GLUTAMATE RECEPTOR GENE EXPRESSION: IP3, cAMP, cGMP FUNCTIONAL REGULATION IN HYPOGLYCAEMIC AND DIABETIC RATS

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Dedicated To My Beloved Parents…

DECLARATION

I hereby declare that the thesis entitled "**Glutamate Receptor Gene Expression: IP3, cAMP, cGMP Functional Regulation in Hypoglycaemic and Diabetic Rats"** 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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ABRREVIATIONS

5-HT	5-Hydroxy tryptamine
7-OH DPAT	7-Hydroxy-2 (di-n-popylamino)-tetralin
AC	Adenylate cyclase
ACh	Acetylcholine
AChR	Acetylcholine receptor
AChE	Acetylcholine esterase
AD	Alzheimers disease
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANS	Autonomic nervous system
ATP	Adenosine triphosphate
BBB	Blood brain barrier
B_{max}	Maximal binding
CA	Cornu Ammonis
CB	Cerebellum
CC	Cerebral cortex
cAMP	Cylic adenosine monophosphate
cAPK	cAMP-dependent protein kinase
cDNA	Complementary deoxy ribonucleic acid
cGMP	Cyclic guanosine monophosphate
CNS	Central Nervous System
CREB	cAMP regulatory element binding protein
CSF	Cerebrospinal fluid
CT	Crossing threshold
DA	Dopamine
DAG	Diacylglycerol
DBH	Dopamine β hydroxylase
DEPC	Di ethyl pyro carbonate
DNA	Deoxy ribonucleic acid

EAA	Excitatory amino acids
EDTA	Ethylene diamine tetra acetic acid
EEG	Electroencephalogram
EPI	Epinephrine
ER	Endoplasmic reticulum
EPSCs	Excitatory postsynaptic current
FCS	Fetal calf serum
GABA	Gamma amino butyric acid
GAD	Glutamic acid decarboxylase
GDH	Glutamate dehydrogenase
GFP	Green fluorescent protein
GLAST	Glutamate/aspartate transporter
GLT	Glutamate transporter
GLUR2	Glutamate Receptor-2
GOD	Glucose oxidase
GPCR	G-protein-coupled receptors
Gq PRC	Gq Protein coupled receptors
GTP	Guanosine triphosphate
HBSS	Hang’s balanced salt solution
IDDM	Insulin dependent diabetes mellitus
IIH	Insulin induced hypoglycaemia
iGluRs	Ionotropic glutamate receptors
IPI	Initial Precipitating Injury
IP3	Inositol 1,4,5-triphosphate
KA	Kainate
K_d	Dissociation constant
LTD	Long term depression
LTP	Long term potentiation
MCI	Mild cognitive impairment

mGLU	Metabotropic Glutamate
(+) MK-801	(+)5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate
MRI	Magnetic resonance imaging
mRNA	Messenger Ribonucleic acid
NADH	Nicotinamide adenine dinucleotide, reduced form
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
NE	Norepinephrine
NGF	Nerve growth factor
NIDDM	Non-insulin dependent diabetes mellitus
NMDA	N-methyl-D-aspartate
nNOS	Neuronal nitric oxide synthase
NOS	Nitric-oxide synthase
NPY	Neuropeptide Y
NSB	Non specific binding
O.D.	Optical density
p	Level of significance
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline Triton X- 100
PCP	Phencyclidine
pCREB	Phosphorylated cAMP regulatory element binding protein
PEG	Poly ethylene glycol
PFC	Prefrontal cortex
Pi	Inorganic phosphate
PIP2	Phospatidyl 4,5-bisphosphate
PKC	Protein kinase C
PKG	Protein kinase G
PLC	Phospolipase C

POD	Peroxidase
RIA	Radioimmunoassay
ROS	Reactive oxygen species
RT-PCR	Reverse-transcription-polymerase chain reaction
S.E.M	Standard error of mean
SMOCCs	Second messenger operated calcium channels
STZ	Streptozotocin
T3	Triiodothyronine
T4	Thyroxine
TCA	Tricarboxylic acid
TM	Transmembrane
TRH	Thyrotropin releasing hormone
TSH	Thyroid stimulating hormone
VICCs	Voltage insensitive calcium channels
VOCC	Voltage sensitive calcium channels

Introduction

Glucose is the primary source of fuel for the cells of the brain. Brain is dependent on a continuous supply of glucose diffusing from the blood into the interstitial tissue within the central nervous system and into neurons themselves. The physiological concentration of glucose in blood is maintained through highly regulated systemic mechanisms. Diabetes Mellitus is a chronic metabolic disorder resulting in hyperglycaemia - high plasma glucose level. Hypoglycaemia - low level of plasma glucose, is a relatively common episode primarily affecting diabetic patients receiving treatment with insulin or other hypoglycaemic drugs and patients suffering from insulinoma. Strict glycemic control with low target ranges invariably carries a risk of inadvertent hypoglycemic episodes. Several studies have nevertheless reported a potentially higher incidence of hypoglycemia in patients under strict glycemic control (Van den Berghe *et al.,* 2005; Krinsley & Grover, 2007; Thomas *et al.,* 2007). Hypoglycaemia impose alterations upon both the central (CNS) and peripheral (PNS) nervous systems. It is one of the most common and serious stress conditions challenging the body homeostasis. Hypoglycaemia can lead to brain damage and long-term cognitive impairment (Wieloch, 1985; Gazit *et al.*, 2003). Severe hypoglycaemia with cognitive dysfunction is three times more common in intensively, rather than conventionally, treated insulin-dependent diabetes mellitus (IDDM) (Maran *et al*., 1994). The hypoglycaemic counter regulatory mechanisms can be blunted irreversibly by disease duration or by acute episodes of prior stress (Ertl & Davis, 2004). Although hypoglycaemia is associated with a number of physiological changes, the most profound effects are seen in the brain, where glucose is the major substrate for energy metabolism. Lack of glucose produces brain damage or even

death if the deficit is prolonged. Children and adults exposed to hypoglycaemia can develop long-term impairment of cognitive function (Blattner, 1968; Hawdon, 1999; Karp, 1989; Ryan *et al.,* 1985; Vannucci & Vannucci, 2001) and are at risk of epilepsy (Kaufman, 1998). Hypoglycaemia can affect all aspects of life, including employment, driving, recreational activities involving exercise and travel and measures should be taken in all of these situations to avoid this potentially dangerous side-effect of insulin therapy (Frier, 2008).

Ageing is the biological process characterized by the progressive and irreversible loss of physiological function accompanied by increasing mortality with advancing age. It is a complex physiological phenomenon associated with a multitude of biological changes at the molecular level, which is eventually manifested at the tissue and organism level. Normal human ageing is associated with a progressive impairment of glucose tolerance. Total glucose stimulated insulin secretion has been described as being not changed, suppressed or increased as the animal ages. Neurotransmitters have been reported to show significant alterations during diabetes resulting in altered functions causing neuronal degeneration. Age related changes in the capacity of β-cell for proliferation affect the insulin production and contribute to a decrease in glucose tolerance with advance in age.

The CNS neurotransmitters play an important role in the regulation of glucose homeostasis. These neurotransmitters mediate rapid intracellular communications not only within the CNS but also in the peripheral tissues. They exert their function through receptors present in both neuronal and non-neuronal cell surface that trigger second messenger signaling pathways (Julius *et al.,* 1989). Neurotransmitters have been reported to show significant alterations during hyperglycaemia resulting in altered functions causing neuronal degeneration (Bhardwaj *et al.,* 1999). Chronic hyperglycaemia during diabetes mellitus is a major initiator of diabetic micro-vascular

complications like retinopathy, neuropathy and nephropathy (Sheetz & King, 2002). Dopaminergic dysfunction in hippocampus (Robinson *et al*., 2009) and glutamatergic dysfunction in cerebral cortex (Joseph *et al.,* 2008) and cerebellum (Joseph *et al.,* 2007) during hypoglycaemia and hyperglycaemia is suggested to contribute to cognitive and memory deficits.

The brain and other tissues require glucose in order to function properly. Studies suggest that acute or chronic hypoglycaemia leads to neurological dysfunction and injury. Hypoglycaemia during insulin therapy causes damage to the brain specifically because that is the organ which cannot withstand glucose deficiency. The functional capability of the brain will deteriorate due to the frequent hypoglycaemic shock. Hypoglycaemia-induced brain injury is a significant obstacle to optimal blood glucose control in diabetic patients. Prolonged insulin-induced hypoglycaemia causes widespread loss of neurons and permanent brain damage with irreversible coma. As in brain injury associated with ischaemia and neurodegenerative conditions, altered neurotransmitter action appears to play a role in hypoglycaemic brain injury (Auer & Seisjo, 1993; Auer, 2004). Pathological studies in humans and animals show that hypoglycaemia-induced neuronal death occurs preferentially in the hippocampus, superficial layers of the cortex and striatum (Auer & Siesjo, 1993; Auer, 2004). Because of the extensive neuronal loss, one of the neurological sequalae associated with hypoglycaemia is cognitive decline. According to clinical studies, significant learning and memory deficits correlate with the frequency of hypoglycaemia not only in patients with type 1 diabetes, but also in the relatively younger group among the population with type 2 diabetes (Dey *et al.*, 1997). Acute neuroglycopenia causes rapid deterioration of cognitive function in humans with and without diabetes. Numerous clinical studies suggest that intensive insulin treatment of type 1 diabetes is associated with an increased frequency of hypoglycaemic coma (Hannonen *et al*.,

2003; Rovet & Ehrlich, 1999) and cognitive impairment (Ryan *et al.*, 1985; Langan *et al*., 1991; Wredling *et al*., 1990).

Glutamate, which is one of the excitatory amino acid neurotransmitters found only in the central nervous system, is believed to play a major role in the pathophysiology of hypoglycaemic brain injury. Numerous reports have documented that excessive glutamate, through NMDA/AMPA receptors, activate the excitotoxic process, which play an important role in the hypoglycaemic brain damage (Choi *et al*., 1998; Lipton & Nicotera, 1998; Duchen, 2000). Excess activation of NMDA receptors by glutamate increases cytoplasmic concentrations of sodium and calcium to levels that exceed the capacity of neuronal homeostatic mechanisms, thereby altering transmembrane ion gradients. Hypoglycaemia specifically increases the sensitivity of NMDA receptors to activation by glutamate, which results in a lower threshold for glutamate induced excitotoxicity (Jane, 1999).

Attention has been focussed on glutamate as a potential mediator of hypoglycaemic brain injury (Aral *et al*., 1998; Cavaliere *et al*., 2001; Marinelli *et al*., 2001). Severe hypoglycaemia triggers a cascade of events in vulnerable neurons that may culminate in cell death even after glucose normalization (Sang *et al*., 2003, 2004, 2005, 2007). Glutamate receptor activation and excitotoxicity has long been recognized as an upstream event in this cascade (Wieloch, 1985). In brain, glutamate accumulation is reported to cause neuronal degeneration (Atlante *et al*., 1997; Berman & Murray, 1996; Budd & Nicholas, 1996).

Despite our advances in the treatment of diabetes, hypoglycaemic episodes are often the limiting factor in achieving optimal blood sugar control. Recent therapeutic strategies aimed at closely controlling elevated glucose levels in diabetic individuals put them at risk for experiencing episodes of hypoglycaemia. Recent reports suggest that if intensive insulin therapy is to be used, great effort must be

taken to avoid hypoglycemia (Bilotta *et al*., 2008). Acute and recurrent hypoglycaemia cause transient or persistent alteration of cognitive functions and can result in seizures or coma. The effects of acute or recurrent episodes of hypoglycaemia on the cells of the CNS are potentially harmful and impose long-lasting damaging effects on the brain. The pathogenesis of hypoglycaemia induced nerve cell injury is largely unknown, but mechanisms that could result in damage to cells of the CNS include excitotoxicity related to a dysregulation of the glutamate-glutamine cycle. To understand the effects of hypoglycaemia on the cells of the CNS, it is essential to characterize the response of CNS cells to reduced glycemic levels, to determine the extent of CNS cell injury induced by hypoglycaemia and to identify the mechanisms involved in hypoglycaemia induced cell or tissue damage in brain. The reports so far stated did not attempt to emphasis the functional role of glutamergic receptor subtypes in hypoglycaemic and hyperglycaemic adult and old brain.

In the present study a detailed investigation on the alterations of glutamate and its receptors in the brain regions of streptozotocin induced diabetic and insulin induced hypoglycaemic adult and old rats were carried out. Glutamate receptor subtypes- NMDAR1, NMDA2B, mGluR5 and GLAST glutamate transporter gene expression in the hypoglycaemic and hyperglycaemic adult and old rat brain were also studied. The molecular studies on the brain damage through glutamergic receptors will elucidate the therapeutic role in the corrective measures of the damage to the brain during hypoglycaemia and hyperglycaemia. This has importance in the management of diabetes and anti-diabetic treatment for better intellectual functioning of the individual.

OBJECTIVES OF THE PRESENT STUDY

1. To quantify glutamate content in the brain regions cerebral cortex (CC), cerebellum (CB), hippocampus and pancreas of hypoglycaemic and diabetic adult and old rats.

2. To study glutamate and NMDA receptors alterations in the brain regions and pancreas of hypoglycaemic and diabetic adult and old rats

3. To study NMDAR1, NMDA2B, mGluR5 glutamate receptor subtypes and GLAST glutamate transporter gene expression in the brain regions and pancreas of hypoglycaemic and diabetic adult and old rats.

4. To study the second messengers IP3, cGMP and cAMP in the brain regions and pancreas of hypoglycaemic and diabetic adult and old rats.

5. To study the expression of NMDAR1, NMDA2B mGluR5 and IP3 receptors using confocal microscope by immunofluorescent receptor specific antibodies in the brain slices of cerebral cortex, cerebellum and pancreatic islets of hypoglycaemic and diabetic adult and old rats.

6. To study the effect of glutamate and dopamine receptor antagonists on intracellular calcium release in pancreatic islets of rats *in vitro* using confocal microscope.

7. To study the behavioural changes in the hypoglycaemic and diabetic adult and old rats using rotarod test.

Literature Review

Glucose is the only fuel that neuronal tissue can use for energy under normal circumstances (Sokoloff, 1981). The brain can neither synthesize nor store more than a few minutes worth of glucose; thus a continuous systemic supply is essential for normal cerebral metabolism (Pardridge, 1983). Chronic changes in the antecedent level of glycaemia (either sustained hyperglycaemia or hypoglycaemia) induce alterations in brain glucose metabolism in rodents (McCall *et al*., 1982; Boyle *et al*., 1994). Diabetes mellitus is a major global health problem that affects more than 185 million people around the world (Amos *et al.,* 1997; Zimmet, 1999; Zimmet *et al.,* 2001). This disease is an increasingly prevalent metabolic disorder in humans and is characterised by hyperglycaemia (Kumar & Clarke, 2002**;** Dunne *et al.,* 2004). The number of diabetic patients is expected to reach 300 million by the year 2025. The projected increase in the number of diabetic patients will strain the capabilities of healthcare providers the world over (Adeghate *et al.,* 2006). The pancreatic hormones have an important role in the regulation of glucose metabolism. The secretion of insulin by ß-cells of the endocrine pancreas is regulated by glucose and other circulating nutrients. It is also modulated by several hormones and neurotransmitters, among which dopamine plays a prominent role.

There is an increased incidence of hypoglycaemia when attempts are made to institute tight glycemic control using currently available regimens of subcutaneous insulin administration in diabetic patients (Cryer, 1994). Tight blood glucose control can reduce the risk of diabetes complications but also increases the risk of hypoglycaemic episodes. Symptomatic hypoglycaemia occurs frequently in insulin-treated patients, and 36% of patients were found in one study to have experienced hypoglycaemic coma in their lifetime (Pramming *et al*., 1991). Upto 10% of patients

practicing conventional insulin therapy and 25% of those practicing intensive therapy suffer at least one episode of severe, temporarily disabling hypoglycaemia, often with seizure or coma, in a given year (Cryer, 1994) and hypoglycaemia causes recurrent and even persistent psychological morbidity in many diabetic patients (Cryer, 1994). Speculation that an adaptation in the CNS might exist in patients with diabetes, depending upon antecedent glycaemia, appeared nearly a decade ago (Cryer & Gerich, 1985; Cryer, 2003). Hypoglycaemia-induced brain injury is a significant obstacle to optimal blood glucose control in diabetic patients (Sang *et al*., 2005). Amiel *et al*., (1988) observed that lower glucose concentrations were required to initiate epinephrine secretion following a period of intensified diabetes management with its attendant increase in hypoglycaemia. Similar hormonal defects with unawareness of symptoms can be induced in patients with diabetes (Dagogo *et al*., 1993; Hepburn *et al*., 1991) and nondiabetics (Veneman *et al*., 1993; Heller & Cryer, 1991; Davis & Shamoon, 1991), some after a solitary episode of hypoglycaemia.

Insulin and sulfonylurea therapy for diabetes mellitus carries the risk of hypoglycaemic brain injury and this risk is a major impediment to optimal glucose regulation in diabetic patients (Davis *et al*., 1998). Depending upon its severity, hypoglycaemia can cause irritability, impaired concentration, focal neurological deficits, seizures, coma and with profound hypoglycaemia, neuronal death (Auer & Siesjo, 1988; McCrimmon *et al*., 1997; Ben-Ami *et al*., 1999). Symptoms of hypoglycaemia result from the actions of hormones and neurotransmitters in the process of restoring blood glucose levels. Declining glucose levels in the brain stimulate the autonomic nervous system, causing epinephrine and norepinephrine to be released from the adrenal medulla. Norepinephrine and acetylcholine from the sympathetic nervous system is also involved in glucose control. Symptoms occur as these hormones and neurotransmitters simultaneously stimulate α-cells in the pancreas

to release glucagon, which consequently induces new glucose production in the liver (Cryer, 1999, 2002 a, b, 2003). In this homeostatic mechanism, rising blood glucose levels shut down the neoglucogenesis activities of autonomic nervous system (Cryer, 1997; Towler *et al*., 1993; McAulay *et al*., 2001, Charles & Goh, 2005). Recent studies indicate that neuronal NADPH oxidase is the primary source of neuronal oxidative stress after hypoglycaemia and the rate of superoxide production is influenced by the blood glucose concentration achieved in the immediate posthypoglycaemic period. Restoring blood glucose to 1–2 mM during the first hour after hypoglycaemia resulted in less superoxide production and less neuronal death than restoration to higher glucose levels (>5 mM). It is suggested that a gradual correction of blood glucose in patients with hypoglycaemic coma is preferable to more rapid correction and hyperglycaemia (Sang *et al*., 2007). Symptoms of hypoglycaemia result primarily from a lowered glucose level in the brain and its effects on the central and autonomic nervous systems (Charles & Goh, 2005). Although hypoglycaemia is associated with a number of physiological changes, the most profound effects are seen in the brain, where glucose is the major substrate for energy metabolism and both local energy store and the supply of alternate sources are limited. The initiating events in hypoglycaemic encephalopathy are not understood completely. But brain injury appears to result from a number of processes that are initiated when blood glucose concentration decrease. Severe hypoglycaemia, whether in patients with type 1 or type 2 diabetes, have debilitating consequences, including seizures or coma or even death (Jane, 1999).

Hypoglycaemia and brain

Hypoglycaemia is a collection of symptoms brought about by an abnormally low plasma glucose level. The brain and other tissues require glucose in order to

function properly. Hypoglycaemia constitutes a unique metabolic brain insult (Auer, 2004). Glucose arrives in the central nervous system (CNS) through the specific brain capillary endothelial transporter, GLUT 1 (Pardridge *et al*., 1990), at a rate that is generally far in excess of the phosphorylation rate by hexokinase (Pardridge, 1983). Therefore, at euglycaemia, glucose transport is not rate-limiting for brain metabolism; but during an acute reduction in the glucose concentration, a level is reached where transport assumes a rate-limiting role. Beyond this critical point, hexokinase is not fully saturated and brain energy metabolism deteriorates. Among the ultimate consequences of neuroglycopenia are initial elevations in epinephrine and glucagon, which serve to increase systemic glucose production and restore glucose provision to the brain. Widespread regions of the brain have been shown to direct this hormonal response during acute CNS fuel deprivation (Frizzell *et al*., 1993). Maintaining cerebral normoglycaemia while inducing systemic hypoglycaemia greatly attenuates this counterregulatory hormone response (Frizzell *et al*., 1993, Biggers *et al*., 1989). The incidence of severe hypoglycaemia in patients with diabetes treated by intensive insulin therapy is two to six times higher as in conventionally treated patients with diabetes. In particular, recurrent hypoglycaemic episodes during the night represent a relevant risk for the patient, because they are often not realized and lead to a deterioration in the awareness for subsequent hypoglycaemic episodes. Recent data show that recurrent hypoglycaemia not only affects neuroendocrine counter regulation but also autonomic and neuroglucopenic symptoms (Minna *et al*., 2005; Kale *et al*., 2006).

Studies suggest that acute or chronic hypoglycaemia leads to neurological dysfunction and injury. The progress of neuronal dysfunction and damage during energy deprivation is a complex process that includes presynaptic and postsynaptic mechanisms (Auer & Siesjo, 1988; Martin *et al.*, 1994). Two main events have been

described when energy levels are reduced: an increased release of excitatory amino acids (EAA) and a reduced concentration of intracellular ATP, which leads to diminished Na^+/K^+-ATPase activity (Benveniste *et al*., 1984; Erecinska & Silver, 1989; Hansen, 1985; Lees, 1991; Roettger & Lipton, 1996). It is well accepted that the excessive stimulation of EAA receptors associated with metabolic inhibition hampers the recovery of $[Na^+]i$ and $[Ca^{2+}]i$ loads and facilitates cell death (Rothman *et al*., 1987; *Novelli et al*., 1988; Monyer *et al*., 1989; Lees, 1991; Rose *et al*., 1998; Cebers *et al.*, 1998). Children and adults exposed to hypoglycaemia can develop long-term impairment of cognitive function (Blattner, 1968; Hawdon, 1999; Karp, 1989; Ryan *et al*., 1985; Vannucci & Vannucci, 2001) and are at risk of epilepsy (Kaufman, 1998). Hypoglycaemia-induced brain injury is a significant obstacle to optimal blood glucose control in diabetic patients. Prolonged insulin-induced hypoglycaemia causes widespread loss of neurons and permanent brain damage with irreversible coma. As in brain injury associated with ischaemia and neurodegenerative conditions, altered neurotransmitter action appears to play a role in hypoglycaemic brain injury (Aral *et al*., 1998; Auer, 1991; Auer & Seisjo, 1993). Attention has been focussed on glutamate as a potential mediator of hypoglycaemic brain injury (Aral *et al*., 1998; Cavaliere *et al*., 2001; Marinelli *et al*., 2001). Severe hypoglycaemia triggers a cascade of events in vulnerable neurons that culminate in cell death even after glucose normalization (Sang *et al*., 2003, 2004, 2005, 2007).

Clinical signs and symptoms of metabolic encephalopathies consist of a generalized depression of cerebral function, including consciousness. The effect on consciousness is a consequence of decreased integrative capacity of the neocortex (Jane, 1999). Arousal of the neocortex and other forebrain structures involved in cognition is mediated by specific brainstem nuclei and their projecting fiber tracts, which together constitute the ascending reticular activating system (ARAS).

Activating pathways ascend from the ARAS *via* thalamic synaptic relays to the neocortex. Metabolic encephalopathies result from alterations of brain chemistry at both neocortical and brainstem ARAS centers (Pulsinelli & Cooper, 1994). If the glucose supply to the brain is not maintained, there is a decrease in cerebral electrical activity, membrane breakdown and release of free fatty acids and altered amino acid metabolism, including increased production of glutamate which is one of the excitatory amino acid neurotransmitter found only in the central nervous system. It is believed to play a major role in the pathophysiology of hypoglycaemic brain injury. There is increasing evidence that specific changes in mitochondrial function also play a major role in the early events leading to hypoglycaemic encephalopathy. Hypoglycaemic brain injury is a common and serious complication of insulin therapy. Not surprisingly, hypoglycaemic brain injury occurs most frequently in patients attempting tight glucose control (Davis *et al*., 1998). Severe hypoglycemia constitutes a medical emergency, involving seizures, coma and death. Studies suggests that suppressing seizures during hypoglycemia decrease subsequent neuronal damage and dysfunction (Abdelmalik *et al.,* 2007). The only treatment for hypoglycaemia is blood glucose repletion and there is no currently available intervention for preventing the neuronal death that develops after hypoglycaemia is corrected.

Hypoglycaemic coma induces a purely neuronal lesion of neo cortex and the hippocampus in rat brain (Wieloch *et al.,* 1984). CT studies show that hypoglycaemia predominantly affects cerebral gray matter in the brain. Analysis of regional cerebral blood flow (CBF) differences identified neuronal activation during hypoglycaemia in bilateral medial prefrontal cortex (Auer & Siesjo, 1993). Hypoglycaemic neuronal death is most pronounced in specific neuron populations: neurons in the hippocampal CA1, subiculum, and dentate granule cell layer; cortical layers 2 and 3 of cerebral cortex; and the dorsolateral striatum (Auer *et al*., 1989; Auer & Siesjo, 1993). These

neurons receive a rich glutamatergic innervation and evidence suggests that hypoglycaemic injury in these neurons is precipitated almost entirely by sustained glutamate receptor activation (excitotoxicity) (Auer *et al*., 1985). The hippocampal neurons in particular are important for learning and memory and patients who survive hypoglycaemic coma are left with significant cognitive impairment (Kalimo & Olsson, 1980; Patrick & Campbell, 1990).

