	55 ± 3 ***	33 ± 3 ***
Hx+G+O	110 ± 6 φφ	108 ± 4 φφφ	72 ± 3 φφφ
Hx+G+E+O	88 ± 4 **	60 ± 5 ***	34 ± 2 ***

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 pups.
**p<0.01, *** p<0.001 when compared to respective Control
φφ p<0.01, φφφ p<0.001when 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

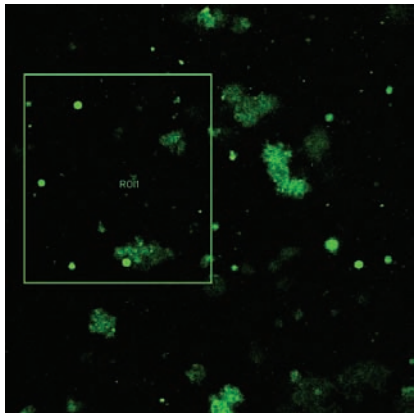
Figure - 82
Effect of Hypoxia and Glucose (4mM) on Ca^{2+} release from neonatal cortical cells *in vitro*



Fluorescence at zero time (T_0)



Fluorescence at 38 sec after hypoxia

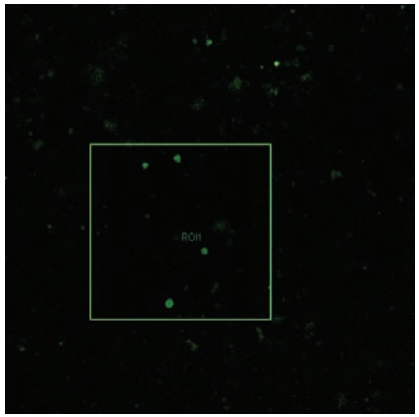


Fluorescence at 53 sec after Glucose supplementation

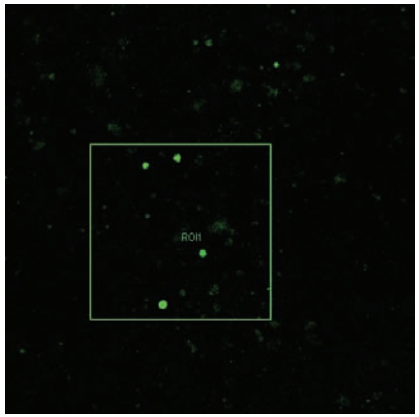
Table - 87
Effect of Hypoxia and Glucose (4mM) on Ca^{2+} release from neonatal cortical cells *in vitro*

Condition	Time in sec	Pixel intensity
-	0	231545
Hypoxia	38	187441
Glucose	53	193459

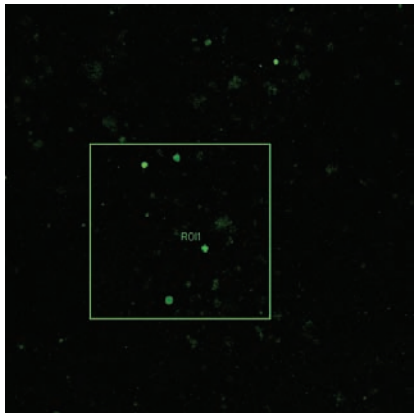
Figure – 83
Effect of DA (10^{-8}M) and Glutamate (10^{-6}M) on Ca^{2+} release from neonatal cortical cells *in vitro*



Fluorescence at zero time (T_0)



Fluorescence at 27 sec after addition of DA

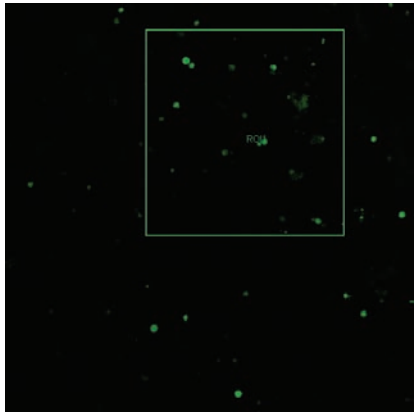


Fluorescence at 53 sec after addition of Glutamate

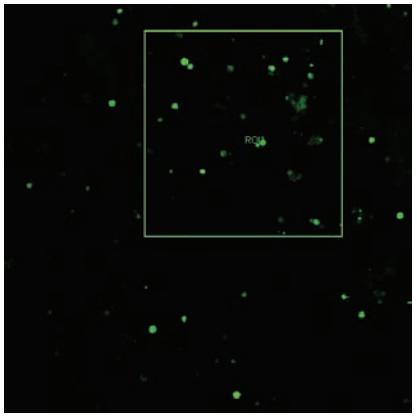
Table – 88
Effect of DA (10^{-8}M) and Glutamate (10^{-6}M) on Ca^{2+} release from neonatal cortical cells *in vitro*