Ageing and the brain

Ageing causes changes to the brain size, vasculature, and cognition (Peters, 2006). The brain shrinks with increasing age and there are changes occuring at the molecular level to morphology. The region specific changes in dendritic branching and spine density are more characteristic of the effects of ageing on neuronal morphology (Sara & Carol, 2006). The brains of individuals, who are cognitively normal, show age-related changes that include an overall reduction in brain volume and weight, which are associated with gyral atrophy and widening of the sulci of the cerebral cortex, and enlargement of the brain ventricles. Microscopically, there are increasing amounts of the age-related pigment, lipofuscin, granulovacuolar degeneration in neurons, hirano bodies, diffused deposits of beta-amyloid in parenchyma, neurofibrillary tangles in hippocampus and amygdala and sparse numbers of senile plaques in these regions and in other cortical areas of the brain (Anderton, 1997). Of these changes, neurofibrillary tangles and senile plaques are the neuropathological hallmark of Alzheimer's disease in which they are more abundant and widespread (Hof *et al*., 1996). Alzheimer's disease has therefore been regarded as accelerated brain ageing. Understanding the molecular basis of plaque and tangle formation is advancing greatly and is the main focus of research into the cellular and molecular changes observed in the ageing brain. The nature of the cognitive and

neurobiological alterations associated with age-related change is substantially different from that seen in the early stages of a dementing illness, such as Alzheimer's disease (Albert *et al*., 1997). The interplay between genetic and environmental factors determines the degree of pathological brain ageing and whether or not individuals develop dementia in later stages.

Neuropathological changes associated with normal brain ageing

The ageing brain shows selective neurochemical changes involving several neuronal cell populations. Ageing and its variants, such as Alzheimer's disease (AD), viewed as the result of alterations in the levels of Abeta, metals, cholinesterase enzymes and neuronal gene expression (Lahiri, 2005). Neurofibrillary tangles and senile plaques are common neuropathological features in both normal brain ageing and Alzheimer's disease. Layer II of the entorhinal cortex is involved with neurofibrillary tangle formation in all of the cases, while the CA1 field of the hippocampus and the subiculum are less consistently affected. Neocortical area 20 is particularly prone to develop neurofibrillary tangles in intellectually preserved elders, whereas other neocortical areas are relatively spared. Substantial senile plaque formation is seen in the neocortex of non-demented cases. Mild cognitive impairment is correlated with neurofibrillary tangle densities in layer II of the entorhinal cortex, and clinically overt Alzheimer's disease with neurofibrillary tangle densities in area 20. In non-demented cases, there is an early development of neurofibrillary tangles in areas usually spared in the course of the degenerative process in younger individuals. These observations demonstrate that mesial and inferior temporal lobe structures are affected more frequently in normal brain ageing. In this respect, neurofibrillary tangle formation in area 20 represent a crucial step of the degenerative process because it precedes the emergence of the neuropsychological deficits characteristic of age related

disorders. In addition, this reveals age-related heterogeneity in the regional vulnerability of the brain region during normal brain ageing (Hof *et al*., 1996).

Neural plasticity in the ageing brain

Aged animals have alterations in the mechanisms of plasticity that contribute to cognitive functions. One functional alteration that could directly affect plasticity is reduced synapse number, which could make it more difficult to attain the sufficient amount of active synapses that is necessary for the network modification. An early electron microscopic investigation at the perforant path–granule cell synapse showed that aged rats have a 27% decrease in axodendritic synapse number in the middle molecular layer of the dentate gyrus compared with young rats (Bondareff & Geinisman, 1976). Moreover, spatial memory deficits have been shown to correlate with a reduction in perforated synapses at the medial perforant path–granule cell synapse (Geinisman *et al*., 1986). The total number of synaptic contacts per neuron was found to be diminished significantly in the middle and inner molecular layer of dentate gyrus of aged rats relative to young adults. Both perforated and non-perforated axospinous synapses showed age-dependent decreases in numbers (Geinisman *et al*., 1992). Cognitive functions that rely on the medial temporal lobe and prefrontal cortex, such as learning, memory and executive function show considerable age-related decline. Several neural mechanisms in these brain areas also seem to be vulnerable during the ageing process. Age-related changes in the medial temporal lobe and prefrontal cortex results in altered functional plasticity contribute to behavioural impairments in the absence of significant pathology (Burke & Barnes, 2006). The subtle changes in neuronal morphology, cell–cell interactions and gene expression that contribute to alterations in plasticity in aged animals disrupt the network dynamics of

aged neurons that ultimately contribute to selective behavioural impairments (Sara & Carol, 2006).

Memory and ageing

Memory is an organism's ability to store, retain, and subsequently retrieve information. Ageing affect memory by changing the way the brain stores information and recall the stored information. Studies comparing the effects of ageing on episodic memory, semantic memory, short-term memory and priming found that episodic memory is greatly impaired in normal ageing (Nilsson, 2003). These deficits are related to impairments seen in the ability to refresh recently processed information (Johnson *et al*., 2002). The ability to encode new memories of events or facts and working memory showed decline in both cross-sectional and longitudinal studies (Hedden & Gabrieli, 2004). In addition, older adults tend to be worse at remembering the source of their information for a particular item or fact (Johnson *et al*., 1993), a deficit that is related to declines in the ability to bind information together in memory (Mitchell *et al*., 2000). In contrast, implicit or procedural memory typically shows no decline with age (Fleischman *et al*., 2004), short-term memory shows little decline (Nilsson, 2003) and semantic knowledge, such as vocabulary improves with age (Verhaeghen, 2003). In addition, the enhancement seen in memory for emotional events is also maintained with age (Mather & Carstensen, 2005). Brain imaging studies have revealed that older adults are more likely to use both hemispheres when completing memory tasks than younger adults (Cabeza *et al*., 2002). In addition, older adults show a positive effect when remembering information, which seems to be a result of the increased focus on regulating emotion seen with age (Mather & Carstensen, 2005; Isaacowitz *et al*., 2006).

In normal ageing, cognitive functions remain unimpaired over the life span whereas sustained decline might represent a pathologic condition (Morris *et al*., 1991; Linn *et al*., 1995). Alzheimer's disease (AD) is the most common cause of dementia demonstrating progressive decline in memory, language and visuospatial abilities. Distinguishing AD from normal ageing has been a recurring nosologic and diagnostic problem (Morris *et al*., 1991; Berg *et al*., 1982). However, memory loss is qualitatively different in normal ageing from the kind of memory loss associated with a diagnosis of Alzheimer's (Budson & Price, 2005). Recent research has identified a transitional state between the cognitive changes of normal ageing and AD; known as mild cognitive impairment (MCI). Many people who experience mild cognitive impairment are at a high risk of developing AD. Several studies have indicated that MCI individuals are at an increased risk for developing AD, ranging from 1% to 25% per year; 24% of MCI patients progressed to AD in 2 years and 20% more over 3 years, whereas a recent study indicated that the progression of MCI subjects was 55% in 4.5 years (Arnáiz & Almkvist, 2003). In neuropathologic studies, Gomez-Isla *et al*., (1996) reported specific neuronal loss in the entorhinal cortex in persons with very mild AD and no change in the same region in the cognitively intact elderly. These observations imply that AD and normal ageing are dichotomous.

Glutamate Receptors

Glutamate is the most prominent neurotransmitter in the body, being present in over 50% of nervous tissue. A large proportion of the glutamate present in the brain is produced by astrocytes through synthesis *de novo* (Hertz *et al*., 1999), but levels of glutamate in glial cells are lower than in neurons, 2–3 mM and 5–6 mM, respectively. During excitatory neurotransmission, glutamate-filled vesicles are docked at a specialized region of the presynaptic plasma membrane known as the active zone.

Packaging and storage of glutamate into glutamatergic neuronal vesicles requires Mg^{2+}/ATP-dependent vesicular glutamate uptake systems, which utilize an electrochemical proton gradient as a driving force. Substances that disturb the electrochemical gradient inhibit this glutamate uptake into vesicles. The concentration of glutamate in vesicle reaches as high as 20–100 mM (Nicholls & Attwell, 1990). In brain tissue, low concentrations of glutamate and aspartate perform as neurotransmitters, but at high concentration these amino acids act as neurotoxins.

It acts through both ligand gated ion channels (ionotropic receptors) and G-protein coupled (metabotropic) receptors. Activation of these receptors is responsible for basal excitatory synaptic transmission and many forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), which are thought to underlie learning and memory. It appears however, that aspartate aminotransferase and glutaminase account for a majority of glutamate production in brain tissue (McGeer *et al*., 1987).

The ionotropic receptors themselves are ligand gated ion channels, i.e. on binding glutamate that has been released from a companion cell, charged ions such as Na^{+} and Ca^{2+} pass through a channel in the centre of the receptor complex. This flow of ions results in a depolarisation of the plasma membrane and the generation of an electrical current that is propagated down the processes (dendrites and axons) of the neuron to the next in line. Metabotropic glutamate (mGlu) receptors are G-protein coupled receptors (GPCRs) that have been subdivided into three groups, based on sequence similarity, pharmacology and intracellular signalling mechanisms. Group I mGlu receptors are coupled to PLC and intracellular calcium signalling, while group II and group III receptors are negatively coupled to adenylyl cyclase.

Glutamate functions as a fast excitatory transmitter in the mammalian brain. Glutamate triggers neuronal death when released in excessive concentrations by over

excitation of its receptors (Vizi, 2000). Glutamate receptor activation and excitotoxicity has long been recognized as an upstream event in this cascade (Wieloch, 1985). In brain, glutamate accumulation is reported to cause neuronal degeneration (Atlante *et al*., 1997; Berman & Murray, 1996; Budd & Nicholas, 1996). The excitatory amino acid glutamate is the most prevalent transmitter in the brain; its effect on postsynaptic receptors is limited by uptake process (Erecinska, 1997) and by diffusion of glutamate from the cleft. The cellular uptake of Glu is driven by the electrochemical gradients of Na^+ and K^+ and is accompanied by voltage and pH changes. In nervous tissue, glutamate dehydrogenase (GDH) appears to function in both the synthesis and the catabolism of glutamate and perhaps in ammonia detoxification (Mavrothalassitis *et al*., 1988). The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA kainite (Choi, 1988). Hypoglycaemia is associated with increased glutamate release (Sandberg *et al*., 1986) and conversely, glutamate toxicity is augmented by hypoglycaemia (Novelli *et al*., 1988).

The majority of excitatory synapses are glutamatergic, in which glutamate transmits the signal through postsynaptic ionotropic [N-methyl-D-aspartic acid (NMDA), -amino-3-hydroxy-5-methysoxazole-4-propionic acid (AMPA), and kainate (KA)] and metabotropic receptors (Bettler & Mulle, 1995). Glutamate 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). Studies have shown that both ionotropic glutamate receptors and glutamate transporters are involved in oxygen-glucose deprivation-induced necrotic cell death in hippocampal slice cultures (Bonde *et al.,* 2005). The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA–kainate (Choi, 1988). The presence of G protein-coupled glutamate receptors

(metabotropic Glu receptors) has been described and since 1991 (Conn & Pin, 1997), 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. In addition, the stimulation of receptors of these three groups directly influences voltage-gated Ca^{2+} and K^{+} channels through their G proteins, but their physiological correlate has not yet defined.

There are several reports of presynaptic localization of Glu receptors and their involvement in transmitter release. The fact that NMDA releases glutamate (Pittaluga *et al*., 1996), DA (Kuo *et al*., 1998) and NE (Pittaluga & Raiteri, 1992) from axon terminals indicates that glutamate released is able to facilitate transmitter release via NMDA receptors (Barnes *et al*., 1994; Desai *et al*., 1994). Montague *et al*., (1994) suggested that glutamate 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., glutamate 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).

The most consistent age-related change in the glutamatergic system is the loss of glutamate receptors. Significant decreases in the mRNA level of glutamate receptors were found in the aged cerebral cortex (Carpenter *et al*., 1992). Among different glutamate receptors, NMDA receptors are preferentially altered in the aged brain. Decrease in NMDA binding was shown in both rodents and mammalian brain (Cohen & Muller, 1992; Wenk *et al*., 1991). mRNA level of both NR1 and NR2B subunits of the NMDA receptors have been shown to decrease preferentially in the

aged cerebral cortex, whereas no age-related change was observed in the NR2A subunit (Magnusson, 2000). The modification of subunit expression alters the receptor composition of NMDA receptor in the aged brain and lead to age-related changes in the binding properties of this receptor (Gallagher *et al*., 1996; Priestley *et al*., 1995) and/or physiological properties such as desensitization (Monyer *et al*., 1992). Binding studies revealed significant decrease in NMDA but not AMPA and kainate receptors (Tamaru *et al*., 1991). These findings support a significant loss of postsynaptic glutamatergic receptors, especially the NMDA subtype, in the aged brain.

NMDA receptors

The discovery of potent and selective agonists and antagonists has resulted in extensive information on the NMDA receptor-channel complex (Wood *et al*., 1990). It consists of four domains:- (1) the transmitter recognition site with which NMDA and L-glutamate interact; (2) a cation binding site located inside the channel where Mg^{2+} can bind and block transmembrane ion fluxes; (3) a PCP binding site that requires agonist binding to the transmitter recognition site, interacts with the cation binding site and at which a number of dissociative anesthetics PCP and ketamine, opiate N-allylnormetazocine (SKF-10047) and MK-801 bind and function as open channel blockers; and (4) a glycine binding site that appears to allosterically modulate the interaction between the transmitter recognition site and the PCP binding site. NMDA is allosterically modulated by glycine, a co-agonist whose presence is an absolute requirement for receptor activation. Molecular cloning has identified to date cDNAs encoding NR1 and NR2A, B, C, D subunits of the NMDA receptor, the deduced amino acid sequences of which are 18% belonging to NR1 and NR2, 55% belonging to NR2A and NR2C or 70% belonging to NR2A and NR2B are identical. Site-directed mutagenesis has revealed that the NR2 subunit carries the binding site for

glutamate within the N-terminal domain and the extracellular loop between membrane segments M3 and M4; whereas the homologous domains of the NR1 subunit carry the binding site for the co-agonist glycine.

Normal functioning of the NMDA receptor complex depends on a dynamic equilibrium among various domain components. Loss of equilibrium during membrane perturbation cause the entire system to malfunction and result in abnormal levels of glutamate in the synaptic cleft (Olney, 1989). An important consequence of NMDA receptor activation is the influx of Ca^{2+} into neurons (MacDermott *et al*., 1986; Murphy & Miller, 1988; Holopainen *et al*., 1989, 1990). Collective evidence suggests that when the membrane is depolarized, the Mg^{2+} block is relieved and the receptor can be activated by glutamate. Activation of the NMDA receptor therefore requires the association of two synaptic events: membrane depolarization and glutamate release. This associative property provides the logic for the role of the NMDA receptor in sensory integration, memory function, coordination and programming of motor activity (Collingridge & Bliss, 1987) associated with synaptogenesis and synaptic plasticity.

Activation of NMDA receptors results in the opening of an ion channel that is nonselective to cations. This allows flow of Na^{+} and small amounts of Ca^{2+} ions into the cell and K^{+} out of the cell (Dingledine *et al.,* 1999; Liu & Zhang, 2000; Cull-Candy *et al.,* 2001; Paoletti & Neyton, 2007). Calcium flux through NMDARs is thought to play a critical role in synaptic plasticity, a cellular mechanism for learning and memory. The NMDA receptor is distinct in that it is both ligand-gated and voltage-dependent. NMDA sensitive ionotropic glutamate receptors probably consist of tetrameric and heteromeric subunit assemblies that have different physiological and pharmacological properties. They are differentially distributed throughout the CNS (Seeburg, 1993; Hollmann & Heinemann, 1994; McBain & Mayer, 1994; Danysz *et*

al., 1995; Parsons *et al*., 1998a). NMDA receptors are probably heteromeric assemblies of four subunits. Each subunit has four hydrophobic regions, although only three form membrane-spanning domains - TM1, TM2, and TM4. TM2 makes a hairpin bend within the membrane and forms the channel pore; the "TM" terminology is therefore inappropriate. Functional NMDA receptor complexes are formed by combinations of NR1 and NR2 subunits, which contain glutamate recognition sites. Alternative splicing at three exons, one in the amino-terminal domain (N1) and two in the carboxyl-terminal domain (C1 and C2), generates eight isoforms for the NR1 subfamily. All heteromeric and homomeric NMDA receptor subtype complexes are permeant to Ca^{2+}, Na^{+}, and K^{+}. The open NMDA channel is blocked by Mg^{2+} and uncompetitive NMDA receptor antagonists, such as (+)MK-801, in a voltage-dependent manner. The speed and voltage observed in this effect depend on the antagonist affinity and the subunit composition. In addition, most NMDA receptors are influenced by Zn^{2+} ions in a voltage-dependent manner, as well as by oxidation/reduction and pH.

The NMDA channel is blocked in a use- and voltage-dependent manner by Mg^{2+}. This means that NMDA receptors are activated only after depolarization of the postsynaptic membrane by, for example, AMPA receptor activation, which relieves the voltage dependent blockade by Mg^{2+}. This biophysical property and their high Ca^{2+} permeability render NMDA receptors inherently suitable for their role in mediating synaptic plasticity underlying learning processes and development (Collingridge & Singer, 1990; Danysz *et al*., 1995). Similar to Mg^{2+}, uncompetitive NMDA receptor antagonists such as ketamine, dextromethorphan, memantine, phencyclidine (PCP) and (+)MK-801 [(+)5-methyl-10,11-dihydro-5*H*-dibenzocyclohepten-5,10-imine maleate] block the NMDA channel in the open state,

although the blocking kinetics and voltage of this effect depend on the antagonist (Rogawski, 1993; Parsons *et al*., 1998b).

To date, two major subunit families, designated NR1 and NR2, have been cloned. Various heteromeric NMDA receptor channels formed by combinations of NR1 and NR2 subunits are known to differ in gating properties, magnesium sensitivity, and pharmacological profile (Sucher *et al*., 1996; Parsons *et al*., 1998b). The heteromeric assembly of NR1 and NR2C subunits, for instance, has much lower sensitivity to Mg^{2+} but increased sensitivity to glycine and very restricted distribution in the brain. *In situ* hybridization has revealed overlapping but different expression profiles for NR2 mRNA. For example, NR2A mRNA is distributed ubiquitously like NR1, with the highest densities occuring in hippocampal regions and NR2B is expressed predominantly in forebrain but not in cerebellum, where NR2C predominates; NR2D is localized mainly in the brainstem (Moriyoshi *et al*., 1991; Monyer *et al*., 1992; Nakanishi, 1992; McBain & Mayer, 1994).

In addition to NR1 and NR2, the NR3A subunit has recently been discovered. This receptor subunit, previously termed chi-1, or NMDAR-L, is a relatively recently identified member of a new class in the ionotropic glutamate receptor family. It attenuates NMDA receptor currents when coexpressed with NR1/NR2 subunits in Xenopus oocytes but has no effect when tested with non-NMDA receptors or when expressed alone (Ciabarra *et al*., 1995; Sucher *et al*., 1995; Das *et al*., 1998). Highest levels are present in the spinal cord, brainstem, hypothalamus, thalamus, CA1 field of the hippocampus and amygdala and this distribution remains the same throughout life. Genetic knockout of NR3A in mice results in enhanced NMDA responses and increased dendritic spines in early postnatal cortical neurons, suggesting that NR3A is involved in the development of synaptic elements by modulating NMDA receptor activity (Das *et al*., 1998).

The highest levels of NR1 mRNA in the adult rat and mouse CNS are in the olfactory bulb and the lowest levels are expressed in the spinal cord. Intermediate levels were found in frontal cortex, hippocampus, cerebellum, and whole brain (Franklin *et al*., 1993; Akazawa *et al*., 1994). Similar findings have been reported with antibodies to NR1 subunits (Petralia *et al*., 1994; Benke *et al*., 1995). mRNA for double-splice variants in the C1/C2 regions, such as NR1011 (NR1A), show an almost complementary pattern with respect to those lacking both of these inserts, such as NR1100 (NR1B). NR1A mRNA are more concentrated in rostral structures such as cortex, caudate and hippocampus, whereas NR1B mRNA are principally found in more caudal regions such as thalamus, colliculi, locus coeruleus and cerebellum (Laurie & Seeburg, 1994; Paupard *et al*., 1997). Others reported that the predominant splice variants in cortex and hippocampus were NR1A without N1 insert, whereas in the cerebellum the major variant was NR1B, containing N1 (Zhong *et al*., 1994). In the hippocampus, NR1A mRNA shows high levels in all regions and is expressed more intensely in CA3 pyramidal neurons (Paupard *et al*., 1997). mRNA for NR1A and NR1B splice forms is found nearly homogeneously throughout the adult CNS, whereas NR1A and NR1B mRNA is scarce, being detected only at very low levels in postnatal cortex and hippocampus (Laurie & Seeburg, 1994; Paupard *et al*., 1997). The predominant splice variant in cultured cortical neurons is NR1A (Zhong *et al*., 1994).

In developing rats, NR1 mRNA levels in cortex and hippocampus increased nearly three-fold from postnatal day 3 to day 15 and approximately doubled from day 15 to day 67 (Franklin *et al*., 1993; Nowicka & Kaczmarek, 1996). In contrast, cerebellum and brainstem showed no change in NMDAR1 mRNA levels between postnatal days 3 and 15 but levels also doubled from day 15 to day 67 (Franklin *et al*., 1993). Similar results were reported by a different group, although levels in the

hippocampus peaked at postnatal day 10 and declined thereafter (Pujic *et al*., 1993). In the hippocampus, NR1A mRNAs dominate at birth and exhibit mature patterns of labeling, with high levels of expression in the CA1 and CA3 regions and the dentate gyrus. In contrast, NR1B mRNAs are initially expressed at lower uniform levels but levels increase more in the CA3 region than in the CA1 region or the dentate gyrus in the second and third postnatal weeks (Paupard *et al*., 1997).

The NMDA receptor antagonists have potential therapeutic applications. NMDA receptors are involved in learning and other forms of plasticity, such as drug dependence and addiction, chronic pain and CNS development, as well as in normal or disturbed synaptic transmission in some areas of the CNS. Activation of NMDA receptors depends not only on the level of synaptic activity but also on other factors, such as agonist affinity, gating kinetics and Mg^{2+} sensitivity. The role of NMDA receptors in various processes depends on the subtype composition and area of the CNS involved. In animals, most NMDA receptor antagonists produce impairment of learning when given at sufficiently high doses before the association phase but not when administered after this phase or during retrieval (Rogawski, 1993; Leeson & Iversen, 1994; Danysz *et al*., 1995; Avenet *et al*., 1996; Parsons *et al*., 1998a).

Functional Effects Mediated *via* the NMDA Receptor Signal Transduction

The NMDA class of glutamate receptors has a critical role in the induction of long-term potentiation (LTP), a synaptic modification that encode some forms of long-term memory. However, NMDA receptor antagonists disrupt a variety of mental processes (Caramanos & Shapiro, 1994; Javitt *et al*., 1996) that are not dependent on long-term memory. They interfere with working memory (Krystal *et al*., 1994; Adler *et al*., 1998) a short-lasting form of memory that is maintained by neuronal activity rather than by synaptic modification. This suggests that there are unknown functions

of the NMDA-receptor channel. Working memory is stored by the maintained firing of a memory - specific subset of neurons in networks of the prefrontal cortex (Funahashi *et al*., 1989). Firing is thought to be maintained by a reverberatory process (Amit *et al*., 1994) in which active neurons selectively excites each other through recurrent connections. The NMDA receptor in the forebrain is thought to modulate some forms of memory formation, with the NR2B subunit being particularly relevant to this process.

Metabotropic glutamate receptor

The metabotropic glutamate receptors or mGluRs are a type of glutamate receptor which are active through an indirect metabotropic process. They are members of the group C family of G-protein-coupled receptors, or GPCRs (Bonsi *et al*., 2005). Like all glutamate receptors, mGluRs bind to glutamate, an amino acid that functions as an excitatory neurotransmitter. The mGluRs perform a variety of functions in the central and peripheral nervous systems: they are involved in learning, memory, anxiety, and the perception of pain (Ohashi *et al*., 2002). They are found in pre- and postsynaptic neurons in synapses of the hippocampus, cerebellum (Hinoi *et al*., 2001) and the cerebral cortex, as well as other parts of the brain and in peripheral tissues (Chu & Hablitz, 2000). Like other metabotropic receptors, mGluRs have seven transmembrane domains that span the cell membrane (Platt, 2007). Unlike ionotropic receptors, metabotropic glutamate receptors are not ion channels. They activate biochemical cascades, leading to the modification of other proteins, as for example ion channels. This can lead to changes in the synapse's excitability, for example by presynaptic inhibition of neurotransmission (Sladeczek *et al*., 1992), or modulation and even induction of postsynaptic responses (Chu & Hablitz, 2000; Endoh, 2004; Bonsi *et al*., 2005; Platt, 2007).

Eight different types of mGluRs, labeled mGluR1 to mGluR8 are divided into groups I, II, and III (Chu & Hablitz, 2000; Hinoi *et al*., 2001; Endoh, 2004; Bonsi *et al*., 2005). Receptor types are grouped based on receptor structure and physiological activity (Ohashi *et al*., 2002). The mGluRs are further divided into subtypes, such as mGluR7a and mGluR7b. The mGluRs in group I, including mGluR1 and mGluR5, are stimulated strongly by the excitatory amino acid analog L-quisqualic acid (Chu & Hablitz, 2000; Bates *et al.,* 2002) Stimulating the receptors causes the associated enzyme phospholipase C to hydrolyze phosphoinositide phospholipids in the cell's plasma membrane (Chu & Hablitz, 2000; Endoh, 2004; Bonsi *et al*., 2005). This leads to the formation of inositol 1,4,5-trisphosphate (IP3) and diacyl glycerol. Due to its hydrophilic character IP3 can travel to the endoplasmic reticulum where it induces, *via* fixation on its receptor, the opening of calcium channels increasing in this way the cytosolic calcium concentrations. The lipophilic diacylglycerol remains in the membrane acting as a cofactor for the activation of protein kinase C. These receptors are also associated with Na^+ and K^+ channels. Their action can be excitatory, increasing conductance, causing more glutamate to be released from the presynaptic cell, but they also increase inhibitory postsynaptic potentials, or IPSPs (Chu & Hablitz, 2000). They can also inhibit glutamate release and can modulate voltage-dependent calcium channels (Endoh, 2004). Group I mGluRs, but not other groups, are activated by 3,5-dihydroxyphenylglycine (DHPG) (Shigemoto *et al.,* 1997) a fact which is useful to experimenters because it allows them to isolate and identify them. The receptors in group II, including mGluRs 2 and 3, and group III, including mGluRs 4, 6, 7, and 8 prevent the formation of cyclic adenosine monophosphate, or cAMP, by activating a G protein that inhibits the enzyme adenylyl cyclase, which forms cAMP from ATP (Chu & Hablitz, 2000; Hinoi *et al.,* 2001; Medical Research Council, 2003; Bonsi *et al*., 2005). These receptors are involved in presynaptic inhibition (Endoh,

2004) and do not appear to affect postsynaptic membrane potential by themselves. Receptors in groups II and III reduce the activity of postsynaptic potentials, both excitatory and inhibitory, in the cortex (Chu & Hablitz, 2000). Different types of mGluRs are distributed differently in cells. One study found that Group I mGluRs are mostly located on postsynaptic parts of cells while groups II and III are mostly located on presynaptic elements (Shigemoto *et al.,* 1997), though they have been found on both pre- and postsynaptic membranes (Endoh, 2004). Also, different mGluR subtypes are found predominantly in different parts of the body. mGluR4 is located only in the brain, in locations such as the thalamus, hypothalamus and caudate nucleus (InterPro, 2008). All mGluRs except mGluR6 are thought to exist in the hippocampus and entorhinal cortex (Shigemoto *et al.,* 1997).

Metabotropic glutamate receptors are known to act as modulators of other receptors. For example, group I mGluRs are known to increase the activity of N-methyl-D-aspartate receptors (Skeberdis *et al*., 2001; Lea *et al.,* 2002), a type of ion channel-linked receptor that is central in a neurotoxic process called excitotoxicity. It has been suggested that mGluRs act as regulators of neurons' vulnerability to excitotoxicity through their modulation of NMDARs, the receptor most involved in that process (Baskys & Blaabjerg, 2005). Excessive amounts of N-methyl-D-aspartate, an agonist for NMDARs, has been found to cause more damage to neurons in the presence of group I mGluR agonists (Bruno *et al*., 1995). Metabotropic glutamate receptors are also thought to affect dopaminergic and adrenergic neurotransmission (Wang & Brownell, 2007). Like other glutamate receptors, mGluRs have been shown to be involved in synaptic plasticity (Endoh, 2004; Bonsi *et al*., 2005) and in neurotoxicity and neuroprotection (Siliprandi *et al.,* 1992; Baskys *et al*., 2005). They participate in long term potentiation and long term depression, and

they are removed from the synaptic membrane in response to agonist binding (Medical Research Council, 2003).

Glutamate mediated excitotoxic cell death

Excitotoxicity is the pathological process by which nerve cells are damaged and killed by glutamate and similar substances. Evidence is gathering that excitatory amino acid (EAA) neurotransmission contribute to neuronal ischemic injury during conditions of metabolic stress (Olney *et al*., 1973; Choi, 1988). Excessive synaptic accumulation of glutamate can cause neuronal overactivation, precipitating a cascade of cellular events that lead ultimately to cell death, a phenomenon termed glutamate excitotoxicity. This occurs when receptors for the excitatory neurotransmitter glutamate such as the NMDA receptor and AMPA receptor are overactivated. Glutamate is a prime example of an excitotoxin in the brain and it is also the major excitatory neurotransmitter in the mammalian CNS (Temple *et al*., 2001). During normal conditions, glutamate concentration can be increased up to 1mM in the synaptic cleft, which is rapidly decreased in the lapse of milliseconds. When the glutamate concentration around the synaptic cleft cannot be decreased or reaches higher levels, the neuron kills itself by a process called apoptosis. Glutamate receptors, including the NMDA subtype and several non-NMDA subtypes, are transiently overexpressed in neonates and infants, in as much as EAAs play a critical role in the development of the central nervous system (McDonald *et al.,* 1990). Hardingham *et al*., (2002) noted that extrasynaptic NMDA receptor activation, triggered by both glutamate exposure or hypoxic/ischemic conditions, activate a CREB (cAMP response element binding protein) shut-off, which in turn, caused loss of mitochondrial membrane potential and apoptosis. Excitotoxins like NMDA and kainic acid which bind to these receptors, as well as pathologically high levels of

glutamate, cause excitotoxicity by allowing high levels of Ca^{2+} (Manev *et al*., 1989) to enter the cell. Ca^{2+} influx into cells activates a number of enzymes, including phospholipases, endonucleases, and proteases such as calpain. These enzymes go on to damage cell structures such as components of the cytoskeleton, membrane and DNA. Reports suggest that calcium influx through NMDA receptors is involved in ROS production and neuronal damage resulting from moderate energy depletion (Hernández-Fonseca *et al.,* 2008). Excitotoxicity is involved in spinal cord injury, stroke, traumatic brain injury and neurodegenerative diseases of the central nervous system such as Multiple sclerosis, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alcoholism and Huntington's disease (Kim *et al.,* 2002) and neurological disorders such as ischemia, cerebral trauma and some chronic neurodegenerative diseases. An excess of glutamate release, or a deficiency in its clearance from the synaptic cleft, which depends mainly on its transport by high affinity carriers, are potential sources for the accumulation of extracellular glutamate.

Glutamate transporters

The concentration of glutamate is regulated to ensure neurotransmission with a high temporal and local resolution. Neuronal damage is associated with excitotoxicity, a type of cell death triggered by the overactivation of glutamate receptors and the loss of calcium homeostasis. The removal of glutamate from the extracellular fluid occurs by uptake and by diffusion (Tong & Jahr, 1994). Failure of glutamate clearance lead to neuronal damage, named excitotoxic damage, due to the prolonged activation of glutamate receptors. Extracellular glutamate must be cleared quickly, perhaps within 1ms, to maintain glutamate below toxic levels (Trotti *et al*., 1998). Glutamate transporters represent the only significant mechanism for the uptake

of extracellular glutamate, and their importance for the long-term maintenance of low non-toxic glutamate concentrations is well documented (Danbolt, 2001).

Excitatory Amino Acid Transporters (EAAT), formerly known as glutamate transporters, belong to the family of neurotransmitter transporters. They serve to terminate the excitatory neurotransmitter signal by removal of glutamate from the neuronal synapse into glia cells. EAATs are membrane-bound pumps that resemble ion channels (Ganel & Rothstein, 1999). In humans as well as in rodents, five subtypes have been identified and named EAAT1-5. Subtypes EAAT1-3 are found in membranes of glial cells (astrocytes, microglia, and oligodendrocytes) as well as in endothelial cells, whereas EAAT4 is located on neurons (Anderson & Swanson, 2000). Finally, EAAT5 is only found in the retina where it is principally localised to photoreceptors and bipolar neurons in the retina (Pow & Barnett, 2000). In rodents, the orthologs for EAAT1-3 are named GLAST, GLT1 and EAAC1 respectively (Shigeri *et al.,* 2004).

When glutamate is taken up into glia cells by the EAATs, it is not reused directly but converted to glutamine and stored vesicles. Subsequently these vesicle are released from Glia cells and glutamine transported back into the presynaptic neuron, converted back into glutamate and store into vesicles by action of the VGLUTs (Pow & Robinson, 1994; Shigeri *et al.,* 2004). This process is named the glutamate-glutamine cycle. Given that glutamate transporters provide the main route by which glutamate is cleared, it is logically predicted that an aberration in transporter expression and function lead to toxic glutamate levels and thus promote neuronal degeneration (Tanaka *et al*., 1997). Recent studies have suggested the involvement of the glutamate transporters in radiation induced neurotoxicity (Martha *et al.,* 2009). Studies in brain autopsy specimens of HIV-1-infected patients have shown that the

expression of EAAT-2 by activated microglia exert a compensatory effect that protects neurons from glutamate neurotoxicity (Xing *et al*., 2009).

Signal transduction through Second Messengers

Inositol 1,4,5-trisphosphate (IP3)

Many biological stimuli, such as neurotransmitters, hormones and growth factors, activate the hydrolysis of phosphatidyl inositol 4,5-bisphosphate (PIP2) in the plasma membrane which is hydrolyzed by phospholipase C (PLC) to produce IP3 and diacylglycerol (DAG). The IP3 mediates Ca^{2+} release from intracellular Ca^{2+} stores by binding to IP3 receptors (IP3R). IP3R are the IP3 gated intracellular Ca^{2+} channels that are mainly present in the endoplasmic reticulum (ER) membrane. 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 response to many stimuli such as neurotransmitters, hormones and growth factors, PIP2 in the plasma membrane is hydrolyzed by PLC to produce IP3 and DAG. IP3 plays a dominant role as a second messenger molecule for the release of Ca^{2+} from intracellular stores, while DAG activates protein kinase C (PKC).

In mammalian cells, there are three IP3R subtypes- IP3R1, IP3R2 and 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, a plasmid vector containing full-length rat IP3R3 linked to green fluorescent protein GFP-IP3R3 was constructed and visualized the distribution of GFP-IP3R3 was constructed in living cells (Morita *et al.*, 2002; 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). 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. Thus, the precise mechanism underlying IP3R clustering remains controversial. Studies by Tojyo *et al.*, (2008) have shown that IP3 binding to IP3R, not the increase in Ca^{2+}, is absolutely critical for IP3R clustering. They also found that depletion of intracellular Ca^{2+} stores facilitates the generation of agonist-induced IP3R clustering.

Group I mGluRs (mGluR1/5 subtypes) are also demonstrated to mainly affect intracellular Ca^{2+} mobilization (Conn & Pin, 1997; Bordi & Ugolini, 1999). To sequentially facilitate intracellular Ca^{2+} release, group I receptors activate the membrane-bound phospholipase C (PLC), which stimulates phosphoinositide turnover by hydrolyzing PIP2 to 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 Monophosphate (cGMP)

cGMP generation has been associated with neurotransmission (Hofmann *et al.*, 2000), vascular smooth muscle relaxation (Fiscus *et al.*, 1985) and inhibition of aldosterone release from adrenal glomerulosa cell suspension (Matsuoka *et al.*, 1985). The most extensively studied cGMP signal transduction pathway is that triggered by nitric oxide (NO) (Bredt & Snyder, 1990). cGMP effects are primarily mediated by the activation of cGMP-dependent protein kinases (PKGs). Two distinct mammalian PKGs- PKG-I and PKG-II- have been identified, as well as two splice variants of PKG-I - PKG-Iα and -Iβ. In the brain, PKG-I is highly expressed in cerebellar Purkinje cells and to a lesser extent, in striatal medium spiny neurons (De Camilli *et al.*, 1984). PKG-II is a membrane-associated protein that is expressed throughout the brain (de Vente *et al.*, 2001). The effects produced by the cGMP signaling pathway modulate drug-induced neural plasticity leading to behavioural alterations (Jouvert *et al.*, 2004).

Activation of the NMDA receptor increases cAMP in the CA1 region of the hippocampus; this increase is mediated through Ca^{2+} calmodulin-dependent adenylyl cyclase (Chetkovich & Sweatt, 1993). The influx of Ca^{2+} also stimulates Ca^{2+} calmodulin-dependent nitric-oxide synthase (NOS) type to produce NO, which stimulates guanylyl cyclase to produce cGMP (Garthwaite, 1991; 2005). Cyclic nucleotide pathways cross talk to modulate each other's synthesis, degradation and actions. Increased cGMP increase the activity of cGMP stimulated PDE2 to enhance hydrolysis of cAMP, or it inhibits the PDE3 family and decreases the hydrolysis of cAMP (Pelligrino & Wang, 1998). cAMP and cGMP are involved in NMDA receptor-mediated signaling in cerebral cortical and hippocampal neuronal cultures. The influx of Ca^{2+} *via* the NMDA receptor stimulates calcium/calmodulin dependent adenylyl cyclase, leading to production of cAMP. This increase in cAMP seems to be

tightly regulated by PDE4. The Ca^{2+} influx also stimulates the production of NO and subsequent activation of guanylyl cyclase, leading to cGMP production (Suvarna & O'Donnell, 2002).

Cyclic Adenosine Monophosphate (cAMP)

The second messenger concept of signaling was born with the discovery of 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 (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*., 1997), cyclic nucleotide gated channels involved in transduction of olfactory and visual signals (Kaupp *et al*., 1989; Goulding *et al*., 1992) and the cAMP-activated guanine exchange factors Epac 1,2 which specifically activate the monomeric G protein Rap (Kawasaki *et al*., 1998).

Brain neurotransmitters and diabetes

Diabetes mellitus is a metabolic disorder that either arrives during the early years of growth (Juvenille diabetes) or later in life called as maturity onset diabetes. It is observed as the body’s inability to effectively regulate the sugar balance which

leads to severe complications such as hyperglycaemia, obesity, neuropathy, nephropathy, retinopathy, cardiopathy, osteoporesis and coma leading to death. Pancreatic damage resulting in the dysfunction of α and β cells causes disordered glucose homeostasis. In diabetic individuals the regulation of glucose levels by insulin is defective, either due to defective insulin production which is called as Insulin Dependent Diabetes Mellitus (IDDM) or due to insulin resistance that is termed as Non Insulin Dependent Diabetes Mellitus (NIDDM).

Diabetes mellitus has been reported to cause degenerative changes in neurons of the central nervous system (Garris, 1990; Lackovic *et al.*, 1990; Bhattacharya & Saraswathi, 1991). Our previous studies demonstrated adrenergic, serotonergic and dopamine D_2 receptor function alterations in the brain of diabetic rats (Abraham & Paulose, 1999; Padayatti & Paulose, 1999; Paulose *et al*., 1999; Eswar *et al*., 2007). The concentration of 5-HT, DA and NE increased in the brain regions of diabetic rats and accumulation of these monoamines is produced by inhibition of monoamine oxidase activity (Salkovic & Lackovic, 1992). Norepinephrine has been reported to increase in several brain regions during diabetes. Ohtani *et al*., (1997) have reported a significant decrease in extracellular concentrations of NE, 5HT and their metabolites in the ventro medial hypothalamus (VMH). Epinephrine (EPI) levels were significantly increased in the striatum, hippocampus and hypothalamus of diabetic rats and these changes were reversed to normal by insulin treatment (Ramakrisha & Namasivayam, 1995). Diabetes is reported to cause a high level of degeneration in neurons in different regions of the brain. Strepozotocin -induced diabetes and acute deficiency of insulin is reported to result in increased concentrations of EPI in the supra chiasmatic nucleus. It is also reported that β-adrenergic receptor populations were decreased in diabetes (Garris, 1995). 5-HT content in the brain is reported to be decreased during diabetes (Jackson & Paulose, 1999; Chu *et al*., 1986; Sumiyoshi *et*

al., 1997). Garris, (1995) reported chronically elevated levels of NE in the brain regions of amydgala, hypothalamus and medulla of diabetic mice. This was proposed to be associated with the expression of the gene causing diabetes mellitus. Hyperglycaemia is reported to alter the noradrenergic and cholinergic nerve components (Akria *et al*., 1994) with decrease in the Na^+ - K^+ ATPase activity in different brain regions (Gurcharan & Sukwinder, 1994). NE, DA and 5-HIAA are reported to be increased in the heart and adrenal gland in STZ rats. In the heart the initial changes in short-term diabetes included an increase in NE concentration but did not persist in the long term diabetic animals. In the adrenal gland there was an initial reduction followed by a steady increase in the concentration of NE and EPI (Cao & Morrison, 2001). Studies of Gireesh *et al.,* (2008a) showed that there is a decrease in total muscarinic and muscarinic M1 receptors during diabetes in the cerebral cortex. A decreased muscarinic M1 receptor gene expression in the hypothalamus, brainstem, and pancreatic islets of diabetic rats was also demonstrated by Gireesh *et al.,* (2008b).

Brain neurotransmitters and hypoglycaemia

Glucose in brain, supplies energy essential for maintenance of the nervous system. During hypoglycaemia, energy dependent mechanisms for restoring normal transmembrane gradients of sodium and calcium cannot operate because of the depletion of ATP and phosphocreatine associated with hypoglycaemia. Excess calcium influx activates cellular phospholipases and proteases, alters mitochondrial metabolism, triggers free radical formation, changes patterns of synaptic transmission, and eventually result in selective neuronal necrosis (Jane, 1999). Deficiency in glucose that results from hypoglycaemic insults trigger neuronal injuries. Balance in ion homeostasis is disturbed, which in turn results in membrane depolarization and massive release of neurotransmitters, including glutamate (Siesjo, 1978; Erecinska &

Silver, 1989). The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA-kainate (Choi, 1988). In addition, neurons impaired of energy metabolism appear to be highly sensitive to excitotoxicity (Wieloch, 1985; Monyer *et al*., 1992; Cebers *et al*., 1998).

Hypoglycaemia causes several-fold elevations in brain extracellular glutamate and aspartate concentrations, and ablation of presynaptic glutamatergic terminals prevent hypoglycaemic neuronal death (Wieloch, 1985; Butcher *et al*., 1987). Pretreatment with glutamate receptor antagonists can also reduce hypoglycaemic neuronal death (Wieloch, 1985), but these agents are less effective when administered after hypoglycaemia has occurred (Nellgard & Wieloch, 1992). An additional limitation to the use of glutamate receptor antagonists in clinical settings is that these agents are themselves neurotoxic (Olney, 1989). Hypoglycaemic neuronal death is not a direct and immediate consequence of low-energy substrate but results instead from a cascade of events precipitated by the lack of substrate. Sustained activation of glutamate receptors has been established as a necessary upstream event in this cascade (Auer & Siesjo, 1993). Because of the extensive neuronal loss, one of the neurological sequalae associated with hypoglycaemia is cognitive decline. According to clinical studies, significant learning and memory deficits correlate with the frequency of hypoglycaemia not only in patients with type 1 diabetes, but also in the relatively younger group among the population with type 2 diabetes (Dey *et al*., 1997; Sang *et al*., 2005). It is reported that moderate prolonged hypoglycaemia results in reduced cardiac vagal outflow in both diabetic patients and nondiabetic subjects (Minna *et al*., 2005).

Clinically, hypoglycaemia results in depression of CNS function, with rostral brain regions being affected before more caudally situated regions. For example, in severe hypoglycaemia associated with isoelectric EEG tracings, cerebral cortical

activity is absent but medullary function persists, as indicated by the maintenance of effective respiratory and cardiovascular activity. Reduced synthesis of neurotransmitters rather than a global cerebral energy deficit explains the neurological symptoms and EEG changes in moderate hypoglycaemia (Butterworth, 1983; 1999). The physiologic disturbances associated with acute hypoglycaemia result in a stress response, with release of catecholamines, glucagon, subsequent lipolysis and glycogenolysis in an attempt to increase substrate availability for normal metabolic processes (Jane, 1999). Protection against epinephrine defects, both without and with antecedent hypoglycaemia in diabetes, is associated with enhancement of adrenal catecholamine-synthesizing enzyme levels. It is reported that 2-D Glucose augmented the turnover of NE, DA and 5-HT under the fasted condition. Insulin perfusion within the medial hypothalamic sites evoked a significant increase in the synthesis and release of DA from the sated rat, but did not alter its turnover. However, in the interval following insulin perfusion, DA and 5-HT turnover were enhanced while the efflux of 5-HT was suppressed.

Pyruvate derived from glucose is the major precursor of the acetyl group. Inhibition of pyruvate oxidation results in reduced ACh synthesis both *in vitro* and *in vivo.* Incorporation of [^{14}C]choline into ACh in brain *in vivo* is decreased in rats with insulin-induced hypoglycaemia. Hypoglycaemia results in decreased synthesis of the neurotransmitter pool of ACh are supported by the observation that administration of the CNS cholinesterase inhibitor physostigmine to hypoglycaemic animals delays the onset of seizures and coma (Gibson & Blass, 1976). It is also reported that the extracelluar concentrations of acethylcholine both in the hippocampus and striatum did not change during hypoglycaemia. Changes of hippocampal cholinergic release is not involved in the mechanism of cognitive impairment during hypoglycaemia (Hiroyuki *et al*., 2006).

Similar findings of an adverse effect of hypoglycaemia on the synthesis of the amino acid neurotransmitters GABA and glutamate have also been reported. Utilization of amino acids such as glutamate and glutamine as alternative energy substrates in moderate to severe hypoglycaemia results in accumulation of aspartate and ammonia in the brain. Hypoglycaemia also produces a transient but substantial increase in extracellular concentrations of glutamate, GABA and dopamine, as measured using *in vivo* cerebral microdialysis (Butcher *et al*., 1987; Butterworth, 1983, 1999). Studies reported that modulation of the GABAergic system in the ventromedial hypothalamus (VMH) alters both glucagon and sympathoadrenal, but not corticosterone, responses to hypoglycaemia. GABAergic inhibitory tone within the VMH modulates glucose counterregulatory responses (Owen *et al*., 2006). Alterations of neurotransmission mediated by ACh, Glu, GABA and/or DA contribute to the neurological signs and symptoms that characterize moderate hypoglycaemia. Enhanced glutamate receptor subtype activity in the cerebellum (Joseph *et al*., 2007) and cerebral cortex (Joseph *et al*., 2008) in insulin induced hypoglycaemiac and streptozotocin induced diabetic rats were reported by studies from our laboratory. Dopaminergic dysfunction in hippocampus during hypoglycaemia and hyperglycaemia contributing to cognitive and memory deficits was recently reported (Robinson *et al*., 2009).

Hypoglycaemia results in cognitive dysfunction. Wredling *et al*., (1990) reported permanent neuropsychological impairment after recurrent episodes of severe hypoglycaemia in diabetic patients. Severe deterioration in cognitive function and personality in patients with long-standing diabetes as a complication of a consequence of insulin treatment is reported (Gold *et al*., 1995). IDDM patients with hypoglycaemia unawareness exhibited more profound cognitive dysfunction during acute hypoglycaemia which persisted for longer following blood glucose recovery

(Gold *et al*., 1995). Severe hypoglycaemia with cognitive dysfunction is three times more common in intensively, rather than conventionally, treated IDDM (Maran *et al*., 1995). In the insulin treated diabetic patients exposed to a spontaneous episode of severe hypoglycaemia, the cognitive decrements and altered mood states noted is persistent and is the consequence of previous exposure to recurrent episodes of severe hypoglycaemia (Strachan *et al*., 2000). Recurrent hypoglycaemia significantly diminished cognitive performance in both diabetic and nondiabetic animals. The diabetic hippocampus adapt to high circulating glucose, with increased susceptibility to reductions in glucose availability. RH diminishes ability to meet the demands of a relatively demanding cognitive challenge during hypoglycaemia (McNay, 2005). Recurrent hypoglycaemia markedly affects hippocampally dependent spatial working memory task. This is accompanied by alterations within the hippocampus, including both ECF glucose and lactate levels during cognitive testing and electrophysiological function. The impact of recurrent hypoglycaemia on cognition is multifaceted and includes both metabolic and electrophysiological components (McNay *et al*., 2006).

Exposure to stress is known to precipitate or exacerbate many neuropsychiatric disorders such as depression, Parkinson's disease, schizophrenia, and others (Schwab & Zieper, 1965; Mazure, 1995). All these disorders involve a working memory deficit caused by prefrontal cortical (PFC) dysfunction (Mattes, 1980; Weinberger *et al*., 1986; Deutch, 1993; Fibiger, 1995). NMDA 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 dopamine neurons is due to an alteration in GABA regulation of the dopamine 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). DA neurons are vulnerable to metabolic stress (Callahan *et al*., 1998). The maintenance of normal energy metabolism in T1DM during hypoglycaemia effect glucose sensing in the brain and contribute to hypoglycaemia-associated autonomic failure (Bischof *et al*., 2006).