Condition	Time in sec	Pixel intensity
-	0	143601
DA	27	181084
Glutamate	53	150626

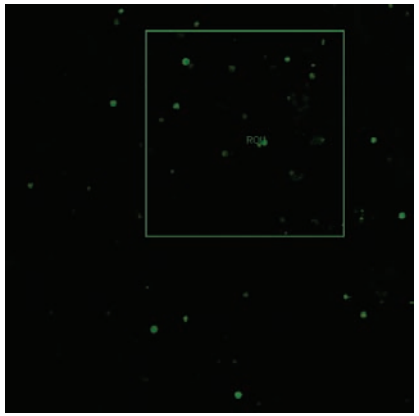
Figure – 84
Effect of DA (10^{-8}M) and Glutamate (10^{-6}M) on Ca^{2+} release from hypoxic neonatal cortical cells *in vitro*



Fluorescence at zero time (T_0)



Fluorescence at 42 sec after addition of DA

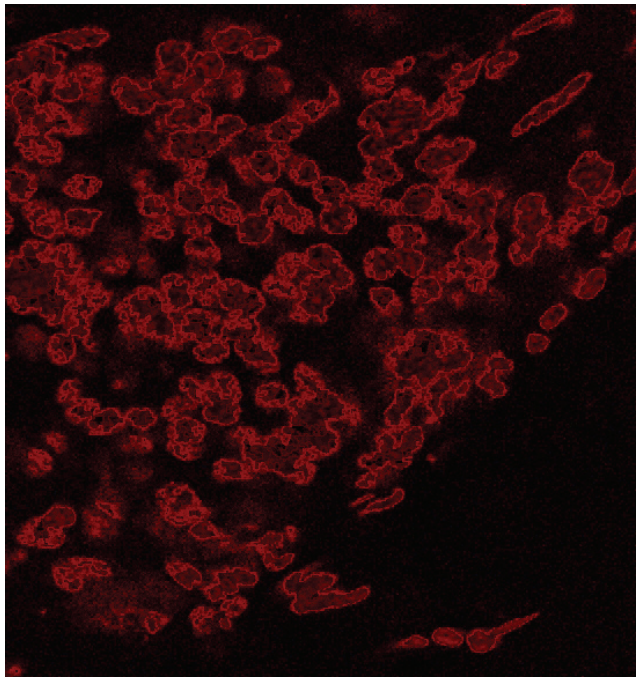


Fluorescence at 30 sec after addition of Glutamate

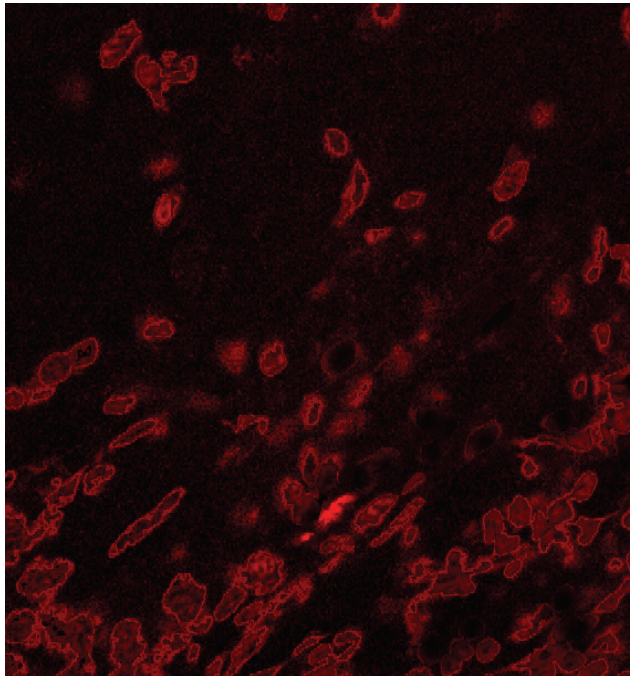
Table – 89
Effect of DA (10^{-8}M) and Glutamate (10^{-6}M) on Ca^{2+} release from hypoxic neonatal cortical cells *in vitro*

Condition	Time in sec	Pixel intensity
-	0	65724
DA	42	69472
Glutamate	30	62892

Figure – 85
Nuclear staining using TO-PRO-3 dye of neonatal brain



Control Brain



Hypoxic Brain