Ageing and diabetes

Diabetes mellitus is a major global health problem that affects more than 185 million people around the world (Amos *et al*., 1997; Zimmet, 1999; Zimmet *et al*., 2001). The disease is an increasingly prevalent metabolic disorder in humans and is characterised by hyperglycemia (Kumar & Clark, 2002; Dunne *et al*., 2004). The number of diabetic patients is expected to reach 300 million by the year 2025. The projected increase in the number of diabetic patients will strain the capabilities of healthcare providers the world over (Adeghate *et al*., 2006). The pancreatic hormones have an important role in the regulation of glucose metabolism. The secretion of insulin by ß-cells of the endocrine pancreas is regulated by glucose and other circulating nutrients. It is also modulated by several hormones and neurotransmitters, among which acetylcholine plays a prominent role. The progression of diabetes is associated with an impaired ability of the neurons in the CNS to release neurotransmitters (Broderick & Jacoby, 1989). Numerous neurochemical studies using both animals and humans have revealed age-related changes in neurotransmitter enzyme activities and receptor binding (McGeer & McGeer, 1982; Hepler *et al*., 1985; Smith, 1988). Neurotransmitters show significant alterations during hyperglycaemia and causes degenerative changes in neurons of the central nervous system (Garris, 1990; Lackovic *et al*., 1990; Bhardwaj, *et al*., 1999). Studies on STZ-induced diabetic rat models have shown similar results which exhibits morphological, behavioural and electrophysiological alterations on diabetes (Jakobsen *et al*., 1987; Biessels *et al*.,

1996; Chabot *et al*., 1997). Learning and memory deficits are associated with Type I and Type II diabetes mellitus (Gispen & Biessels, 2000) and brain morphological abnormalities have been found in diabetic patients, mainly in the cortical area (Dejgaard *et al*., 1991). STZ-induced diabetes results in structural alterations of mAChRs in the brain (Latifpour *et al*., 1991) which in turn alters cholinergic nerve components (Akria *et al*., 1994) with decrease in the Na^+, K^+-ATPase activity (Gurcharan & Sukwinder, 1994). Studies of Latifpour and McNeill, (1984) on long-term STZ-induced diabetes reported large reduction in muscarinic receptor densities as compared with their age-matched controls. Ageing and diabetes are intimately related at a molecular level and hence diabetes is able to provide the link between disease treatment and the prevention of age-related diseases. If specific molecular pathways controlling the rate of ageing are modulated genetically, then perhaps they are modulated pharmacologically (Geesaman, 2006). These insights ultimately have an important impact on the discovery and development of drugs to both treat and prevent a wide range of diseases

Ageing and hypoglycaemia

The increasing proportion of elderly persons in the global population, and the implications of this trend in terms of increasing rates of chronic diseases such as diabetes mellitus, continue to be a cause for concern for clinicians and healthcare policy makers. The diabetes of the elderly subjects has two forms: diabetes of long duration, manifesting itself in younger or medium ages and senile diabetes, appearing above the age of 65 years. The diagnosis and treatment of diabetes in the elderly is challenging, as age-related changes alter the clinical presentation of diabetic symptoms. There are numerous reasons to maintain blood glucose levels below 11.1 nmol/L (200 mg/dl) in older persons and there are a number of changes often seen

with advancing age that persons and there are a number of changes often seen with advancing age that interfere with the management of diabetes mellitus, e.g. hypodipsia, anorexia, visual disturbance, altered renal and hepatic function, depression, impaired basoreceptor response and multiple medications (Morley & Perry, 1991). Combination therapy of insulin with oral hypoglycaemic agents is not recommended in this group of patients. Combination therapy of insulin with oral hypoglycaemic agents is not recommended in this group of patients. The decline in cognitive function, especially on challenging tasks, associated with aging is well known and relatively well-characterised. Recent evidence has provided strong support for the view that reduced ability to provide and regulate fuel supply, i.e., glucose, to the aged brain is a major cause of such decline (McNay, 2005). Inability to regulate glucose also defines diabetes and both diabetes and the recurrent hypoglycemia seen in intensively insulin-treated diabetic patients also affect cognition. As type 2 diabetes progresses in older persons, polypharmacy intensification is required to achieve adequate glycaemic control with the attendant increased risk of adverse effects as a result of age-related changes in drug metabolism. The recently available oral glucose lowering agents in the market along with the newer types of insulin are used in elderly diabetic patients. The effect of aging on metabolism and drug elimination kinetics must, however, be taken into consideration. In particular, it should be borne in mind that the risk of hypoglycemia is more deleterious in the elderly and should be avoided (Oiknine & Mooradian, 2003). A better compliance is obtained, being a fundamental aspect in the elderly diabetics and a reduction of the number and severity of the hypoglycemia, which are the most important aspects in the elderly diabetes (Motta *et al.,* 2008). The recent introduction of the incretins, a group of intestinal peptides that enhance insulin secretion after ingestion of food, as novel oral antihyperglycaemic treatments may prove significant in older persons (Abbatecola *et al*., 2008).

Glutamate receptors in diabetes and hypoglycaemia

Neurodegeneration results from over activation of NMDA receptors causing excitotoxicity proposed to be responsible for certain neurological diseases. Excess activation of NMDA receptors by glutamate increases cytoplasmic concentrations of sodium and calcium to levels that exceed the capacity of neuronal homeostatic mechanisms, thereby altering transmembrane ion gradients. Diabetes mellitus induces cognitive impairment and defects of long-term potentiation in the hippocampus as indicated by behavioural and electrophysiological analysis. Considered to be an important mechanism of learning and memory in mammals, long-term potentiation is known to require regulation of the glutamate receptor properties. According to many studies, defects of long-term potentiation in the hippocampus of diabetic animals are due to abnormal glutamate receptors. Earlier studies explained that changes in glutamate receptors account for modifications of long-term potentiation in various models of diabetes mellitus. Deficits in long-term potentiation during chronic diabetes arise from dysfunction of the NMDA subtype of glutamate receptors in early stages of the disease (Trudeau *et al*., 2004). Previous studies demonstrated that disruption of glutamate homeostasis occurs in the diabetic retina (Qing & Donald, 2002). Binding properties of brain glutamate receptors of STZ induced rats and the possible role of AMPA receptors in cognitive deficits during diabetes is reported by Gagne *et al*., (1997). Altered glutamatergic neurotransmission and calcium homeostasis contribute to retinal neural cell dysfunction and apoptosis in diabetic retinopathy. Elevated Glucose is reported to change the expression of ionotropic Glutamate receptor subunits and impairs calcium homeostasis in retinal neural cells (Ana *et al*., 2006). It is suggested that enzymes of the glutamate system respond differently towards diabetes or deprivation of food and diabetes affect the glutamate uptake system in glial cells (Galanopoulos *et al*., 1988). Recent studies suggest that glutamate plays a

pivotal role in the processing of sensory information in the spinal cords of patients with diabetic neuropathy. Abnormal expression of multiple glutamate receptors is involved in the development of diabetic neuropathy (Tomiyama *et al*., 2005).

Studies reported that neurons impaired of energy metabolism are highly sensitive to excitotoxicity (Simon *et al*., 1984; Wieloch, 1985; Monyer *et al*., 1989; Cebers *et al.*, 1998). Pathophysiological mechanisms responsible for neuronal cell death in hypoglycaemia include the involvement of glutamate excitotoxicity. Hypoglycaemia specifically increases the sensitivity of NMDA receptors to activation by glutamate, which result in a lower threshold for glutamate induced excitotoxicity (Jane, 1999). Severe and prolonged hypoglycaemia results in increased release of glutamate in the brain, leading to membrane depolarization. This is followed by cerebral energy failure and neuronal cell death. Glutamate neurotoxicity is thus implicated in the pathogenesis of hypoglycaemia induced neuronal death and Ca^{2+} calmodulin-dependent protein kinase II appears to be one of the intracellular targets for glutamate neurotoxicity in hypoglycaemia (Hu *et al*., 1995). Hypoglycaemia causes several-fold elevations in brain extracellular glutamate concentrations and pretreatment with glutamate receptor antagonists prevent hypoglycaemic neuronal death (Nellgard & Wieloch, 1992; Sandberg *et al*., 1986; Wieloch, 1985).

Inositol 1, 4, 5-trisphosphate (IP3) and activation of calcium release

Cytosolic Ca^{2+} is a focal point of many signal transduction pathways and modulates a diverse array of cellular activities ranging from fertilization to cell death (Berridge *et al*., 2000). In most cell types, the major internal [Ca^{2+}] stores are the endoplasmic reticulum/sarcoplasmic reticulum (ER/SR). One mechanism for mobilizing such stores involves the phosphoinositide pathway. The binding of many hormones to specific receptors on the plasma membrane leads to the activation of an

enzyme (phosphoinositidase C) that catalyses the hydrolysis of phospholipids to produce the intracellular messenger inositol 1,4,5-trisphosphate (IP3). Although derived from a lipid, IP3 is water soluble and diffuses into the cell interior where it encounters IP3 receptors (IP3Rs) on the ER/SR. The binding of IP3 changes the conformation of IP3Rs such that an integral channel is opened, thus allowing the [Ca^{2+}] stored at high concentrations in the ER/SR to enter the cytoplasm. A critical feature of IP3Rs is that their opening is regulated by the cytosolic Ca^{2+}concentration. This sensitivity to cytosolic [Ca^{2+}] allows them to act as Ca^{2+}-induced calcium release (CICR) channels that promote the rapid amplification of smaller trigger events.

Factors affecting insulin regulation from pancreatic β-cells

D-Glucose is the major physiological stimulus for insulin secretion. The mechanism of glucose induced insulin release is not completely understood. Phosphorylation of glucose to glucose-6-phosphate serves as the rate limiting step in glucose oxidation (Schuit, 1996). Glucokinase acts as a glucose sensor during this process. An increased ATP/ADP ratio is believed to close K^+-ATP channel at the plasma membrane, resulting in decreased K^+ efflux and subsequent depolarisation of the β-cell (Dunne, 1991). Depolarisation activates voltage-dependent Ca^{2+} channels, causing an influx of extracellular Ca^{2+} (Liu *et al.*, 1998). Although intracellular Ca^{2+} activates protein kinases such as Ca^{2+} and calmodulin dependent protein kinase (Breen & Aschcroft, 1997), it remains unclear how increase in intracellular Ca^{2+} leads to insulin release. Intracellular Ca^{2+}stores appear to regulate a novel plasma membrane current [Ca^{2+} release activated non-selective cation current], whose activity control glucose activated secretion. Glucose induced insulin secretion is also partly dependent upon the activation of typical isoforms of PKC within the β-cell (Harris *et al.*, 1996). It is suggested that PKC is tonically active and effective in the

maintenance of the phosphorylated state of the voltage-gated L-type Ca^{2+} channel, enabling an appropriate function of this channel in the insulin secretory process (Arkhammar *et al*., 1994). Glucose is an important regulator of various β-cell processes including insulin biosynthesis and release. Glucose, over short intervals stimulates insulin biosynthesis at the level of translation (Permut & Kipnis, 1972). Studies have shown that preproinsulin mRNA levels rise 4-10 fold in response to glucose stimulation. Studies of insulin gene expression in primary cultures of rat islets transfected Insulin I gene 5'-flanking sequence suggested that metabolic signal from glucose influx is transmitted through the insulin enhancer (German *et al.,* 1990).

ROLE OF NEUROTRANSMITTERS IN INSULIN REGULATION

Epinephrine and Norepinephrine

Epinephrine and norepinephrine has an antagonistic effect on insulin secretion and glucose uptake (Renstrom *et al*., 1996; Porte, 1967). They also inhibit insulin - stimulated glycogenesis through inactivation of glycogen synthase and activation of phosphorylase with consequent accumulation of glucose-6-.phosphate. In addition, it has been reported that epinephrine enhances glycolysis through an increased activation of phosphofructokinase. EPI and NE at low concentrations bind and activate β-adrenergic receptors which in turn stimulate the insulin secretion from pancreatic islets and at high concentration they can bind to α_{2A} receptors and inhibit insulin secretion (Lacey *et al*., 1993). Previous studies had shown that in diabetic condition α_{2A} receptors are more activated which brought out the insulin inhibition and in turn hyperglycaemia (Lacey *et al*., 1993). Rat islet cell membrane is equipped with α_{2A}-adrenoceptors (Filipponi *et al*., 1986) which are linked to adenylyl cyclase inhibiting insulin secretion.

NE and EPI, the flight and fright hormones are released in all stress conditions and are the main regulators of glucose turnover in strenuous exercise (Simartirkis *et al*., 1990). In severe insulin induced hypoglycaemia, a 15 to 40 -fold increase of epinephrine plays a pivotal role in increasing glucose production independently of glucagon (Gauthier *et al*., 1980). In humans, adrenaline stimulates lipolysis, ketogenesis, thermogenesis and glycolysis and raises plasma glucose concentrations by stimulating both glycogenolysis and gluconeogenesis. It is already known that, when used in high doses *in vivo* or *in vitro*, EPI reduces the insulin response to stimulators (Malaisse, 1972). *In vitro* studies with yohimbine showed that the insulin secretion from the pancreatic islets increased significantly suggesting that when the α_2-adrenergic receptors are blocked, it enhances islet cell proliferation and insulin secretion. Our previous studies demonstrated the role of α and β-adrenergic receptors in the insulin secretion (Ani *et al*., 2006a; b; c). We also reported the effect of NE in DA mediated insulin secretion (Eswar *et al*., 2006).

Acetylcholine

Acetylcholine is the neurotransmitter of the parasympathetic system. Cholinergic receptors are classified as ionotropic nicotinic receptor and metabotropic muscarinic receptor. Acetylcholine increases insulin secretion through muscarinic receptors in pancreatic islet cells (Tassava *et al*., 1992; Greenberg & Pokol, 1994). Muscarinic receptors are classified as M_1, M_2, M_3, M_4 and M_5. They are G protein coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions that interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects. Expression studies have revealed the presence of M_1 and M_3 receptors in the pancreas. Acetylcholine is reported to be involved in the activation of glucose transport in the chromaffin cells. The cholinergic activation affecting this process is coupled with calmodulin and

protein kinase C (Serck-Hanssen *et al*., 2002). It is reported that the role of acetylcholine in insulin secretion is mediated through M_1 and M_3 receptors (Renuka *et al*., 2004; 2005; 2006).

γ-Aminobutyric acid

Gamma aminobutyric acid (GABA) is the major inhibitory neurotransmitter in central nervous system. GABA is reported to be present in the endocrine pancreas at concentrations comparable with those found in central nervous system. The highest concentration of GABA within the pancreatic islet is confined to β-cells (Sorenson *et al*., 1991). Glutamate decarboxylase, the primary enzyme that is involved in the synthesis of GABA, has been identified as an early target antigen of the T-lymphocyte mediated destruction of pancreatic β-cells causing insulin-dependent diabetes mellitus (Baekkeskov *et al*., 1990). The brain GABAergic mechanisms also play an important role in glucose homeostasis. Recent studies reported the regulatory role of GABA during pancreatic regeneration (Balaram *et al.,* 2007; 2008). Also, we reported the role of GABA in hepatocyte proliferation (Biju *et al*., 2002). GABA through its receptors has been demonstrated to attenuate the glucagon and somatostatin secretion from pancreatic α-cells and δ-cells respectively (Gaskins *et al*., 1995). GABA which is present in the cytoplasm and in synaptic-like microvesicles is co-released with insulin from β-cells in response to glucose (Reetz *et al*., 1991). GABA inhibits islet α and δ-cell hormonal secretion in a paracrine manner. GABA release is decreased in diabetes resulting in the enhancement of glucagon secretion from α-cells leading to hyperglycaemia. GABA is involved in the maintenance of glucose homeostasis and inhibition of central $GABA_A$ receptors increasing the plasma glucose concentration (Lang, 1995). Thus, any impairment in the GABAergic mechanism in central nervous system and/or pancreatic islets is important in the pathogenesis of metabolic stress.

Serotonin

Brain serotonin content decreased during diabetes (Jackson & Paulose, 1999). This decrease is reported to be due to a decrease in uptake of tryptophan through the blood brain barrier (BBB) (Fernstrom & Wurtman, 1971; Fernstrom & Wurtman, 1972; Madras *et al*., 1974) and a decrease in rate of 5-HT synthesis (Carndall *et al*., 1981). The turnover rate of 5-HT to 5-HIAA in diabetic rats was also reported to be lower (Kwok & Juorio, 1987; Sandrini *et al*., 1997). A decrease in brain 5-HT lead to an up regulation of 5-HT_{2A} receptors of cerebral cortex and brain stem which in turn inhibit insulin secretion due to increased sympathetic activity (Jackson & Paulose, 1999). Our ealrier studies reported the role of serotonin in cell proliferation (Sudha & Paulose, 1998). The functional regulation of brain 5-HT during pancreatic regeneration is also reported (Mohanan *et al.*, 2005a, b; 2006).

Central glutamatergic regulation of glucose homeostasis

Although the role of glutamate as a signaling molecule is well established in the central nervous system, a similar role in the periphery has only recently been suggested. Inagaki *et al*., (1995) and Weaver *et al*., (1996) have detected functional glutamate receptors in the pancreatic islets of Langerhans. Pancreas is composed of four major cell types: the insulin-secreting β-cell, the glucagon-secreting α-cell, the pancreatic polypeptide-secreting PP cell and the somatostatin-secreting delta cells. The electrically excitable β-cells are stimulated to secrete insulin in response to changes in serum glucose concentrations. Secretion of insulin and the three other major peptide hormones found in islets is also believed to be affected by other metabolic and neuronal signals (Boyd, 1992; Ashcroft *et al*., 1994). Bertrand *et al.,* (1992; 1993) have shown that AMPA receptor agonists can potentiate both insulin and glucagon secretion from a perfused pancreas preparation and that oral or intravenous

glutamate can increase insulin secretion and glucose tolerance *in vivo* (Bertrand *et al*., 1995).

The precise role of a glutamatergic signaling system in islet physiology or pathology is not completely understood. Glutamate also subserves communication between islets and the central nervous system. Glucose-stimulated insulin release is Ca^{2+}-dependent, perhaps because Ca^{2+} couples the process of stimulus recognition to that of insulin discharge (Douglas, 1968; Milner & Hales, 1970; Matthews, 1970; Malaisse, 1973; Malaisse *et al.*, 1974). Although several studies have indicated that glucose alters the state of Ca^{2+} in the pancreatic cells, the nature of the changes and the mechanisms by which they occur are poorly understood (Hellman *et al.*, 1976).

Triiodotyhronine (T3) regulation on diabetes and ageing

Diabetes mellitus and thyroid diseases are the two common endocriniopathies seen in the adult population. Insulin and thyroid hormones being intimately involved in cellular metabolism and excess/ deficit of either of these hormones could result in the functional derangement of the other. In euthyroid individuals with diabetes mellitus, the serum T3 levels, basal TSH levels and TSH response to thyrotropin releasing hormone (TRH) is influenced by the glycemic status (Schlienger *et al*., 1982). Alterations in serum T3 levels have been described in association with energy deprivation (Vagenakis *et al*., 1975; Eisenstein *et al*., 1978) wasting illnesses (Burke & Eastman, 1974)., the neonatal period (Larsen, 1972) and the use of such drugs as propylthiouracil (Oppenheimer *et al*., 1972), dexamethasone (Chopra *et al*., 1975) and propranolol (Roszkowska *et al*., 1974; Tevaarwerk & Boyd, 1977; Tevaarwerk *et al.*, 1978). An increase in the serum T3 level has been reported in response to long-term growth hormone administration in growth-hormone-deficient children (Sato *et al*., 1977). Fasting appears to inhibit 5'-monodeiodination, causing a decrease in the rate

of conversion of T4 to T3 and an increase in the reverse T Concentration. Poorly controlled diabetes, both Type 1 and Type 2, induce a "Low T3 state" characterized by low serum total and free T3 levels, increase in reverse T3 (rT3) but near normal serum T4 and TSH concentrations. Low serum T3 is due to reduced peripheral conversion of thyroxine (T4) to tri-iodothyronine (T3) *via* 5' monodeiodination reaction. Studies indicate that long term diabetic control determines the plasma T3 levels. TSH responses and low T3 state normalized with improvement in glycaemic status but even with good diabetes control, the normal nocturnal TSH peak is not restored in C-peptide negative patients i.e., those with totally absent pancreatic β cell function (Coiro *et al*., 1997). Studies show decreased insulin secretion (Ahren *et al*., 1985) as well as normal or increased levels of insulin is reported in the peripheral and portal circulation in hyperthyroidism (Dimitriadis *et al*., 1985). Long term thyrotoxicosis has been shown to cause beta cell dysfunction resulting in reduced pancreatic insulin content, poor insulin response to glucose and decreased rate of insulin secretion (Bech *et al*., 1996).

In hyperthyroidism, the endogenous glucose production is greatly increased by a variety of mechanisms: (a) an increase in the availability of gluconeogenic precursors in the form of lactate, glutamine and alanine from skeletal muscles and glycerol from adipose tissue, (b) an increase in the concentration of plasma FFA stimulating hepatic gluconeogenesis (Dimitriadis & Raptis, 2001); (c) an increase in glycogenolysis due to inhibition of glycogen synthesis resulting in hepatic glucose output even in fed state (Holness & Sugden, 1987); (d) an upregulation of GLUT-2 glucose transporters protein expression in the hepatocyte plasma membrane. This permits increased glucose efflux to occur without intracellular glucose accumulation which would limit hepatic glucose production (Mokuno *et al*., 1990); and (e) an increased secretion and exaggerated effects of glucagon and adrenaline on liver cells

(Dimitriadis & Raptis, 2001). In skeletal muscle, there is a preferential increase in glucose uptake and lactate formation relative to glucose oxidation and storage in hyperthyroid state. This is due to increase in both basal and insulin stimulated GLUT1 and GLUT-4 transporters (Haber *et al*., 1995), increased responsiveness of glycogenolysis to beta adrenergic stimulation (Dimitriadis & Raptis, 2001), increased activity of hexokinase and 5- phosphofructokinase and decreased sensitivity of glycogen synthesis to insulin (Dimitriadis *et al*., 1997). In hypothyroidism, the synthesis and release of insulin is decreased (Ahren *et al*., 1985). The rate of hepatic glucose output is decreased probably due to reduced gluconeogenesis. A post receptor defect has been proposed to explain the decrease in insulin stimulated glucose utilization in peripheral tissues (Dimitriadis & Raptis, 2001). A reduced secretion of thyroid hormones with age has been documented in humans and animals with no substantial increase in TSH secretion, which is indicative of an age-related impairment of the pituitary sensitivity to the negative control exerted by thyroid hormones. Studies in young animals of both sexes showed an inverse correlation between the density of pituitary T3 receptors and plasma TSH whereas in old animals an age-related impairment of T3 action was reported on the thyrotrophs or changes pertaining to others factors modulating TSH secretion (Donda *et al*., 1987).

Calcium imaging

The Langerhans' islet is another example of the presence of peripheral glutamatergic systems (Satin & Kinard, 1998). Intracellular free Ca^{2+} concentration plays a pivotal role in the regulation of various cellular functions as an intracellular messenger system. After stimulation of islets with AMPA or kainate, intracellular Ca^{2+} increased by way of activation of voltage-gated Ca^{2+} channels (Inagaki *et al.,* 1995; Weaver *et al*., 1999), resulting in an elevated level of insulin secretion through

increased exocytosis of insulin granules in β-cells (Bertrand *et al.,* 1992). Since the development of digital video imaging of Ca^{2+} novel findings including Ca^{2+} oscillations (Berridge & Galione, 1988; Berridge, 1991) 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).

Cerebellar dysfunction and hypoglycaemia

The cerebellum is known to be resistant to hypoglycaemia, and selective cerebellar dysfunction caused by hypoglycaemia has not been reported. In a case of episodic bilateral cerebellar dysfunction caused by hypoglycaemia, quantitative dynamic PET study demonstrated decreased glucose uptake-to-utilization ratio and increased leak of glucose in the cerebellum indicating that cerebellum is not invariably resistant to hypoglycaemia (Kim *et al*., 2005). Motor learning, a process by which an animal learns to perform a motor skill more accurately and efficiently through practice, plays an essential role in human life. Unlike explicit memory such as recognition memory and spatial memory, motor learning is characterized by slow development, without the requirement of conscious recall, and in general being lifetime-lasting (Llinas & Welsh, 1993; Tulving & Markowitsch, 1998; Eichenbaum, 2000). Based on the role of the cerebellum in motor activities such as fine motor movement and motor coordination as well as the computational network within the neural circuitries, cerebellar motor learning was first postulated by Albus, (1971) and

Marr, (1969). It is well accepted that motor learning undergoes a typical form of use-dependent plasticity in the brain (Kleim *et al.,* 2003; Butefisch *et al*., 2004). At the same time, it has been well established that the NMDA receptor (NMDAR) plays a central role in synaptic plasticity (Nakanishi, 1992). Accumulating evidence has indicated that NMDAR also plays a role in motor learning (Takehara *et al.,* 2004; Llansola *et al.,* 2005; Dang *et al.,* 2006). It was reported recently that NMDA receptors in cerebellar granule cells helped in voluntary motor training on motor learning in the mouse (Jiao *et al*., 2008).

Hypoglycaemic brain injury is a common and serious complication of insulin therapy and occurs most frequently in patients attempting tight glucose control (Davis *et al*., 1998). Neuronal death resulting from hypoglycaemia is the result of a series of events triggered by reduced glucose availability and the normalization of blood glucose levels does not necessarily block or reverse this cell death process once it has begun. Elimination of hypoglycaemia from the lives of people with diabetes and long term maintenance of euglycaemia will undoubtedly require glucose-regulated insulin replacement or secretion. Pending that ultimate goal, there is a critical need to develop therapeutic approaches that minimize both hyper- and hypoglycaemia. The only treatment for hypoglycaemia is blood glucose repletion and there is no currently available intervention for preventing the neuronal death that develops after hypoglycaemia is corrected. Recurrent hypoglycaemia in IDDM has become even more a major focus of research and clinical interest. The brain regions most vulnerable to hypoglycaemia are important for learning and memory. Accordingly, patients who recover from severe hypoglycaemia are left with difficulties in cognition, particularly short-term memory, out of proportion to gross motor disability (Langan *et al*., 1991). The preservation of neuron cell bodies is not always accompanied by normal synaptic activity and function (Li *et al*., 2003). Several lines of evidence

suggest that dopamine is associated with mechanisms underlying the neurobiologic response to metabolic stress.

Studies on the functional regulation of glutamate through NMDA receptors during diabetes and hypoglycaemia during adult and old will lead to a better understanding of the cognitive and memory function due to neuronal damage in the brain. The present study will be carried out to elucidate hypoglycaemic and diabetic effect on brain cellular function of glutamate through NMDA receptors.

Materials and Methods

Chemicals used and their sources

Biochemicals used in the present study were purchased from Sigma Chemical Co., St. Louis, USA. All other reagents were of analytical grade purchased locally.

Biochemicals

Streptozotocin, Glutamate, (+)MK-801 [(+)5-methyl-10,11-dihydro-5*H*-dibenzocyclohepten-5,10-imine maleate, collagenase type XI and bovine serum albumin fraction V, ethylene diamine tetra acetic acid (EDTA), Tris HCl, calcium chloride, HEPES - [n′ (2-hydroxy ethyl)] piperazine-n′-[2-ethanesulfonic acid], glycine, foetal calf serum (heat inactivated), citric acid, RPMI-1640 medium and paraformaldehyde were purchased from Sigma Chemical Co., St. Louis, USA. All other reagents were of analytical grade purchased locally from SRL, India.

Radiochemicals

L-[G-^{3}H]Glutamic acid (Sp. Activity 49.0 Ci/mmol) was purchased from Amersham Life Science, UK. (+)-[3-^{3}H] MK-801 (Sp. Activity 27.5 Ci/mmol) was purchased from Perkin Elmer Nen Life and Analytical Sciences, Boston, MA, USA. The [^{3}H] IP3, [^{3}H] cGMP and [^{3}H] cAMP Biotrak Assay Systems were purchased from G.E Healthcare UK Limited, UK. Radioimmunoassay kits for insulin and Triiodothyronine (T3) assay were purchased from Bhabha Atomic Research Centre (BARC), Mumbai, India

Molecular Biology Chemicals

Tri-reagent kit was purchased from Sigma Chemical Co., St. Louis, USA. ABI PRISM High Capacity cDNA Archive kit, Primers and Taqman probes for Real-Time PCR were purchased from Applied Biosystems, Foster City, CA, USA. NMDAR1 (Rn_00433800), NMDA2B (Rn00561352_m1), mGluR5 (Rn00566628_m1) and GLAST (Rn00570130_m1) primers were used for the gene expression studies using real time PCR.

Confocal Dyes

Ca^{2+} fluorescent dye- fluo 4-AM (Molecular Probes, Eugene, OR, USA), rat primary antibody for NMDAR1 (No: 556308, BD Pharmengin™), NMDA2B (No: 610416, BD Pharmengin™), mGluR5 (No: AB7130F, Chemicon) and secondary antibody of either FITC (No: AB7130F, Chemicon) or Rhodamine dye (No: AP307R Chemicon) were used for the calcium imaging and immunohistochemistry studies using confocal microscope.

Animals

Adult male Wistar rats of different age groups were purchased from Kerala Agriculture University, Mannuthy and Amrita Institute of Medical Sciences, Kochi and used for all experiments. They were housed in separate cages under 12 hours light and 12 hours dark periods and were maintained on standard food pellets and water *ad libitum*.

Induction of diabetes and hypoglycaemia

Both adult (30 weeks) and old (90 weeks) rats were divided into the following groups as i) Control ii) Diabetic iii) Insulin induced hypoglycaemia in diabetic rats (D+IIH) and iv) Insulin induced hypoglycaemia in control rats (C+IIH). Each group consisted of 6-8 animals.

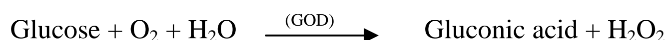
Diabetes was induced by a single intrafemoral dose, 55 mg/kg body weight, of STZ prepared in citrate buffer, pH 4.5 (Arison *et al*., 1967; Hohenegger

& Rudas, 1971). The D+IIH group received daily 2 doses, 10 Unit/Kg body weight of regular human insulin (Actrapid) and C+IIH received daily 2 doses, 1.5 Unit/Kg body weight of regular human insulin (Flanagan *et al*., 2003). D+IIH and C+IIH group had daily two episodes of insulin-induced hypoglycaemia for 10 days. Control rats were injected with citrate buffer.

Estimation of Blood Glucose

Blood glucose was estimated by GOD-POD glucose estimation kit (Biolab Diagnostics Pvt. Ltd.). The glucose was estimated at 0, 30, 60, 120, 180, 240, 300 min after the insulin administration. The spectrophotometric method using glucose oxidase-peroxidase reactions was as follows:

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



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

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

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

Estimation of Circulating Insulin by Radioimmunoassay

Principle of the assay

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

Assay Protocol

Standards, ranging from 0 to 200µU/ml, insulin free serum and insulin antiserum (50µl each) were added together and the volume was made up to 250µl with assay buffer. Samples of appropriate concentration from experiments were used for the assay. They were incubated overnight at 2°C. Then 50µl [^{125}I]insulin was added and incubated at room temperature for 3 hrs. 50µl second antibody was added along with 500µl of PEG. The tubes were then vortexed and incubated for 20 minutes and they were centrifuged at 1500 x g for 20 minutes. The supernatant was aspirated out and the radioactivity in the pellet was determined in a gamma counter.

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

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

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

Estimation of Circulating Triiodothyronine (T3) by Radioimmunoassay

Principle of the assay

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

Assay Protocol

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

A standard curve was plotted with $\%B/B_o$ on the Y-axis and T3 concentration (ng /ml) on the X-axis of a log-logit graph. $\%B/B_o$ was calculated as:

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

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

Tissue preparation

The rats were maintained in hypoglycemic condition for 10 days and were killed on the 11th day by decapitation after the experimental set up. The brain regions - cerebral cortex and cerebellum were dissected out quickly over ice according to the procedure of Glowinski and Iversen, (1966) and pancreas were dissected out quickly over ice. Hippocampus was dissected out quickly over ice according to the procedure of Heffner *et al*., (1980). 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 Glutamate

Glutamate content in the brain regions - cerebral cortex, cerebellum, hippocampus and pancreas of control experimental adult and old rat groups were quantified by displacement method using modified procedure of Enna and Snyder, (1976). Tissues were homogenized in 20 volumes of 0.32 M sucrose, 10 mM Tris/HCl and 1 mM $MgCl_2$ buffer, pH 7.4, with a polytron homogenizer. The homogenate was centrifuged twice at 27,000 x g for 15 min. The supernatant was pooled and used for the assay. The incubation mixture contained 1 nM [^{3}H] glutamate with and without glutamate at a concentration range of 10^{-9} M to 10^{-4} M. The unknown concentrations were determined from the standard displacement curve using appropriate dilutions and calculated for nmoles/g wt. of the tissue.

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

Glutamate Receptor Binding Studies Using [^{3}H]Glutamate

Membranes were prepared according to the modified method of Timothy *et al.,* (1984). The brain regions - cerebral cortex, cerebellum, hippocampus and pancreas were homogenized in 20 volumes of 0.32 M sucrose, 10 mM Tris-HCl and 1 mM $MgCl_2$ buffer, pH 7.4, with a polytron homogenizer. The homogenate

was centrifuged twice at 1,000 x g for 15 min at 4°C and the pellets were discarded. The supernatants were pooled and centrifuged at 27,000 x g for 15 min. The resulting pellet was lysed in a 10 mM Tris-HCl buffer, pH 7.4, for 30 min and centrifuged at 27,000 x g for 15 min. The resultant pellet was washed three times in 10 mM Tris/HCl buffer, pH 7.4, and centrifuged at 27,000 x g for 15 min. All steps were carried out at 4°C.

Membranes were incubated in 0.25 ml reaction mixture containing 25 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$ and 20 nM to 350 nM of [^{3}H]Glutamate containing 0.2 mg to 0.3 mg protein concentrations. Incubation was carried out at 30°C for 15 min and the reaction was stopped by centrifugation at 27,000 x g for 15 min. The pellet and the wall of the tube were quickly and carefully washed with ice-cold distilled water. 0.1% SDS and scintillation fluid were added to the dry pellet and radioactivity incorporated was determined with a Wallac scintillation counter. All the assays were carried out in triplicate. Nonspecific binding was determined by adding 350 μM nonradioactive glutamate to the reaction mixture in a parallel assay. Specific binding was considered to be the difference between total and nonspecific binding.

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

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). The brain regions - cerebral cortex, cerebellum, hippocampus and pancreas were homogenized in a 0.32 M sucrose buffer solution containing 10 mM HEPES, 1 mM EDTA buffer, pH 7.0. The homogenate was centrifuged at 1,000 × g for 10 min and the supernatant was centrifuged at 40,000 × g for 1 h. The pellet was resuspended and homogenized in 10 mM HEPES buffer containing 1.0 mM EDTA, pH 7.0 and centrifuged at

40,000 × g for 1 h. The final pellet was suspended in 10 mM HEPES, 1 mM EDTA buffer, pH 7.0 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 0.25 to 50 nM at 23°C in an assay medium containing 10 mM HEPES, pH 7.0, 200 - 250 µg of protein, 100 µM glycine and 100 µM glutamate. After 1 h of incubation, the reaction was stopped by filtration through GF/B filters and washed with HEPES buffer pH 7.0. Specific [^{3}H] MK-801 binding was obtained by subtracting nonspecific binding in the presence of 100 µM unlabeled MK-801 from the total binding. Bound radioactivity was counted with cocktail-T in a Wallac 1409 a liquid scintillation counter

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 GLUTAMATE RECEPTOR SUBTYPES - NMDAR1, NMDA2B, mGluR5 AND GLAST GLUTAMATE TRANSPORTER IN DIFFERENT BRAIN REGIONS AND PANCREAS OF CONTROL AND EXPERIMENTAL ADULT AND OLD RATS.

Preparation of RNA

RNA was isolated from the different brain regions - cerebral cortex, cerebellum, hippocampus and pancreas of control and experimental adult and old rats using the Tri reagent from Sigma Chemical Co., St. Louis, USA.

Isolation of RNA

Tissue (25-50 mg) homogenates were made in 0.5 ml 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 5 minutes. 100µl of chloroform was added to it, mixed vigorously for 15 seconds and allowed to stand at room temperature for 15 minutes. The tubes were then centrifuged at 12,000 x *g* for 15 minutes 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 250 µl of isopropanol was added and the tubes were allowed to stand at room temperature for 10 minutes. The tubes were centrifuged at 12,000 x *g* for 10 min at 4ºC. RNA precipitated as a pellet on the sides and bottom of the tube. The supernatants were removed and the RNA pellet was washed with 500 µl 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. 2 µl of RNA was made up to 1 ml and absorbance was measured at 260 nm and 280 nm in spectrophotometer (Shimadzu UV-1700 pharmaSPEC). For pure RNA preparation the ratio of absorbance at 260/280 was ≥ 1.7. The concentration of RNA was calculated as 1 OD at 260 = 42µg.

cDNA Synthesis

Total cDNA synthesis was performed using ABI PRISM cDNA Archive kit in 0.2 ml microfuge tubes. The reaction mixture of 20 μl contained 0.2 μg 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 10 minutes and 37°C for 2 hours using an Eppendorf Personal Cycler. The primers and probes were purchased from Applied Biosystems, Foster City, CA, USA designed using Primer Express Software Version (3.0).

Real -Time PCR Assay

Real Time PCR assays were performed in 96-well plates in an 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. 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 20 μl contained 25 ng of total RNA-derived cDNAs, 200 nM each of the forward primer, reverse primer and PCR analyses were conducted with gene-specific primers and fluorescently labelled Taqman probes of NMDAR1 (Rn_00433800), NMDA2B (Rn00561352_m1), mGluR5 (Rn00566628_m1) and GLAST (Rn00570130_m1). Endogenous control (β-actin) was labeled with a reporter dye (VIC). 12.5 μl of TaqMan 2X Universal

PCR Master Mix was taken and 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 AND PANCREAS OF CONTROL AND EXPERIMENTAL ADULT AND OLD RATS *IN VIVO*

The brain regions - cerebral cortex, cerebellum, hippocampus and pancreas of control and experimental adult and old rats were homogenised in a polytron homogeniser in 50 mM Tris-HCl buffer, pH.7.4, containing 1 mM EDTA to obtain a 15% homogenate. The homogenate was then centrifuged at 40,000 x g for 15 min. 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 25 pmoles/tube, [^{3}H]IP3 and binding protein were added together and the volume was made up to 100 µl with assay buffer. Samples of appropriate concentration from experiments were used for the assay. The tubes were then vortexed and incubated on ice for 15 minutes and they were centrifuged at 2000 x g for 10 minutes at 4°C. The supernatant was aspirated out and the pellet was resuspended in water and incubated at room temperature for 10 minutes. 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:

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

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

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

The brain regions - cerebral cortex, cerebellum, hippocampus and pancreas of control and experimental adult and old rats were homogenised in a polytron homogeniser with cold 50 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA to obtain a 15% homogenate. The homogenate was then centrifuged at 40,000 x g for 15 min 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 was 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 resuspended 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 4.0 pmoles/tube and [^{3}H]cGMP were added together and the volume was made up to 100 µl 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 90 minutes at 2 - 8°C. Ammonium sulphate was added to all tubes, mixed and allowed to stand for 5 minutes in ice bath. The tubes were centrifuged at 12000 x g for 2 minutes at room temperature. The supernatant was aspirated out and the pellet was resuspended in water and decanted immediately into

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

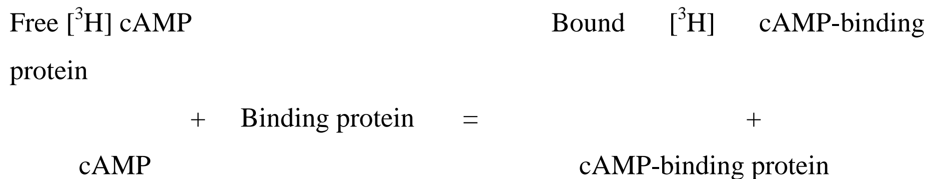
A standard curve was plotted with Co/Cx on the Y-axis and cGMP concentration (pmoles/tube) on the X-axis of a linear graph paper. Co - the cpm bound in the absence of unlabelled cGMP; Cx - the cpm bound in the presence of standard/unknown cGMP. cGMP concentration in the samples were determined by interpolation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

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

The brain regions - cerebral cortex, cerebellum, hippocampus and pancreas of control and experimental adult and old rats were homogenised in a polytron homogeniser with cold 50 mM Tris-HCl buffer, pH 7.4, containing 1 mM 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 was 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 was 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.



Separation of the protein bound cAMP from unbound nucleotide was 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 samples were 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 2 hours. 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 was calculated.

ROTAROD TEST

Rotarod has been used to evaluate motor coordination by testing the ability of rats to remain on revolving rod (Dunham & Miya, 1957). The control and experimental adult and old rats were subjected to rotarod test after 10 days of hypoglycaemic shock once daily for 15 days. Rotarod has been used to evaluate motor co-ordination by testing the ability of rats to remain on revolving rod. The apparatus has a horizontal rough metal rod of 3 cm diameter attached to a motor

with variable speed. This 70 cm long rod was divided into four sections by wooden partitions. The rod was placed at a height of 50 cm 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, 25 and 50 rpm after 10 days of treatment in all groups of rats.

ISOLATION OF PANCREATIC ISLETS

Pancreatic islets were isolated from male adult and old Wistar rats by standard collagenase digestion procedures using aseptic techniques (Howell & Taylor, 1968). The islets were isolated in HEPES-buffered sodium free Hanks Balanced Salt Solution (HBSS) (Pipeleers *et al.*, 1985) with the following composition: 137 mM Choline chloride, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1 mM KH_2PO_4, 14.3 mM $KHCO_3$ and 10 mM HEPES**.** Autoclaved triple distilled water was used in the preparation of the buffer.

The pancreas from both adult and old rats was aseptically dissected out into a sterile petridish containing ice cold HBSS and excess fat and blood vessels were removed. The pancreas was cut into small pieces and transferred to a sterile glass vial containing 2 ml collagenase type XI solution (1.5 mg/ml in HBSS, pH 7.4). The collagenase digestion was carried out for 15 minutes at 37°C in an environmental shaker with vigorous shaking (300rpm/minute). The tissue digest was filtered through 500 µm nylon screen and the filtrate was washed with three successive centrifugations and resuspensions in cold HBSS. The pancreatic islet preparation having a viability of >90% as assessed by Trypan Blue exclusion which was chosen for experiments.

CALCIUM IMAGING STUDIES USING CONFOCAL MICROSCOPE

Pancreatic islets were prepared from adult rats by collagenase digestion method as mentioned earlier. The isolated islets were incubated for 4 hours at

room temperature in 1 ml of calcium free RPMI medium containing 5 µM of Ca^{2+} fluorescent dye, fluo 4-AM (Molecular Probes, Eugene, OR) to monitor the changes in the intracellular Ca^{2+} and 1 mM, 4 mM and 20 mM of glucose. 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 de-esterification of intracellular AM esters. The 35 mm plates, containing pancreatic islet cells were placed on the stage of a Leica TCS SP5 laser scanning confocal microscope equipped with a HC PL FLUOTAR 20.0x 0.50 dry objective (NA 0.5). Fluo 4-AM was excited with 514 nm 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 508.4 nm – 571.5 nm. The images were continuously acquired before and after addition of 10^{-5} M Glutamate, 10^{-5} M MK801 (NMDA receptor antagonist), 10^{-5} M Dopamine and 10^{-5} M Sulpiride (Dopamine D_2 receptor antagonist), at time intervals of 26.35, 104.1 and 173.9 seconds. Time series experiments were performed collecting 512x512 pixel images at 400 Hz. 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.

NMDAR1, NMDA2B, mGluR5 AND IP3 RECEPTOR EXPRESSION STUDIES IN THE CEREBRAL CORTEX AND CEREBELLUM OF CONTROL AND EXPERIMENTAL RATS USING CONFOCAL MICROSCOPE

Control and experimental rats were deeply anesthetized with ether. The rat was transcardially perfused with PBS, pH- 7.4, followed by 4% paraformaldehyde in PBS (Chen *et al*., 2007). After perfusion the brains were dissected and

immersion fixed in 4% paraformaldehyde for 1 hr and then equilibrated with 30% sucrose solution in 0.1 M PBS, pH- 7.0. 40 µm sections were cut using Cryostat (Leica, CM1510 S). The sections were treated with PBST (PBS in 0.01% Triton X-100) for 20 min. Brain slices were incubated overnight at 4°C with either rat primary antibody for NMDAR1 (No: 556308 BD Pharmengin™, diluted in PBST at 1: 500 dilution), NMDA2B (No: 610416 BD Pharmengin™, diluted in PBST at 1: 500 dilution), mGluR5 (No: AB7130F, Chemicon, diluted in PBST at 1: 500 dilution) (polyclonal or monoclonal). After overnight incubation, the brain slices were rinsed with PBST and then incubated with appropriate secondary antibody of either FITC (No: AB7130F, Chemicon, diluted in PBST at 1: 1000 dilution) or Rhodamine dye (No:AP307R Chemicon, diluted in PBST at 1: 1000 dilution). The sections were observed and photographed using confocal imaging system (Leica SP 5).

NMDAR1, NMDA2B, mGluR5 AND IP3 RECEPTOR EXPRESSION STUDIES IN THE PANCREAS OF CONTROL AND EXPERIMENTAL RATS USING CONFOCAL MICROSCOPE

Pancreatic islets were prepared from adult rats by collagenase digestion method as mentioned earlier. The islets were seeded in culture wells and allowed to adhere to the plate. The islets were rinsed with PBS and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH- 7.0., for 30 minutes on ice. After fixation the islets were washed thrice with blocking buffer containing 0.1 M phosphate buffer, pH- 7.0., 0.1% Triton X and 10% BSA. Then the islets were incubated with primary antibody for NMDAR1 (No: 556308 BD Pharmengin™, diluted in PBST at 1: 500 dilution), NMDA2B (No: 610416 BD Pharmengin™,, diluted in PBST at 1: 500 dilution), mGluR5 (No: AB7130F, Chemicon, diluted in PBST at 1: 500 dilution) (polyclonal or monoclonal) prepared in blocking buffer with 1% serum and incubated overnight at 4°C. After the incubation the islets were washed thrice with blocking buffer. Then the islets were incubated with

appropriate secondary antibody of either FITC (No: AB7130F, Chemicon, diluted in PBST at 1: 1000 dilution) or Rhodamine dye (No:AP307R Chemicon, diluted in PBST at 1: 1000 dilution) diluted in blocking buffer with 1% serum and incubated at room temperature in dark for two hours. After incubation the islets were rinsed with blocking buffer and were observed and photographed using confocal imaging system (Leica SP 5).

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). Relative Quantification Software was used for analyzing Real-Time PCR results.

Results

BODY WEIGHT AND BLOOD GLUCOSE LEVEL OF EXPERIMENTAL RATS

Streptozotocin induced diabetic adult and old rats showed a significant ($p<0.001$) decrease in body weight after 10 days compared to their respective control. Insulin induced hypoglycaemia in adult and old diabetes (D+IIH) and control (C+IIH) rats showed no significant change in the body weight compared to their respective control (Table-1, 2; Fig-1, 2).

Diabetic adult and old rats showed a significant ($p<0.001$) increase in blood glucose compared to their respective control. There was a significant ($p<0.001$) decrease in blood glucose level of D+IIH and C+IIH adult and old rats compared to their respective diabetic and control rats (Table-3; Fig-3). Insulin administration to diabetic adult and old rats decreased blood glucose level significantly ($p<0.001$) below 50mg/dL after 3 hours and in control adult and old rats after 1 hour. The decreased glucose level reversed to diabetic level after 5 hours and control level after 2 hours respectively (Fig-4, 5).

CIRCULATING INSULIN LEVEL IN THE PLASMA OF CONTROL AND EXPERIMENTAL ADULT AND OLD RATS

Diabetic adult and old rats showed a significant ($p<0.001$) decrease in circulating insulin level compared to their respective control. There was a significant ($p<0.001$) increase in circulating insulin level of D+IIH and C+IIH adult and old rats compared to their respective diabetic and control rats. The adult and old C+IIH rats showed a significant ($p<0.01$) and ($p<0.001$) increase respectively in circulating insulin level compared to the respective D+IIH group (Table-4; Fig-6).

TRIIODOTHYRONINE (T3) CONTENT IN SERUM OF CONTROL AND EXPERIMENTAL ADULT AND OLD RATS

Diabetic adult and old rats showed a significant ($p<0.001$) decrease in T3 content compared to their respective controls. There was a significant increase in T3 content in adult ($p<0.01$) and old ($p<0.001$) D+IIH compared to their respective control rats. Also, the T3 content increased significantly ($p<0.001$) in D+IIH adult and old rats compared to their respective diabetic rats. The C+IIH adult and old rats showed significant ($p<0.001$) increase in the T3 content compared to their respective diabetic and control rats. The old C+IIH rats showed a significant ($p<0.001$) increase in T3 content compared to the old D+IIH group (Table-5; Fig-7).

GLUTAMATE CONTENT IN DIFFERENT BRAIN REGIONS AND PANCREAS OF CONTROL AND EXPERIMENTAL ADULT AND OLD RATS

Cerebral Cortex

Glutamate content in the cerebral cortex was significantly ($p<0.001$) increased in diabetic, D+IIH and C+IIH adult rats compared to control adult rats. In old experimental rats the glutamate content in the cerebral cortex was significantly increased in diabetic ($p<0.01$), D+IIH ($p<0.001$) and C+IIH ($p<0.001$) rats compared to control old rats. There was a significant ($p<0.001$) increase in glutamate content of D+IIH and C+IIH adult and old rats compared to their respective diabetic rats. The adult and old C+IIH rats showed a significant ($p<0.001$) increase in the glutamate content compared to the respective D+IIH group (Table-6; Fig-8).

Cerebellum

Glutamate content in the cerebellum was significantly ($p<0.001$) increased in diabetic, D+IIH and C+IIH adult and old rats compared to their respective control group. The adult D+IIH group showed a significant ($p<0.001$) increase in glutamate content compared to diabetic adult rats. The adult and old C+IIH rats showed a significant ($p<0.001$) increase in the glutamate content compared to the respective diabetic and D+IIH group (Table-7; Fig-9).

Hippocampus

Glutamate content in the hippocampus was significantly ($p<0.001$) increased in diabetic, D+IIH and C+IIH adult and old rats compared to their respective control group. The adult and old D+IIH group showed a significant ($p<0.001$) and ($p<0.01$) increase respectively in glutamate content compared to diabetic adult and old rats. The adult and old C+IIH rats showed a significant ($p<0.001$) increase in the glutamate content compared to the respective diabetic group. Also, a significant increase in glutamate content in hippocampus was observed in the adult ($p<0.001$) and old ($p<0.01$) C+IIH rats compared to the respective D+IIH group (Table-8; Fig-10).

Pancreas

Glutamate content in the pancreas was significantly ($p<0.001$) increased in diabetic, D+IIH and C+IIH adult and old rats compared to their respective control group. The adult and old D+IIH group showed a significant ($p<0.001$) and ($p<0.01$) increase respectively in glutamate content compared to diabetic adult and old rats. The adult and old C+IIH rats showed a significant ($p<0.001$) increase in the glutamate content compared to the respective diabetic and D+IIH group (Table-9; Fig-11).

GLUTAMATE AND NMDA RECEPTOR CHANGES IN THE BRAIN REGIONS AND PANCREAS OF CONTROL AND EXPERIMENTAL ADULT AND OLD RATS

Cerebral Cortex

Scatchard analysis of glutamate receptors using [^{3}H]Glutamate against glutamate

Scatchard analysis of [^{3}H]glutamate against glutamate in cerebral cortex of adult and old diabetic, D+IIH and C+IIH groups of rats showed a significant ($p<0.001$) increase in B_{max} compared to their respective control rats. D+IIH and C+IIH adult and old rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective diabetic group. Both adult and old C+IIH rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective D+IIH rats. There was no significant change in K_d in all experimental groups in both adult and old rats (Table-10, 11; Fig-12, 13).

Scatchard analysis of NMDA receptors using [^{3}H]MK801 against MK801

Scatchard analysis of [^{3}H]MK801 against MK801 in cerebral cortex of adult rats showed a significant increase in B_{max} in diabetic ($p<0.05$), D+IIH ($p<0.001$) and C+IIH ($p<0.001$) rats compared to control rats. In the old cerebral cortex there was a significant ($p<0.001$) increase in the B_{max} in diabetic, D+IIH and C+IIH rats compared to the control group. Both adult and old D+IIH and C+IIH rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective diabetic rats. There was a significant increase in B_{max} in adult ($p<0.01$) and old ($p<0.001$) C+IIH rats compared to the respective D+IIH rats. A significant ($p<0.001$) increase in K_d was observed in

the old C+IIH rats compared to old control, diabetic and D+IIH rats (Table-12, 13; Fig-14, 15).

Real-Time PCR analysis of NMDAR1 receptors

The Real-Time PCR analysis in the cerebral cortex showed a significant ($p<0.001$) increase in the expression of NMDAR1 receptor mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. There was a significant increase in expression of NMDAR1 receptors in adult ($p<0.001$) and old ($p<0.01$) D+IIH rats compared to the respective diabetic rats. Both adult and old C+IIH rats showed a significant ($p<0.001$) increase in expression of NMDAR1 receptors compared to the respective diabetic and D+IIH rats (Table-14, 15; Fig-16, 17).

Real-Time PCR analysis of NMDA2B receptors

The Real-Time PCR analysis in the cerebral cortex showed a significant ($p<0.001$) increase in the expression of NMDA2B receptor mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. There was a significant ($p<0.01$) increase in expression of NMDA2B receptors in adult and old D+IIH rats compared to the respective diabetic rats. C+IIH rats showed a significant ($p<0.001$) increase in expression of NMDA2B receptors in both adult and old compared to the respective diabetic rats. Also, a significant increase in the expression of NMDA2B receptors was observed in adult ($p<0.05$) and old ($p<0.01$) C+IIH rats compared to the respective D+IIH group (Table-16, 17; Fig-18, 19).

Real-Time PCR analysis of mGluR5 receptors

The Real-Time PCR analysis in the cerebral cortex showed a significant ($p<0.001$) increase in the expression of mGluR5 receptor mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. There was a significant ($p<0.001$) increase in expression of mGluR5 receptors in adult and old D+IIH and C+IIH rats compared to the respective diabetic rats. C+IIH rats showed a significant ($p<0.001$) increase in expression of mGluR5 receptors in both adult and old rats compared to the respective D+IIH rats (Table-18, 19; Fig-20, 21).

Real-Time PCR analysis of GLAST glutamate transporter

The Real-Time PCR analysis in the cerebral cortex showed a significant ($p<0.001$) decrease in the expression of GLAST glutamate transporter mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. There was a significant decrease in expression of GLAST glutamate transporter in adult ($p<0.01$) and old ($p<0.001$) D+IIH rats compared to the respective diabetic rats. C+IIH rats showed a significant ($p<0.001$) decrease in expression of GLAST glutamate transporter in both adult and old rats compared to the respective diabetic and D+IIH rats (Table-20, 21; Fig-22, 23).

Cerebellum

Scatchard analysis of glutamate receptors using [^{3}H]Glutamate against glutamate

Scatchard analysis of [^{3}H]glutamate against glutamate in cerebellum of adult and old diabetic, D+IIH and C+IIH groups of rats showed a significant ($p<0.001$) increase in B_{max} compared to their respective control. D+IIH and C+IIH adult and old rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective

diabetic group. Both adult and old C+IIH rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective D+IIH rats. The K_d showed a significant ($p<0.01$) increase in adult diabetic, D+IIH and C+IIH groups of rats compared to control adult rats. The D+IIH and C+IIH groups of adult rats showed a significant ($p<0.001$) increase in K_d compared to diabetic adult rats. Also, the C+IIH adult rats showed a significant ($p<0.01$) increase in K_d compared to D+IIH adult rats (Table-22, 23; Fig-24, 25).

Scatchard analysis of NMDA receptors using [^{3}H]MK801 against MK801

Scatchard analysis of [^{3}H]MK801 against MK801 in cerebellum of adult and old rats showed a significant ($p<0.001$) increase in B_{max} in diabetic, D+IIH and C+IIH rats compared to their respective control rats. D+IIH and C+IIH adult and old rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective diabetic group. Both adult and old C+IIH rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective D+IIH rats. There was no significant change in K_d in all experimental groups in both adult and old rats (Table-24, 25; Fig-26, 27).

Real-Time PCR analysis of NMDAR1 receptors

The Real-Time PCR analysis in the cerebellum showed a significant ($p<0.001$) increase in the expression of NMDAR1 receptor mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. There was a significant ($p<0.001$) increase in expression of NMDAR1 receptors in adult and old D+IIH and C+IIH rats compared to the respective diabetic rats. Both adult and old C+IIH rats showed a significant ($p<0.001$) increase in expression of NMDAR1 receptors compared to the respective D+IIH rats (Table-26, 27; Fig-28, 29).

Real-Time PCR analysis of NMDA2B receptors

The Real-Time PCR analysis in the cerebellum showed a significant ($p<0.001$) increase in the expression of NMDA2B receptor mRNA in adult diabetic, D+IIH and C+IIH rats compared to control adult rats. The Real-Time PCR analysis in the cerebellum showed a significant increase in the expression of NMDA2B receptor mRNA in old diabetic ($p<0.05$), D+IIH ($p<0.001$) and C+IIH ($p<0.001$) rats compared to control old rats. There was a significant ($p<0.01$) increase in expression of NMDA2B receptors in adult D+IIH rats compared to the respective diabetic rats. C+IIH rats showed a significant increase in expression of NMDA2B receptors in both adult ($p<0.01$) and old ($p<0.001$) compared to the respective diabetic rats. Also, a significant ($p<0.01$) increase in the expression of NMDA2B receptors was observed in adult and old C+IIH rats compared to the respective D+IIH group (Table-28, 29; Fig-30, 31).

Real-Time PCR analysis of mGluR5 receptors

The Real-Time PCR analysis in the cerebellum showed a significant ($p<0.001$) increase in the expression of mGluR5 receptor mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. There was a significant ($p<0.001$) increase in expression of mGluR5 receptors in adult and old D+IIH and C+IIH rats compared to the respective diabetic rats. C+IIH rats showed a significant ($p<0.001$) increase in expression of mGluR5 receptors in both adult and old rats compared to the respective D+IIH rats (Table-30, 31; Fig-32, 33).

Real-Time PCR analysis of GLAST glutamate transporter

The Real-Time PCR analysis in the cerebral cortex showed a significant ($p<0.001$) decrease in the expression of GLAST glutamate transporter mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. There was a significant ($p<0.001$) decrease in expression of GLAST glutamate transporter in both adult and old D+IIH rats compared to the respective diabetic rats (Table-32, 33; Fig-34, 35).

Hippocampus

Scatchard analysis of glutamate receptors using [^{3}H]Glutamate against glutamate

Scatchard analysis of [^{3}H]glutamate against glutamate in hippocampus of adult and old diabetic, D+IIH and C+IIH groups of rats showed a significant ($p<0.001$) increase in B_{max} compared to their respective controls. D+IIH and C+IIH adult and old rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective diabetic group. Both adult and old C+IIH rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective D+IIH rats. There was no significant change in K_d in all experimental groups in both adult and old rats (Table-34, 35; Fig-36, 37).

Scatchard analysis of NMDA receptors using [^{3}H]MK801 against MK801

Scatchard analysis of [^{3}H]MK801 against MK801 in hippocampus of adult and old rats showed a significant ($p<0.001$) increase in B_{max} in diabetic, D+IIH and C+IIH rats compared to their respective control rats. D+IIH and C+IIH adult and old rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective diabetic group. Both adult and old C+IIH rats showed a significant ($p<0.001$) increase

in B_{max} compared to the respective D+IIH rats. There was no significant change in K_d in all experimental groups in both adult and old rats (Table-36, 37; Fig-38, 39).

Real-Time PCR analysis of NMDAR1 receptors

The Real-Time PCR analysis in the hippocampus showed a significant ($p<0.001$) increase in the expression of NMDAR1 receptor mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. There was a significant ($p<0.001$) increase in expression of NMDAR1 receptors in adult and old D+IIH and C+IIH rats compared to the respective diabetic rats. A significant increased expression of NMDAR1 receptors in adult ($p<0.001$) and old ($p<0.05$) C+IIH rats was observed compared to the respective D+IIH rats (Table-38, 39; Fig-40, 41).

Real-Time PCR analysis of NMDA2B receptors

The Real-Time PCR analysis in the hippocampus showed a significant ($p<0.001$) increase in the expression of NMDA2B receptor mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. There was a significant ($p<0.001$) increase in expression of NMDA2B receptors in old D+IIH rats compared to the respective diabetic rats. C+IIH rats showed a significant ($p<0.001$) increase in expression of NMDA2B receptors in both adult and old compared to the respective diabetic rats. Also, a significant ($p<0.001$) increase in the expression of NMDA2B receptors was observed in old C+IIH rats compared to the D+IIH old rats (Table-40, 41; Fig-42, 43).

Real-Time PCR analysis of mGluR5 receptors

The Real-Time PCR analysis in the hippocampus showed a significant ($p<0.001$) increase in the expression of mGluR5 receptor mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. The mGluR5 receptors showed a significant increase in expression of in both adult ($p<0.001$) and old ($p<0.05$) D+IIH rats compared to the respective diabetic rats. C+IIH rats showed a significant ($p<0.001$) increase in expression of mGluR5 receptors in both adult and old compared to the respective diabetic rats. Also, a significant ($p<0.001$) increase in the expression of mGluR5 receptors was observed in old C+IIH rats compared to the D+IIH old rats (Table-42, 43; Fig-44, 45).

Real-Time PCR analysis of GLAST glutamate transporter

The Real-Time PCR analysis in the hippocampus showed a significant ($p<0.001$) decrease in the expression of GLAST glutamate transporter mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. The GLAST glutamate transporter showed a significant decrease in expression of in both adult ($p<0.01$) and old ($p<0.001$) D+IIH rats compared to the respective diabetic rats. In C+IIH group GLAST glutamate transporter showed a significant decrease in expression of in both adult ($p<0.01$) and old ($p<0.001$) rats compared to the respective D+IIH rats (Table-44, 45; Fig-46, 47).

Pancreas

Scatchard analysis of glutamate receptors using [^{3}H]Glutamate against glutamate

Scatchard analysis of [^{3}H]glutamate against glutamate in pancreas of adult and old diabetic, D+IIH and C+IIH groups of rats showed a significant ($p<0.001$)

increase in B_{max} compared to their respective control. D+IIH and C+IIH adult and old rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective diabetic groups. Both adult and old C+IIH rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective D+IIH rats. There was no significant change in K_d in all experimental groups in both adult and old rats (Table-46, 47; Fig-48, 49).

Scatchard analysis of NMDA receptors using [^{3}H]MK801 against MK801

Scatchard analysis of [^{3}H]MK801 against MK801 in pancreas of adult and old rats showed a significant ($p<0.001$) increase in B_{max} in diabetic, D+IIH and C+IIH rats compared to their respective control rats. D+IIH and C+IIH adult and old rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective diabetic groups. Both adult and old C+IIH rats showed a significant ($p<0.001$) increase in B_{max} compared to the respective D+IIH rats. There was no significant change in K_d in all experimental groups in both adult and old rats (Table-48, 49; Fig-50, 51).

Real-Time PCR analysis of NMDAR1 receptors

The Real-Time PCR analysis in the pancreas showed a significant ($p<0.001$) increase in the expression of NMDAR1 receptor mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. The NMDAR1 receptor expression showed a significant ($p<0.001$) increase in both adult and old D+IIH and C+IIH rats compared to their respective diabetic rats. A significant ($p<0.001$) increased expression of NMDAR1 receptors in adult C+IIH rats was observed compared to the adult D+IIH rats (Table-50, 51; Fig-52, 53).

Real-Time PCR analysis of NMDA2B receptors

The Real-Time PCR analysis in the pancreas showed a significant ($p<0.001$) increase in the expression of NMDA2B receptor mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. There was a significant ($p<0.001$) increase in expression of NMDA2B receptors in old D+IIH rats compared to the respective diabetic rats. C+IIH rats showed a significant ($p<0.001$) increase in expression of NMDA2B receptors in both adult and old compared to the respective diabetic rats. Also, a significant increase in the expression of NMDA2B receptors was observed in adult ($p<0.001$) and old ($p<0.05$) C+IIH rats compared to the respective D+IIH rats (Table-52, 53; Fig-54, 55).

Real-Time PCR analysis of mGluR5 receptors

The Real-Time PCR analysis in the pancreas showed a significant ($p<0.001$) increase in the expression of mGluR5 receptor mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. The mGluR5 receptors showed a significant increase in expression of in both adult ($p<0.001$) and old ($p<0.05$) D+IIH rats compared to the respective diabetic rats. C+IIH rats showed a significant ($p<0.001$) increase in expression of mGluR5 receptors in both adult and old compared to the respective diabetic rats. Also, a significant ($p<0.01$) increase in the expression of mGluR5 receptors was observed in old C+IIH rats compared to the D+IIH old rats (Table-54, 55; Fig-56, 57).

Real-Time PCR analysis of GLAST glutamate transporter

The Real-Time PCR analysis in the pancreas showed a significant ($p<0.001$) decrease in the expression of GLAST glutamate transporter mRNA in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. The

GLAST glutamate transporter showed a significant decrease in expression of in both adult ($p<0.05$) and old ($p<0.001$) D+IIH rats compared to the respective diabetic rats. C+IIH rats showed a significant ($p<0.001$) decrease in expression of GLAST glutamate transporter in both adult and old compared to the respective diabetic rats. In C+IIH group GLAST glutamate transporter showed a significant decrease in expression of in both adult ($p<0.001$) and old ($p<0.01$) rats compared to the respective D+IIH rats (Table-56, 57; Fig-58, 59).

SECOND MESSENGER ASSAYS

IP3, cGMP and cAMP content in the cerebral cortex of control and experimental adult and old rats

The IP3 content in the cerebral cortex was significantly ($p<0.001$) increased in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. The IP3 content showed a significant increase in both adult ($p<0.05$) and old ($p<0.001$) D+IIH rats compared to the respective diabetic rats. The C+IIH adult and old rats showed a significant ($p<0.001$) increase in the IP3 content when compared to their respective diabetic and D+IIH rats (Table-58, 59; Fig-60, 61).

The cGMP content in the cerebral cortex was significantly ($p<0.001$) increased in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. The cGMP content showed a significant ($p<0.001$) increase in adult and old D+IIH and C+IIH rats compared to the respective diabetic rats. Also, C+IIH rats showed a significant increase in the cGMP in adult ($p<0.01$) and old ($p<0.001$) rats when compared to their respective D+IIH rats (Table-58, 59; Fig-60, 61).

The cAMP content in the cerebral cortex was significantly ($p<0.001$) increased in both adult and old diabetic, D+IIH and C+IIH rats compared to their

respective control rats. The cAMP content showed a significant ($p<0.001$) increase in both adult and old D+IIH and C+IIH rats compared to the respective diabetic rats. Also, C+IIH rats showed a significant ($p<0.001$) increase in the cAMP in adult and old rats when compared to their respective D+IIH rats (Table-58, 59; Fig-60, 61).

IP3, cGMP and cAMP content in the cerebellum of control and experimental adult and old rats

The IP3 content in the cerebellum was significantly ($p<0.001$) increased in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. The IP3 content showed a significant ($p<0.001$) increase in adult and old D+IIH and C+IIH rats compared to the respective diabetic rats. The C+IIH adult and old rats showed a significant ($p<0.001$) increase in the IP3 content when compared to their respective D+IIH rats (Table-60, 61; Fig-62, 63).

The cGMP content in the cerebellum was significantly ($p<0.001$) increased in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. The cGMP content showed a significant increase in adult ($p<0.05$) and old ($p<0.01$) D+IIH rats compared to the respective diabetic rats. The cGMP content showed a significant ($p<0.001$) increase in adult and old C+IIH rats compared to the respective diabetic rats. Also, C+IIH rats showed a significant increase in the cGMP in adult ($p<0.01$) and old ($p<0.05$) rats when compared to their respective D+IIH rats (Table-60, 61; Fig-62, 63).

The cAMP content in the cerebellum was significantly ($p<0.001$) increased in adult diabetic, D+IIH and C+IIH rats compared to control rats. The cAMP content was significantly increased in old diabetic ($p<0.01$), D+IIH ($p<0.001$) and C+IIH ($p<0.001$) rats compared to control rats. The cAMP content showed a significant increase in both adult ($p<0.001$) and old ($p<0.01$) D+IIH rats compared to the diabetic

rats. The cAMP content showed a significant ($p<0.001$) increase in adult and old C+IIH rats compared to the respective diabetic rats. Also, C+IIH rats showed a significant increase in the cAMP in adult ($p<0.001$) and old ($p<0.05$) rats when compared to their respective D+IIH rats (Table-60, 61; Fig-62, 63).

IP3, cGMP and cAMP content in the hippocampus of control and experimental adult and old rats

The IP3 content in the hippocampus was significantly ($p<0.001$) increased in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. The IP3 content showed a significant increase in adult ($p<0.01$) and old ($p<0.001$) D+IIH rats compared to the respective diabetic rats. The C+IIH adult and old rats showed a significant ($p<0.001$) increase in the IP3 content when compared to their respective diabetic and D+IIH rats (Table-62, 63; Fig-64, 65).

The cGMP content in the hippocampus was significantly increased in adult diabetic ($p<0.05$), D+IIH ($p<0.001$) and C+IIH ($p<0.001$) rats compared to control adult rats. The cGMP content in the hippocampus was significantly ($p<0.001$) increased in old diabetic, D+IIH and C+IIH rats compared to control old rats. The cGMP content showed a significant increase in adult ($p<0.01$) and old ($p<0.001$) D+IIH rats compared to the respective diabetic rats. The cGMP content showed a significant ($p<0.001$) increase in adult and old C+IIH rats compared to the respective diabetic rats. Also, C+IIH rats showed a significant increase in the cGMP in adult ($p<0.001$) and old ($p<0.05$) rats when compared to their respective D+IIH rats (Table-62, 63; Fig-64, 65).

The cAMP content in the hippocampus was significantly ($p<0.001$) increased in adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. In the old D+IIH rats the cAMP content showed a significant ($p<0.001$) increase

compared to the old diabetic rats. The cAMP content showed a significant ($p<0.001$) increase in adult and old C+IIH rats compared to the respective diabetic and D+IIH rats (Table-62, 63; Fig-64, 65).

IP3, cGMP and cAMP content in the pancreas of control and experimental adult and old rats

The IP3 content in the pancreas was significantly ($p<0.001$) increased in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. The IP3 content showed a significant ($p<0.001$) increase in adult and old D+IIH and C+IIH rats compared to the respective diabetic rats. Also, C+IIH adult and old rats showed a significant ($p<0.001$) increase in the IP3 content when compared to their respective D+IIH rats (Table-64, 65; Fig-66, 67).

The cGMP content in the pancreas was significantly ($p<0.001$) increased in both adult and old diabetic, D+IIH and C+IIH rats compared to their respective control rats. The cGMP content showed a significant ($p<0.001$) increase in adult and old D+IIH and C+IIH rats compared to the respective diabetic rats. Also, C+IIH adult and old rats showed a significant ($p<0.001$) increase in the cGMP content when compared to their respective D+IIH rats (Table-64, 65; Fig-66, 67).

The cAMP content in the pancreas was significantly ($p<0.001$) increased in adult diabetic, D+IIH and C+IIH rats compared to adult control rats. The cAMP content significantly increased in old diabetic ($p<0.001$), D+IIH ($p<0.05$) and C+IIH ($p<0.01$) rats compared to old control rats. The cAMP content showed a significant ($p<0.001$) increase in adult and old D+IIH and C+IIH rats compared to the respective diabetic rats. The cAMP content showed a significant ($p<0.001$) increase in adult C+IIH rats compared to the adult D+IIH rats (Table-64, 65; Fig-66, 67).

Rotarod Performance of control and experimental groups of adult rats

Rotarod experiments showed a significant ($p<0.001$) decrease in the retention time on the rotating rod in adult diabetic, D+IIH and C+IIH rats group compared to control at 10 rpm, 25 rpm and 50 rpm. The D+IIH and C+IIH adult rats showed significantly ($p<0.001$) decreased retention time compared to adult diabetic group at 10 rpm, 25 rpm and 50 rpm. The C+IIH adult rats showed significantly ($p<0.001$) decreased retention time compared to adult D+IIH group at 10 rpm, 25 rpm and 50 rpm (Table-66; Fig-68).

Rotarod Performance of control and experimental groups of old rats

Rotarod experiments showed a significant ($p<0.001$) decrease in the retention time on the rotating rod in old diabetic, D+IIH and C+IIH rats group compared to control at 10 rpm, 25 rpm and 50 rpm. The D+IIH and C+IIH old rats showed significantly ($p<0.001$) decreased retention time compared to old diabetic group at 10 rpm, 25 rpm and 50 rpm. The C+IIH old rats showed significantly ($p<0.001$) decreased retention time compared to old D+IIH group at 10 rpm, 25 rpm and 50 rpm (Table-67; Fig-69).

CONFOCAL STUDIES

Cerebral Cortex

NMDAR1 receptor antibody staining in control and experimental groups of rats

The NMDAR1 receptor antibody staining in the cerebral cortex showed significant ($p<0.001$) increase in the NMDAR1 receptor in diabetic, D+IIH and C+IIH rats group compared to control. There was significant ($p<0.001$) increased expression of NMDAR1 receptors in the cerebral cortex of D+IIH and C+IIH rats group

compared to diabetic rats. The C+IIH rats showed significant ($p<0.001$) increased NMDAR1 receptor expression compared to D+IIH rats (Table-68; Fig-70).

NMDA2B receptor antibody staining in control and experimental groups of rats

The NMDA2B receptor antibody staining in the cerebral cortex showed significant ($p<0.001$) increase in the NMDA2B receptor in diabetic, D+IIH and C+IIH rats group compared to control. There was significant ($p<0.001$) increased expression of NMDA2B receptors in the cerebral cortex of D+IIH and C+IIH rats group compared to diabetic rats. The C+IIH rats showed significant ($p<0.001$) increased NMDA2B receptor expression compared to D+IIH rats (Table-68; Fig-71).

mGluR5 receptor antibody staining in control and experimental groups of rats

The mGluR5 receptor antibody staining in the cerebral cortex showed significant ($p<0.001$) increase in the mGluR5 receptor in diabetic, D+IIH and C+IIH rats group compared to control. There was significant ($p<0.001$) increased expression of mGluR5 receptors in the cerebral cortex of D+IIH and C+IIH rats group compared to diabetic rats. The C+IIH rats showed significant ($p<0.001$) increased mGluR5 receptor expression compared to D+IIH rats (Table-68; Fig-72).

IP3 receptor antibody staining in control and experimental groups of rats

The IP3 receptor antibody staining in the cerebral cortex showed significant ($p<0.001$) increase in the IP3 receptor in diabetic, D+IIH and C+IIH rats group compared to control. There was significant ($p<0.001$) increased expression of IP3 receptors in the cerebral cortex of D+IIH and C+IIH rats group compared to diabetic rats. The C+IIH rats showed significant ($p<0.001$) increased IP3 receptor expression compared to D+IIH rats (Table-68; Fig-73).

Cerebellum

NMDAR1 receptor antibody staining in control and experimental groups of rats

The NMDAR1 receptor antibody staining in the cerebellum showed significant ($p<0.001$) increase in the NMDAR1 receptor in diabetic, D+IIH and C+IIH rats group compared to control. There was significant ($p<0.001$) increased expression of NMDAR1 receptors in the cerebellum of D+IIH and C+IIH rats group compared to diabetic rats. The C+IIH rats showed significant ($p<0.001$) increased NMDAR1 receptor expression compared to D+IIH rats (Table-69; Fig-74).

NMDA2B receptor antibody staining in control and experimental groups of rats

The NMDA2B receptor antibody staining in the cerebellum showed significant ($p<0.001$) increase in the NMDA2B receptor in diabetic, D+IIH and C+IIH rats group compared to control. There was significant ($p<0.001$) increased expression of NMDA2B receptors in the cerebellum of D+IIH and C+IIH rats group compared to diabetic rats. The C+IIH rats showed significant ($p<0.001$) increased NMDA2B receptor expression compared to D+IIH rats (Table-69; Fig-75).

mGluR5 receptor antibody staining in control and experimental groups of rats

The mGluR5 receptor antibody staining in the cerebellum showed significant ($p<0.001$) increase in the mGluR5 receptor in diabetic, D+IIH and C+IIH rats group compared to control. There was significant ($p<0.001$) increased expression of mGluR5 receptors in the cerebellum of D+IIH and C+IIH rats group compared to diabetic rats. The C+IIH rats showed significant ($p<0.001$) increased mGluR5 receptor expression compared to D+IIH rats (Table-69; Fig-76).

IP3 receptor antibody staining in control and experimental groups of rats

The IP3 receptor antibody staining in the cerebellum showed significant ($p<0.001$) increase in the IP3 receptor in diabetic, D+IIH and C+IIH rats group compared to control. There was significant ($p<0.001$) increased expression of IP3 receptors in the cerebellum of D+IIH and C+IIH rats group compared to diabetic rats. The C+IIH rats showed significant ($p<0.001$) increased IP3 receptor expression compared to D+IIH rats (Table-69; Fig-77).

Pancreas

NMDAR1 receptor antibody staining in control and experimental groups of rats

The NMDAR1 receptor antibody staining in the pancreas showed significant ($p<0.001$) increase in the NMDAR1 receptor in diabetic, D+IIH and C+IIH rats group compared to control. There was significant ($p<0.001$) increased expression of NMDAR1 receptors in the pancreas of D+IIH and C+IIH rats group compared to diabetic rats. The C+IIH rats showed significant ($p<0.001$) increased NMDAR1 receptor expression compared to D+IIH rats (Table-70; Fig-78).

NMDA2B receptor antibody staining in control and experimental groups of rats

The NMDA2B receptor antibody staining in the pancreas showed significant ($p<0.001$) increase in the NMDA2B receptor in diabetic, D+IIH and C+IIH rats group compared to control. There was significant ($p<0.001$) increased expression of NMDA2B receptors in the pancreas of D+IIH and C+IIH rats group compared to diabetic rats. The C+IIH rats showed significant ($p<0.001$) increased NMDA2B receptor expression compared to D+IIH rats (Table-70; Fig-79).

mGluR5 receptor antibody staining in control and experimental groups of rats

The mGluR5 receptor antibody staining in the pancreas showed significant ($p<0.001$) increase in the mGluR5 receptor in diabetic, D+IIH and C+IIH rats group compared to control. There was significant ($p<0.001$) increased expression of mGluR5 receptors in the pancreas of D+IIH and C+IIH rats group compared to diabetic rats. The C+IIH rats showed significant ($p<0.001$) increased mGluR5 receptor expression compared to D+IIH rats (Table-70; Fig-80).

IP3 receptor antibody staining in control and experimental groups of rats

The IP3 receptor antibody staining in the pancreas showed significant ($p<0.001$) increase in the IP3 receptor in diabetic, D+IIH and C+IIH rats group compared to control. There was significant ($p<0.001$) increased expression of IP3 receptors in the pancreas of D+IIH and C+IIH rats group compared to diabetic rats. The C+IIH rats showed significant ($p<0.001$) increased IP3 receptor expression compared to D+IIH rats (Table-70; Fig-81).

CALCIUM IMAGING

Effect of 10^{-5}M Dopamine, 10^{-5}M Sulpiride on calcium release from Pancreatic Islets in the presence of 1mM, 4mM and 20mM Glucose

Dopamine at 10^{-5} M significantly ($p<0.001$) inhibited calcium release from the pancreatic islets in the presence of 1 mM glucose. Dopamine D_2 receptor antagonist sulpiride at 10^{-5} M significantly ($p<0.001$) reversed the inhibition from the pancreatic islets in the presence of 1 mM glucose (Table-71; Fig-82). In the normoglycaemic condition, 4mM glucose, 10^{-5} M dopamine significantly ($p<0.001$) increased the calcium release. Dopamine D_2 receptor antagonist sulpiride at 10^{-5} M significantly

($p<0.001$) inhibited the calcium release from the islet cells in the presence of 10^{-5} M dopamine (Table-72; Fig-83). In the hyperglycaemic condition, 20 mM glucose, 10^{-5} M dopamine significantly ($p<0.001$) increased the calcium release. Dopamine D_2 receptor antagonist sulpiride at 10^{-5} M significantly ($p<0.001$) inhibited the calcium release from the islet cells in the presence of 10^{-5} M dopamine (Table-73; Fig-84).

Effect of 10^{-5}M glutamate, 10^{-5}M MK801 on calcium release from Pancreatic Islets in the presence of 1mM, 4mM and 20mM Glucose

Glutamate at 10^{-5}M significantly ($p<0.001$) increased calcium release from the pancreatic islets in the presence of 1mM glucose. NMDA receptor antagonist MK801 at 10^{-5} M significantly ($p<0.001$) inhibited the release from the pancreatic islets in the presence of 1 mM glucose (Table-74; Fig-85). In the normoglycaemic condition, 4 mM glucose, 10^{-5} M glutamate significantly ($p<0.001$) increased the calcium release. NMDA receptor antagonist MK801 at 10^{-5} M significantly ($p<0.001$) inhibited the calcium release from the islet cells in the presence of 10^{-5} M glutamate and 4 mM glucose (Table-75; Fig-86). In the hyperglycaemic condition, 20 mM glucose, 10^{-5} M glutamate significantly ($p<0.001$) increased the calcium release. NMDA receptor antagonist MK801 at 10^{-5} M significantly ($p<0.001$) inhibited the calcium release from the islet cells in the presence of 10^{-5} M glutamate 20 mM glucose (Table-76; Fig-87).

Discussion

Glycemic control, a worthwhile goal for people with diabetes, is limited by the barrier of hypoglycemia (Cryer, 2008; Cryer, 2009). Hypoglycaemia constitutes a unique metabolic brain insult. Hypoglycaemic brain injury is a common and serious complication of insulin therapy and occurs most frequently in patients attempting tight glucose control (Davis *et al*., 1998). Hypoglycemia causes brain fuel deprivation that, if unchecked, results in functional brain failure that is typically corrected after the plasma glucose concentration is raised (Cryer, 2007). Rarely, it causes sudden, presumably cardiac arrhythmic death or, if it is profound and prolonged, brain death (Cryer, 2007). Several experimental models have been described which provide information on the etiology of IDDM. Streptozotocin is a toxic agent selective to pancreatic β-cells that induces IDDM by causing the β-cell destruction (Like & Rossini, 1976; Paik *et al*., 1980). Increased blood glucose and decreased body weight during diabetes is similar with previous reports as a result of the marked destruction of insulin secreting pancreatic islet β-cells by streptozotocin (Junod *et al.,* 1969). Hypoglycemia can cause recurrent morbidity in many people with type 1 diabetes and also in some with advanced type 2 diabetes (Zammitt & Frier, 2005; Cryer, 2009). Hyperglycaemia occurs as a result of increased glycogenolysis, decreased glycogenesis, increased gluconeogenesis, impaired glucose transport across membranes and almost complete suppression of the conversion of glucose into fatty acids *via* acetyl-CoA. Hyperglycaemic state during diabetes is due to the increased gluconeogenic pathway, which is physiologically less sensitive to the inhibition by insulin (Burcelin *et al*., 1995). During diabetes there is decrease in body weight as a result of altered metabolic function.

Administration of 1.5 U/Kg of regular insulin produced a fall in glucose level below 50mg/dL after 1hour in C+IIH rats (Joseph *et al*., 2008). The minimum required dose to produce irreversible severe hypoglycaemia was 0.5 units/kg (Abdul-Ghani *et al*., 1989). In D+IIH rats, administration of 10U/Kg of insulin decreased the blood glucose level below 50 mg/dL after 3hours (Joseph *et al*., 2008). It is well recognized that the glucose level is the primary determinant of the hormonal and metabolic counter regulatory responses to insulin induced hypoglycaemia. Falling plasma glucose concentrations elicit a sequence of responses that normally prevent or rapidly correct hypoglycemia (Cryer, 2001; Cryer, 2009). A single episode of very mild hypoglycaemia (56 mg/dL) causes a reduction of neuroendocrine counter regulation that is readily discernible about 24 h later. A similar effect of a single hypoglycaemic episode has been shown in healthy (Hvidberg *et al*., 1996) and diabetic (Dagogo *et al*., 1993) humans. The risk increases with a history of hypoglycemia and an increased number of years of insulin treatment and age (Cryer *et al*., 2003; Donnelly *et al*., 2005). Hypoglycaemia is the most common metabolic complication occurring in older people with type 2 diabetes (Abdelhafiz & Sinclair, 2009). Studies determined the incidence and risk factors for developing severe hypoglycaemia among persons aged 80 yr or older, with diabetes mellitus were more (Greco & Angileri, 2004; Chelliah & Burge, 2004). The glycemic levels during antecedent hypoglycaemia in those studies were 46–50 mg/dL. The plasma glucose level during antecedent hypoglycaemia has been shown to be a major determinant of the effects on subsequent counter regulation (Davis *et al*., 1997). Heller and Cryer, (1991) reported a reduced counter regulatory response 18 h after one mild hypoglycaemic episode (plasma glucose, 54 mg/dL). In fully developed (*i.e.,* C-peptide negative) type 1 diabetes, circulating insulin concentrations do not decrease as plasma glucose concentrations decline in response to therapeutic (exogenous)

hyperinsulinemia (Dagogo *et al*., 1993; Cryer, 2009). Recurrent episodes of hypoglycaemia have been demonstrated to reduce subsequent endocrine counter regulation (Heller & Cryer, 1991; Davis & Shamoon**,** 1991; Davis *et al*., 1992; Widom & Simonson, 1992; Veneman *et al*., 1993; George *et al*., 1995**;** Davis *et al*., 1997). The prolonged effects of even mild hypoglycaemia on subsequent counter regulation underline the importance of scrupulously avoiding even mild hypoglycaemic episodes in patients with diabetes. The body weights of D+IIH and Cl+IIH rats showed no significant change compared to control.

CENTRAL NERVOUS SYSTEM ALTERATIONS OF GLUTAMATE DURING HYPOGLYCAEMIA AND DIABETES

Diabetes mellitus is a metabolic disorder that not only causes a decrease in efficiency of the pancreatic β-cells to secrete insulin but also is accompanied by altered monoamine levels and their turnover rates in the CNS (Garris, 1990; Lackovic *et al*., 1990; Bhattacharya & Saraswathi, 1991). Glucose is the major source of brain energy and is essential for maintaining normal brain and neuronal function. Hypoglycemia causes impaired synaptic transmission. Oxidative stress plays an important role in tissue damage caused by hypoglycemia and diabetes, which results in deterioration in glucose homeostasis caused by these metabolic disorders. Hypoglycaemia is associated with increased glutamate release (Sandberg *et al.,* 1986) and conversely, glutamate toxicity in neurons is augmented by hypoglycaemia (Novelli *et al*., 1988). Hyperglycaemia is reported to be a major factor that damages the CNS monoaminergic activity as a result of neuronal degeneration in different regions of the brain. Onset of diabetes has been reported to inhibit the firing of dopaminergic neurons (Saller, 1984) with alteration in its metabolism. Our previous studies reported increased monoamine content in the plasma and platelet of diabetic

patients (Jackson *et al*., 1997). We reported increased NE in the brainstem of young diabetic rats while the NE content decreased in old diabetic rats (Abraham & Paulose, 1999). EPI content increased in adult diabetic rats without any change in NE. NE to EPI turn over showed a significant increase during diabetes (Pius & Paulose, 1999). There is a significant reduction in the cortical and brainstem 5-HT content of diabetic rats (Jackson & Paulose, 2000). Alterations of central neurotransmission and environmental factors change the relative contribution of sympathetic outflow to the pancreas, liver, adrenal medulla and adipose tissues, leading to the modulation of glucose and fat metabolism (Nonogaki, 2000). Studies revealed that during severe energy deprivation following hypoglycemia and diabetes, mitochondrial free radicals scavenger system is down regulated, which leads to reactive oxygen species (ROS) generation (Singh *et al*., 2004). Neurotransmitter alterations in the brain of insulin induced hypoglycaemic rats are poorly studied.

Glucose is known to serve as the major substrate for cerebral energy under normal conditions (Siesjo, 1978). Recent evidence suggests a direct correlation between glucose utilization and cognitive function (McNay *et al.,* 2000). Considerable evidence suggests that oxidative stress plays an important role in tissue damage associated with hypoglycemia and other metabolic disorders. The altered brain neurotransmitters metabolism, cerebral electrolyte contents and impaired blood-brain barrier function may contribute to CNS dysfunction in hypoglycemia (Bhardwaj *et al*., 1999). Reports suggest that glucose deprivation-induces damage by enhancing the formation of energy-yielding products and extracellular load of glutamate (Geng *et al*., 1997). Age-related cognitive impairments are associated with structural and functional changes in the cerebral cortex. It was previously demonstrated in the rat that excitatory and inhibitory pre- and postsynaptic changes occur with respect to age

and cognitive status; however, in aged cognitively impaired animals (Majdi *et al.*, 2007).

In the CNS, glutamate is an important factor for maintaining calcium homeostasis; it is the most abundant excitatory neurotransmitter and it is widely distributed. Glutamate is associated with various brain functions, such as synaptic plasticity, learning and long-term potentiation (Collingridge & Singer, 1990). Attention has been focussed on glutamate as a potential mediator of hypoglycaemic brain injury (Aral *et al.*, 1998; Cavaliere *et al.*, 2001; Marinelli *et al.*, 2001). We observed an increase in the glutamate content in the cerebral cortex, cerebellum, hippocampus and pancreas of both adult and old diabetic and hypoglycaemic rats. The increase in the glutamate content was more prominent in the adult and old hypoglycaemic group. Glutamate is essential for synaptic communication in the CNS, but inadequate regulation of extracellular glutamate and glutamate receptor agonists cause toxicity in the nervous system (Olney, 1989; Choi, 1992; Coyle & Puttfarcken, 1993; Greene & Greenamyre, 1996; Doble, 1999) leading to neurodegenerative disorders. Prolonged insulin-induced hypoglycemia (IIH) causes widespread loss of neurons and permanent brain damage with irreversible coma (Kleihues *et al.*, 1986). The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA kainate (Choi, 1988). Acute hypoglycemia was found to enhance the excitotoxic effects of glutamate in the newborn (McGowan *et al.*, 1995). Our previous studies also reported that GDH enzyme activity enhanced during diabetes and did not completely reverse even after insulin administration (Preetha *et al.*, 1996; Aswathy *et al.*, 1998). Studies using young and old diabetic rats clearly revealed that GDH activity regulation is essential to avoid diabetic associated brain glutamate toxicity (Biju & Paulose 1998). Studies from our laboratory have shown that an increased glutamate dehydrogenase activity

producing increased glutamate content in the cerebellum and thereby leading to glutamate toxicity (Joseph *et al*., 2007). Recent studies have shown that the abnormal accumulation of glutamate and NO plays a key mechanism of axonal degeneration in disorders such as multiple sclerosis (Ouardouz *et al*., 2009). It was shown by Larsen *et al*., (2006) that glucose deprivation caused 77% of the neurons are lost due to glutamate excitotoxicity. It was also shown that the main process implied in the neuronal cell death responsible for aging and the related neurodegenerative diseases are started by neurotrophic factors, hypoxia, hypoglycemia, excitotoxicity and oxygen and nitrogen free radicals (Peinado *et al*., 2000). Hypoglycemic brain damage associated with high levels of the excitatory amino acids aspartate and glutamate in the newborn piglets and adult pigs were demonstrated by Darling *et al*., (2001). Our studies on the cerebral cortex showed that the increased glutamate content increased brain damage during hypoglycemia compared with hyperglycemia which is suggested to contribute to cognitive and memory function (Joseph *et al*., 2008).

GLUTAMATE RECEPTOR ALTERATIONS AND FUNCTIONAL REGULATION IN CONTROL AND EXPERIMENTAL RATS

Cerebral Cortex

The cerebral cortex is the seat of our highest forms of intelligence. It plays a central role in many complex brain functions including memory, attention, perceptual awareness, thought, language and consciousness. One study has found positive association between the cortical thickness and intelligence (Katherine *et al*., 2007). Another study has found that the somatosensory cortex is thicker in migraine sufferers (Alexandre *et al*., 2007). All three ionotropic glutamate receptors exhibit a ubiquitous distribution in the brain, the NMDA receptors being particularly abundant in the

forebrain (Ozawa *et al*., 1998). Although all receptors have pivotal roles in brain functions, the NMDA receptors have received special attention in development and aging. They are involved in cell migration, growth and differentiation in the developing brain (Vallano, 1998). Furthermore, important roles have been assigned to them in cognitive functions, learning and memory (Kito *et al*., 1990). They also execute neuronal cell death (Szatkowski & Attwell, 1994), this possibly being relevant to aging of the brain and degenerative neurological diseases. Immunohistochemical studies have previously identified positive staining for presynaptic mGlu5 receptors in the rat cerebral cortex (Romano *et al*., 1995). The mGluR5 is reported to mediate a G-protein-dependent release of intracellular calcium stores (Valenti *et al*., 2002). Moreover, NMDA receptor function is inhibited by a rise in intracellular calcium (Rosenmund *et al*., 1995). Yu *et al*., (1997) pointed out that mGlu5 mediated direct inhibition *via* G-proteins also leads to NMDA receptor inhibition. NR2B is expressed selectively in the forebrain, with high levels in the cerebral cortex, hippocampal formation, septum, caudate-putamen, olfactory bulbs and thalamus. An increased NMDA2B mRNA level was found in the postmortem brain of Huntington's disease patients showing neuronal degeneration due to glutamate excitotoxicity (Arzberger *et al*., 1997). Metabotropic glutamate receptors (mGluRs) have various functions on neuronal excitability in the CNS (Pin & Duvoisin, 1995). Group I mGluRs are positively coupled to phosphoinositide hydrolysis and the mobilization of intracellular Ca^{2+} leading to excitotoxic cell death. Metabotropic glutamate regulates synaptic glutamate release both in *in vitro* (Herrero *et al*., 1994) rat brain slices (Croucher *et al*., 1997; Thomas *et al*., 2000) and *in vivo* (Patel & Croucher, 1997). The role of NMDA2B and mGluR5 receptors in hypoglycemic brain damage is not reported before.

Our findings report an increase in total glutamate and NMDA receptors function in the cerebral cortex with no significant change in K_d. This increased B_{max} observed shows the increased number of receptors with no change in the affinity of the receptors which was shown from the K_d. The increased receptor acitivity observed from the Scatchard plot was supported by the gene expression studies of NMDAR1, NMDA2B and mGluR5 glutamate receptor subtypes. Severe hypoglycemia with brain dysfunction limits intensified therapy in patients with insulin dependent diabetes mellitus, despite evidence that such therapy reduces the risk of chronic complications of the disease (Maran *et al*., 1994). Severe hypoglycemia causes neuronal death and cognitive impairment. Deficits in long-term potentiation during chronic diabetes arise from dysfunction of the NMDA subtype of glutamate receptors in early stages of the disease (Trudeau *et al*., 2004). The NMDA receptor is expressed in the cerebral cortex and hippocampus and is important in learning and memory. Recent studies reported that abnormal expression of NMDA receptor is involved in the development of diabetic neuropathy (Tomiyama *et al*., 2005). Among them, the neurotransmitter receptor NMDA shows strong age-related reduction of expression (Lu *et al*., 2004). Acute hypoglycemia was found to enhance the excitotoxic effects of glutamate in the newborn (McGowan *et al*., 1995). Previous studies reported that changes in the protein expression of the NMDA receptor subunits occur during the ageing process and it was greater than the changes seen in mRNA expression (Magnusson *et al*., 2002). Ageing does not affect all brain regions equally. Some regions seem to be more sensitive to ageing than others (Horiuchi & Saitoe, 2005; Lu *et al*., 2004). The brain regions - cerebral cortex and hippocampus of diabetic rats is suggested to be more vulnerable to glutamate toxicity *via* NMDA receptor activation. An age-related increase in mGlu1 receptor mRNA levels was found in thalamic nuclei, hippocampal CA3 with parallel increases in mGlu1a receptor protein expression (Simonyi *et al*.,

2005). Exposure to acute hypoglycemia in newborn piglets showed increased glutamate binding sites of cerebral NMDA receptors (McGowan *et al*., 2002). It has been shown from previous studies that NMDA receptors, as well as mGluRs, play important roles in the cascade of biochemical reactions resulting in death of neuronal cells *in vivo* (Tsintsadze *et al*., 2001). Studies have reported that rats subjected to severe hypoglycemia showed deficits in the Morris water maze test, a standard measure of learning and spatial memory (Sang *et al*., 2005). We studied the glutamate content, the total glutamate and NMDA receptor kinetics and the gene expression of NMDA2B and mGluR5 glutamate receptor subunits in the cerebral cortex of hypoglycemic and hyperglycemic rats. Also, the immunohistochemistry studies using confocal microscope for the expression of NMDAR1, NMDA2B, mGluR5 and IP3 receptors confirmed the Scatchard analysis and real time PCR results. Our results showed an increased glutamate content and glutamate receptor gene expression in the cerebral cortex of both hypoglycemic and diabetic rats. Our study is focused on the hypoglycemic shock usually happening in an insulin or antihyperglycemic therapy to diabetic patients. This frequent hypoglycemic shock is going to reduce the supply of glucose to the brain which will have deleterious effect to the functioning of brain cells. Our results suggest that glutamate receptor alterations found in the brain regions contributes to cognitive and memory deficits during diabetes and hypoglycaemia as a function of age. It is observed that there is occurrence of seizures in hypoglycemic state which is due to the decreased glucose (energy) for the brain cells to function (Yoshikawa *et al*., 2003; Gordon, 2006). Evidence suggests that hypoglycemic neuronal death involves excitotoxicity and DNA damage (Sang *et al*., 2007). It is widely accepted that energy deprivation causes a neuronal death that is mainly determined by an increase in the extracellular level of glutamate (Marinelli *et al*., 2001). Our studies on the cerebral cortex showed that the increased glutamate content

increased brain damage during hypoglycemia compared with hyperglycemia which is suggested to contribute to cognitive and memory function (Joseph *et al*., 2008).

The extracellular concentration of the excitatory neurotransmitter L-glutamate in the CNS must be kept low to ensure a high signal to noise ratio during synaptic activation (Katagiri *et al*., 2001) and to prevent excitotoxicity due to excessive activation of glutamate receptors (Mangano & Schwarcz, 1983; Rosenberg & Aizenman, 1989; Rosenberg *et al*., 1992; Rothstein *et al*., 1996; Tanaka *et al*., 1997; Wang *et al*., 1998) and this function is served by glutamate transporter proteins. Glutamate uptake into neurons and glia cells is important for termination of glutamatergic transmission. Glutamate transporters are essential for the maintenance of low extracellular levels of glutamate. Studies in brain autopsy specimens of HIV-1-infected patients have shown that the expression of EAAT-2 by activated microglia exert a compensatory effect that protects neurons from glutamate neurotoxicity (Xing *et al*., 2009). We observed a reduced expression of GLAST glutamate transporter which shows a reduced uptake of the extracellular glutamate which activated the glutamate receptor subtypes-NMDAR1, NMDA2B and mGluR5.

The present study showed that the second messengers- IP3, cGMP and cAMP were up regulated in the cerebral cortex of the entire experimental group of rats. All of glutamate receptors couple positively to phospholipase C *via* guanine nucleotide binding proteins (G-proteins) whereby they stimulate phosphoinositide hydrolysis generating a second messenger cascade consisting of diacylglycerol and inositol 1,4,5 trisphosphate (Berridge, 1987). Ng *et al*., (2004) reported that up regulation of glutamate receptors and calcium-binding proteins in the diabetic retina. Jo *et al*., (2008) demonstrated that NMDA and mGluR receptors mediate calcium release by stimulating IP3 and PKC. It was also reported that activation of the first-group mGluR (including mGluR1 and mGluR5) results in stimulation of metabolism of

inositol phosphates and results in mobilization of the intracellular calcium (Schoepp & Conn, 1993). Excessive stimulation of glutamate receptor/ion channel complexes triggers calcium flooding and a cascade of intracellular events that results in apoptosis and/or necrosis (Johnston, 2005). Prolonged stimulation of glutamate receptor subtypes, followed by intracellular Ca^{2+} overload and activation of specific genes, results in synthesis of enzymes involved in cell stress response (Caccamo *et al*., 2004). Excessive Ca^{2+} overload in cells have been reported to cause apoptosis. Boehning *et al*., (2003) demonstrated a small amount of cytochrome C released from mitochondria binds to and promote Ca^{2+} conductance through IP3 in the endoplamic reticulum membrane. The 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. Elevation of intracellular calcium can lead to cell death (Choi, 1994). Studies of Abdul-Ghani *et al*., (1996) revealed that mGluR-mediated the production of IP3 and the mobilization of intracellular Ca^{2+}. Increase in intranuclear Ca^{2+} that leads to altered transcription of apoptotic genes and activation of nuclear endonucleases resulting in hypoxia-induced programmed neuronal death (Mishra & Delivoria-Papadopoulos, 2004).

cGMP mediates physiological effects in the cardiovascular, endocrinological, and immunological systems as well as in CNS. In the CNS, activation of the NMDA receptor induces Ca^{2+}-dependent NOS and NO release, which then activates soluble guanylate cyclase for the synthesis of cGMP. Both compounds appear to be important mediators in long-term potentiation and long-term depression and thus play an important role in the mechanisms of learning and memory. Altered modulation of cGMP levels in brain seems to be responsible for the impairment of cognitive function (Erceg *et al*., 2005). cGMP has been implicated in the regulation of many essential functions in the brain, such as synaptic plasticity, phototransduction, olfaction and

behavioural state. Studies have reported that activation of N-methyl-d -aspartate receptors causes increase in cGMP in hyperammonemic rats (Cauli *et al*., 2008). Calcium flux through the NMDA receptor activates neuronal nitric oxide synthase (nNOS), which produces NO. In neurons activated by nitric oxide produces cGMP (Serulle *et al*., 2008). In the present work we observed an increase in cGMP in the cerebral cortex of hypoglycaemic and diabetic group with more prominent up regulation in the hypoglycaemic group. The NO/cGMP/PKG-mediates pathological mechanism that leads to hyperexcitability and sensitizes neurons to excitotoxic damage in neurodegenerative disorders (González-Forero *et al*., 2007). Our study showed the increased cAMP content in the cerebral cortex of hypoglycaemic and diabetic rats. Up regulation of cAMP activates cAMP dependent protein kinase resulting in phosporylation.

The receptor analysis, gene expression and immunohistochemistry studies implicate a role for glutamate receptor subtypes in the manifestation of the cognitive and memory deficits associated with hypoglycaemia and diabetes in adult and old rats. The decreased glutamate transporter GLAST expression reduces the reuptake of the extracellular glutamate which was confirmed from the glutamate content analysis. The increased glutamate content and the receptor activity enhanced the second messengers- IP3, cGMP and cAMP which lead to the calcium influx and neurodegeneration. The enhanced glutamate receptors were more prominent in hypoglycemic group which is of significance in this study This increased brain damage observed during hypoglycemia compared with hyperglycemia is suggested to contribute to cognitive and memory function.

Cerebellum

Experimental evidence indicate the involvement of the cerebellum in variety of human mental activities including language (Fiez *et al*., 1996), attention (Allen *et al*., 1997), cognitive affective syndromes (Schmahmann & Sherman, 1998), fear and anxiety caused by threats of pain (Ploghause *et al*., 1999), thirst sensation and fear for air hunger (Parsons *et al*., 2001) and motor relearning (Imazumi *et al*., 2004; Hermann *et al*., 2004; Jiao *et al*., 2008). The cerebellum is known to be resistant to hypoglycaemia. Studies from our laboratory have demonstrated that cerebellum is susceptible to hypoglycaemia (Joseph *et al*., 2007). Some of the most frequent signs of cerebellar hypoplasia include poor fine motor skills, hypotonia and autistic features (Wassmer *et al*., 2003). The cerebellar vermis integrates and processes the inputs from the vestibular, visual and proprioceptive systems to coordinate muscle timing as a result of which the centre of gravity stays within the limits of stable upright standing (Diener *et al*., 1989). Damage to the cerebellum, in particular the vermis (Baloh *et al*., 1998) results in more postural sway than in control subjects (Ho *et al*., 2004, Marvel *et al*., 2004). Decreased postural stability would correspond with abnormalities of the vermis observed in autistic subjects (Gowen & Miall, 2005). Unlike explicit memory such as recognition memory and spatial memory, motor learning is characterized by slow development, without the requirement of conscious recall, and in general being lifetime-lasting (Llinas & Welsh, 1993; Tulving & Markowitsch, 1998; Eichenbaum, 2000). Based on the role of the cerebellum in motor activities such as fine motor movement and motor coordination as well as the computational network within the neural circuitries, cerebellar motor learning was first postulated by Marr (1969) and Albus (1971).

Recent studies have shown the involvement of NMDA receptor subunits-NMDAR1, NMDA2B in the cerebellum in motor learning in mouse (Jiao *et al*.,

2008). Our investigation revealed an increase in the glutamate content and glutamate receptor number. The Scatchard analysis of NMDA receptors also showed an increased NMDA receptor number with no significant change in the affinity of the receptors. Studies of Yan and Rivkees, (2006) reported that hypoglycaemia inhibits oligodendrocyte development and myelination and that hypoglycemia triggers apoptotic cell death in oligodendrocyte precursor cells in the cerebellum. It is thought that the combination of extracellular glutamate accumulation and mitochondrial damage is involved in neuronal death associated with brain ischemia and hypoglycemia and some neurodegenerative diseases such as Huntington's disease (Bittigau & Ikonomidou, 1997). Reports have suggested that the accumulation of endogenous extracellular glutamate after inhibition of its transporters induces a stimulation of mitochondrial respiratory chain activity, which leads to ROS production and GSH deficiency in a manner dependent on NMDA receptor activation (García *et al.,* 2005). Earlier studies have conclude that during severe energy deprivation following hypoglycemia and diabetes, mitochondrial free radicals scavenger system is down regulated, which leads to reactive oxygen species (ROS) generation. High levels of ROS in turn activate the processes leading to DNA damage (Singh *et al.,* 2004). Metabotropic glutamate (mGlu) regulate synaptic glutamate release both in *in vitro* (Herrero *et al.,* 1994) rat brain slices (Croucher *et al*., 1997) and *in vivo* (Patel & Croucher, 1997). We also observed an increase in the gene expression of NMDAR1, NMDA2B, mGluR5 receptor sutypes. Activation of mGluRs modulates NMDA receptor activity and is implicated in synaptic transmission and activity-dependent synaptic plasticity (Pin & Duvoisin, 1995; Conn & Pin, 1997; Nicoletti *et al*., 1999).

The cerebellum is known to be resistant to hypoglycaemia, and selective cerebellar dysfunction caused by hypoglycaemia has not been reported. In a case of

episodic bilateral cerebellar dysfunction caused by hypoglycaemia, quantitative dynamic PET study demonstrated decreased glucose uptake-to-utilization ratio and increased leak of glucose in the cerebellum indicating that cerebellum is not invariably resistant to hypoglycaemia (Auer 2004; Kim *et al*., 2005). It is widely accepted that energy deprivation causes a neuronal death that is mainly determined by an increase in the extracellular level of glutamate (Marinelli *et al*., 2001). Glutamate which causes excitotoxic neuronal damage, increases calcium influx through NMDA receptors in post synaptic neurons, leading to phospholipase A_2 mediated arachidonic acid release (Miriam *et al*., 1996). Our previous studies on cerebellum also reported that GDH enzyme activity enhanced during diabetes and did not completely reverse after insulin administration (Preetha *et al*., 1996; Aswathy *et al*., 1998). Studies using young and old diabetic rats clearly revealed that in cerebellum GDH activity regulation is essential to avoid diabetic associated brain glutamate toxicity (Biju & Paulose, 1998). Increased number of glutamate receptor activity leading to glutamate excitotoxicity and neuronal degeneration were reported from our lab (Joseph *et al*., 2007).

One of the major causes of neuronal death in neurodegenerative disease is excitotoxicity from the neurotransmitter glutamate. This form of cell death arises from either excess levels of glutamate due to decreased astrocyte clearance or due to increased susceptibility. Several glutamate transporters have been characterized, the Na^+–dependent glutamate/ aspartate transporter, GLAST being the major uptake system within the cerebellum (Danbolt, 2001). We also observed a decrease in the GLAST glutamate transporter expression in both adult and old diabetic and hypoglycaemic rats. Previous studies from our lab showed the increased glutamate production *via* increased glutamate dehydrogenase enzyme activity (Joseph *et al*., 2007). The present study showed the increased glutamate content and NMDA receptor

number and gene expression in the cerebellum. The protective role of GLAST glutamate transporter in the multiple sclerotic cerebellum was reported by Mitosek-Szewczyk *et al*., (2008).

It has been shown from previous studies that NMDA receptors, as well as metabotropic glutamate receptors, play important roles in the cascade of biochemical reactions resulting in death of neuronal cells *in vivo* (Tsintsadze *et al*., 2001). Pharmacological tools now allow for the examination of the role of metabotropic glutamate receptors (mGluRs) in the development of sensitization (Spooren *et al*., 2000). mGluRs regulate synaptic transmission by modulating calcium and potassium channels and the activity of ionotropic glutamate receptors. mGluR5 receptors modulate NMDA receptor function because both receptors have been linked as signaling partners (O'Leary *et al*., 2000; Movsesyan *et al*., 2001; Kotecha *et al*., 2003). The activation of mGluR5 receptors leads to the potentiation of NMDA currents (Bleakman *et al*., 1992; Cerne & Randic, 1992), possibly through the activation of protein kinase C and the subsequent increase in intracellular Ca^{2+}, thereby acting as an indirect agonist of NMDA receptors (Benquet *et al*., 2002; Fujii *et al*., 2004). NMDA receptor activation in the cerebellum leads to an increase in the Ca^{2+} also *via* IP3 receptors. Our results also show an increase in the IP3, cGMP and cAMP content in the cerebellum of hypoglycaemic and diabetic adult and old rats. The immunohistochemistry study done using confocal microscope also showed an increased expression of NMDAR1, NMDA2B, mGluR5and IP3 receptors. Suvarna and 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 induce cGMP formation in the cultured cerebellar granule cells. Increased IP3 activation leads to Ca^{2+} influx which in turn activates neuronal nitric oxide synthase (nNOS) to produce NO (Garthwaite, 2005). NO activates soluble guanylyl cyclase

(sGC) to generate increased levels of cGMP which in turn activates protein kinase G (PKG) (Garthwaite, 2005) in the cerebellum. cGMP modulates phosphorylation in cerebellum by changing the relationship between cGMP-dependent protein kinase and type 2 inhibitor content (Biggio *et al*., 1977). The ability of rats to learn a Y-maze conditional discrimination task depends on the function of the glutamate–nitric oxide–cGMP pathway in brain (Piedrafita *et al*., 2007). Infusion of D-serine (1 mM) enhanced (150-200%) extracellular cGMP in the cerebellum with no age-related differences (Vallebuona & Raiteri, 1995). cGMP-dependent signal transduction in hippocampus and cerebellum is insufficient in senescent brain and have functional consequences in disturbances of learning and memory processes (Chalimoniuk & Strosznajder, 1998). Group I mGluRs couple positively to phospholipase C, the activation of which leads to stimulation of protein kinase C and release of intracellular Ca^{2+} (Conn & Pin, 1997), or to adenylyl cyclase, activation of which stimulates cAMP formation (Aramori & Nakanishi, 1992; Joly *et al*., 1995).

Rotarod test has been previously used to examine motor in-coordination (Cendelín *et al*., 2008). The rotarod experiment demonstrated the impairment in the motor function and coordination in the hypoglycaemic and diabetic adult and old rats. All the experimental rats showed lower fall off time from the rotating rod when compared to control suggesting impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture and at the same time adjust their limb movements on the metallic rod and is indicative of cerebellar dysfunction. Many other brain regions have been associated with timing tasks including the dorsal lateral premotor cortex, inferior parietal lobe, supplementary motor area, superior temporal gyrus, caudal putamen, ventrolateral thalamus and inferior frontal gyrus (Rao *et al*., 1997; Jancke *et al*., 2000; Lewis & Miall, 2003). However, increased timing variance has been observed in patients with cerebellar disorders (Ivry *et al*.,

1988). Loss of coordination of motor movement, inability to judge distance and timing, incapacity to perform rapid alternating movements and hypotonia has been reported during cerebellar damage (Gowen & Miall, 2005). Poor limb - eye coordination in patients with cerebellar dysfunction has been earlier report (Van Donkelaar & Lee, 1994). Studies conducted in subjects with cerebellum lesion, showed deficits in learning associated with component movement (motor learning) from deficits in performing compound movement (such as motor coordination) (Mussa-Ivaldi & Bizzi, 2000; Krakauer & Shadmehr, 2006). The lower fall of time shown by our experimental rats confirmed the cerebellar dysfunction. Thus the upregulation of glutamate receptor activity in the cerebellum causing the increase in second messengers which mediates the Ca^{2+} overload in the cells, leads to neurodegeneration. The enhanced NMDA receptors were more prominent in hypoglycaemic group which is of significance in this study suggesting that hypoglycaemia is causing more damage to the brain at the molecular level than the hyperglycaemic condition.

To summarize, our findings suggest dysfunction of the diabetic and hypoglycaemic cerebellum in both adult and old rats that is a reflection of cerebellar glutamatergic abnormality. The receptor analysis and gene expression studies along with the behavioural data implicate a role for glutamate, NMDA and mGlu5 receptors in the modulation of neuronal network excitability *via* changes in IP3, cGMP and cAMP. These neurofunctional deficits are one of the key contributors to motor deficits and stress associated with insulin induced hypoglycaemia and diabetes. The enhanced neurodegeneration in hypoglycaemia is suggested to have more impairment of the motor learning and memory ability which has clinical significance in the diabetes treatment.

Hippocampus

The effect of hypoglycaemic episodes is visible in brain regions associated with memory, especially the hippocampus. The hippocampal formation contains a rich glutamatergic and GABA-ergic input, GABA-ergic interneurones containing peptide co-transmitters and the glutamatergic perforant pathway interconnects with entorhinal cortex, subiculum, CA1, CA3 fields and dentate gyrus (Ottersen & Storm-Mathisen, 1984). Potentiation, defined as an increase in synaptic efficacy, is readily induced by high frequency stimulation (HFS) of the synapses between the Schaffer collaterals and the pyramidal cells in the hippocampus CA1 area (Collingridge & Bliss, 1995; Malenka & Nicoll, 1999). The excitatory synapse in the stratum radiatum of the CA1 area of the hippocampus has a number of features that have been attributed to various aspects of memory encoding (Martin *et al.,* 2000). In this study, we focused on the glutamate receptor, which is abundantly expressed throughout the hippocampal formation. Our results showed increased glutamate content in the hippocampus of diabetic, D+IIH and C+IIH rats compared to control. The hippocampus contains a high concentration of NMDA receptors. Our experiments revealed an increased glutamate and NMDA receptor number in adult and old hypoglycaemic and diabetic rats. The expression studies also showed an increased expression of NMDAR1, NMDA2B and mGluR5 receptors in the hippocampus of experimental rats. These particular receptors are vulnerable to hypoglycaemic episodes. Studies of Ennis *et al*., (2008) suggested that hippocampus is vulnerable to hypoglycemia-induced neuronal death. Also, studies suggest that children with type I diabetes who experience hypoglycaemia exhibit impairment of hippocampal-dependent memory (Hershey *et al*., 1999). When hippocampal cultures were deprived of glucose, massive release of lactate dehydrogenase (LDH), an indicator of neuronal death, occurred *via* NMDA receptor activation (Geng *et al*., 1997). Neurons impaired of energy metabolism are

highly sensitive to excitotoxicity (Simon *et al*., 1984; Wieloch, 1985; Monyer *et al*., 1992; Cebers *et al*., 1998). Hippocampal region of the brain is particularly vulnerable to the adverse effects of hypoglycaemia (Abdelmalik *et al*., 2007). Pathological studies in humans and animals have shown that hypoglycaemia-induced neuronal death occurs preferentially in the hippocampus, superficial layers of the cortex and striatum (Wieloch *et al*., 1985; Auer 2004; Camacho & Massieu, 2006). It is reported that profound hypoglycaemia selectively damages CA1 and the dentate gyrus of the hippocampus (Tasker *et al*., 1992). The selectively greater reduction in hippocampal cerebral blood flow (CBF) indicate severe impairment in glucose metabolism at moderate levels of hypoglycaemia in these structures as compared with the remainder of the brain (Denise *et al*., 2004). Hippocampal neurons receive a rich glutamergic innervation and evidence suggests that hypoglycaemic injury in these neurons is precipitated almost entirely by sustained glutamate receptor activation (Auer *et al*., 1985). Tanaka *et al.,* (2008) reported that absence of glucose, insulin accelerated the neuronal cell death both in the CA1 and DG. They also concluded that insulin has a double-edged effect on the neuronal cell death dependent on glucose concentration and that the CA1 and the DG have a different sensitivity to insulin in terms of cell survival. Recent reports suggest that both hypoglycaemia and hyperglycaemia have adverse effects on the brain neuronal structural changes and impaired long-term spatial memory (Malone *et al*., 2008). Long-term potentiation of neuronal activity in the hippocampus is thought to be a substrate for learning and memory. Gasparova *et al*., (2008) revealed that prolonged exposure to hypoglycaemic state influenced induction of LTP in the hippocampus and that it had deleterious effects on learning and memory. Ageing process affects NMDA receptors more in the intermediate hippocampus than the dorsal hippocampus (Magnusson *et al*., 2006). The dysfunction in hippocampal LTP, an electrophysiological model of synaptic plasticity thought to

subserve learning and memory processes is associated with diabetic conditions (Di Mario *et al*., 1995; Biessels *et al*., 1996). Abdelmalik *et al*., (2007) suggested that suppressing seizures during hypoglycemia using NMDA antagonist, decrease subsequent neuronal damage and dysfunction in hippocampus.

Based on extensive supportive experimental data, the release of high levels of glutamate by neurons is thought to be the underlying mechanism for the initiation of hypoglycaemic neurodegeneration. Quintana *et al.,* (2006) reported that transient anoxia/hypoglycaemia is associated with a marked enhancement of excitatory transmission with an increased synthesis of excitatory receptor subunits in organotypic hippocampal slice cultures. Our experimental results support the earlier reports. The immunohistochemistry experiments in the present work supported the gene expression studies of NMDAR1, NMDA2B and mGluR5 receptors. This up regulation will increase the glutamate receptor activity and molecular cascades inside the cells. Our experiments also demonstrated decreased expression of GLAST glutamate transporter in the hippocampus of experimental rats compared to control. This decreased expression of glutamate transporter will lead to the decreased clearance of glutamate from the extracellular space and we report in our present study that glutamate content is high in the hippocampus of experimental group compared to control. Up regulation of NMDA receptor and down regulation of glutamate transporter expression suggests a response to altered synaptic glutamate levels (Lyon *et al*., 2008). It was found that GLAST glutamate transporter down regulation is involved in cell swelling in hippocampus during hypoglycaemia (Han *et al.,* 2004).

Our experimental results also showed an increase in the IP3, cGMP and cAMP content in the hippocampus of experimental adult and old rats compared to control. Inositol 1, 4, 5-trisphosphate receptors (IP3Rs) mediate calcium release and they are involved in many biological processes and IP3R activity regulators will

contribute to neuronal functions (Kawaai *et al*., 2009). Ambient glutamate release from astrocytes occurs in a Ca^{2+}-independent manner (Cavelier & Attwell, 2005; Malarkey & Parpura, 2008). Astrocytes in the hippocampus release calcium from intracellular stores intrinsically and in response to activation of G(q)-linked G-protein-coupled receptors through the binding of inositol 1,4,5-trisphosphate to its receptor (Foskett *et al*., 2007). Our data showed an increased expression of IP3 receptors in all the experimental conditions which will lead to increased secretion of glutamate. IP3 induced Ca^{2+} overload will cause neurodegeneration (Zhu *et al*., 2006). . Increase in intranuclear Ca^{2+} that leads to altered transcription of apoptotic genes and activation of nuclear endonucleases, result in hypoxia-induced programmed neuronal death (Mishra & Delivoria-Papadopoulos, 2004).

To summarize our results in hippocampus we observed an increased glutamate and NMDA receptor activity. A decreased glutamate transporter expression and increased glutamate content was observed. Enhanced NMDA receptor functions activated the second messenger cascade and our study showed increased IP3, cGMP and cAMP content. Glutamatergic neurotransmission is critically involved in many aspects of complex behaviour and cognition. Up regulated glutamatergic activity mediates neurodegeneration in the hippocampus. Thus our study showed impairment in the hippocampal glutamate system during hypoglycaemia and diabetes. This glutamatergic dysfunction in the hippocampus was intense in hypoglycaemia compared to diabetes which contribute to towards cognitive and memory deficits.

Pancreas

Insulin secretion from the pancreatic islets is controlled by the central nervous system through sympathetic and parasympathetic nerves (Burr *et al*., 1976; Campfield & Smith, 1980; Ahren, 2000). Recent studies from our laboratory described the

regulatory role of the sympathetic and parasympathetic systems in pancreatic regeneration (Renuka *et al*., 2004, 2005; Mohanan *et al*., 2005a, b). Pancreatic islets receive innervations from both divisions of the autonomic nervous system, and pancreatic endocrine secretion is partly controlled by the autonomic nervous system (Liu *et al*., 2001). Anatomical studies suggest that the vagal efferent fibers originating from the nucleus ambiguus and dorsal motor nucleus of the brainstem directly innervate the pancreas (Bereiter *et al*., 1981) and have a role in neurally mediated insulin release (Azmitia & Gannon, 1986). Our laboratory has reported that dopamine differentially regulates glucose induced insulin secretion in the pancreatic islets, an effect mediated by pancreatic DA D2 receptors (Eswar *et al*., 2006). Studies from our laboratory suggests that the down-regulation of DA D2 receptors could influence the regulation of insulin secretion by releasing epinephrine and norepinephrine from the adrenal medulla, which leads to the inhibition of insulin secretion in the pancreas (Eswar *et al*., 2007).

The present study showed an up regulation in the glutamate content, glutamate and NMDA receptor activity in the experimental groups. It was shown in previous study that enhanced GDH produced glutamate, a second messenger of insulin secretion (Anno *et al.,* 2004). Glutamate receptor agonists induce various cellular responses outside the CNS, such as a rise in intracellular calcium concentration in rat pituitary cells and stimulation of growth hormone secretion (Lindstrom & Ohlsson, 1992), stimulation of insulin and glucagons secretion from rat endocrine pancreas (Bertrand *et al.,* 1992; Bertrand *et al.,* 1993) and contractions of the myenteric plexus-longitudinal muscle of guinea pig ileum (Shannon & Sawyer, 1989). These pharmacological studies suggest the presence of glutamate receptors in peripheral tissues, including endocrine tissues. Two important findings were reported regarding a relationship between glutamate metabolism and insulin secretion. A new

form of persistent hyperinsulinemia with hypoglycemia of the infant (PHHI) was demonstrated to be caused by an excessive activity of glutamate dehydrogenase, which produces glutamate (Stanley *et al.,* 1998; Stanley *et al.,* 2000; Macmullen *et al*., 2001). Second, glutamate produced via α-ketoglutarate from glucose was reported to enhance insulin secretion under conditions of clamped cytosolic Ca^{2+} and ATP at high levels (Macmullen *et al*., 2001). Although the direction of metabolic flux between glutamate and α-ketoglutarate upon stimulation with glucose has been controversial in β-cells (Gao *et al*., 1999; MacDonald & Fahien, 2000), these results raised a novel postulation that glutamate play a role in transducing secretory signals from glucose metabolism to secretory vesicles and that this pathway involve in modulation of secretory vesicle pH, the acidity of which is thought to be generated mainly by vacuolar-type H^+-ATPase (Hutton & Peshavaria, 1982; Hutton, 1989; Bode *et al*., 1996; Nelson & Harvey, 1999). An increase in β-cell glutamate is an important messenger in the amplification of insulin secretion by glucose (Bertrand *et al*., 2002). We observed an increase in the gene expression of NMDAR1, NMDA2B and mGluR5 receptors in the pancreas of adult and old experimental rats compared to their control rats. Molnár *et al.,* (1995) and Inagaki *et al.,* (1995) reported the presence of NMDA receptor subunits in the pancreatic islets and also that the glutamate receptor ligands and NMDA increased insulin secretion. Studies have reported that glutamate, transmitted from α-cells and neurons, stimulates insulin secretion through activation of ionotropic glutamate receptors in β-cells (Inagaki *et al.,* 1995). Glutamate receptors classified into the ionotropic glutamate receptors, functioning as ion channels and the metabotropic glutamate receptors, coupled to intracellular second messenger systems (Nakanishi, 1992b). We also have found an increase in the second messenger- IP3, cGMP and cAMP content in the pancreas. It has been demonstrated by Cabrera *et al*., (2008) that glutamate acts on iGluRs, resulting in membrane depolarization, opening

of voltage-gated Ca^{2+} channels, increase in cytoplasmic free Ca^{2+} concentration. Our studies also support this. The activation of second messengers enhanced the Ca^{2+} release from pancreatic islets which was demonstrated from our *in vitro* studies. IP_3 mediates rapid mobilization of Ca^{2+} from the endoplasmic reticulum, whereas diacylglycerol stimulates protein kinase C (Berridge *et al*., 2003). Recent studies by Diederichs, (2008) demonstrate that Ca^{2+} release stimulated by IP3 increased insulin secretion. Activation of the GLP-1-R on β- cells initiates a complex series of signalling events that include cAMP production, membrane depolarization, an increase of intracellular calcium concentration and exocytosis (Thorens, 1992; Holz *et al*., 1993, 1995, 1999; Gromada *et al*., 1995, 1998a, b; Bode *et al*., 1999; Nakazaki *et al*., 2002; Eliasson *et al*., 2003). Kang *et al*., (2005) demonstrated that cAMP production, promote Ca^{2+} mobilization in pancreatic β- cells and thereby increase insulin release.

Glutamate acts as an intracellular messenger that couples glucose metabolism to insulin secretion (Maechler & Wollheim, 1999). Glutamate produced *via* α-ketoglutarate from glucose was reported to enhance insulin secretion under conditions of clamped cytosolic Ca^{2+} and ATP at high levels (Macmullen *et al*., 2001). Our experiments showed that the glutamate content and the NMDA receptor activity were increased in both hypoglycaemic and diabetic condition. The GLAST glutamate transporter expression was decreased in both hypoglycaemic and diabetic condition. Previous studies reported that an increased islet content of L-glutamate is necessary, but not sufficient, to allow its net conversion into 2-oxoglutarate and its further metabolism in the Krebs cycle or the GABA shunt. This and the subsequent stimulation of insulin secretion, requires activation of GDH by L-leucine (Li *et al*., 2006). The insulin secretion stimulated by glutamate was blocked by an inhibitor of vacuolar type H^+-ATPase or by an inhibitor of vesicular glutamate transporter (Gao *et*

al., 1999; MacDonald *et al*., 2000). Decreased GLAST activity during diabetes could account for the inhibition of insulin secretion. It was also reported that the enhanced glutamate activity during insulin induced hypoglyceamia in pancreas also enhanced glucagon release (Cabrera *et al*., 2008).

Effect of dopamine on Ca^{2+} release from pancreatic islets *in vitro*

The control of insulin secretion by the pancreatic β-cell is achieved through a complex metabolic cascade converting glucose and other nutrients into signals leading to appropriate insulin release (Wollheim, 2000). Neurotransmitters especially catecholamines play an important role in insulin secretion. Dopamine is reported to modulate insulin secretion in the pancreatic islets (Nogueira *et al*., 1994; Eswar *et al*., 2006). Dopamine in the islets is essential for maintaining the equilibrium of insulin secretion.

In hypoglycaemic condition, dopamine significantly inhibited insulin secretion by pancreatic islets. Dopamine at high concentration is reported to inhibit insulin secretion from the islets (Nogueira *et al*., 1994). Our results also showed inhibition of Ca^{2+} release from pancreatic islets at 10^{-5} M concentration of dopamine in hypoglycaemic condition. In hyperglycemic condition, we observed a significant stimulation of Ca^{2+} release from pancreatic islets at 10^{-5} M concentration of dopamine. Previous studies from our laboratory also showed a maximum inhibition of insulin secretion at high concentration of dopamine in hypoglycaemic condition. In hyperglycemic condition a significant stimulation of insulin secretion at low concentration of dopamine and inhibition at high concentration was observed (Robinson, 2007). Thus the present study also supports that the concentration of dopamine is very critical for glucose homeostasis.

Modulation of insulin secretion by dopamine depends on specific receptor-receptor interactions. Our study showed that dopamine D2 receptor antagonist sulpiride significantly blocked the inhibitory action of dopamine on Ca^{2+} release from pancreatic islets at hypoglycaemic condition and stimulatory action of dopamine during hyperglycaemic condition. Studies have reported that dopamine D_2 receptors are expressed in pancreatic cells and inhibit glucose induced insulin secretion (Blanca *et al*., 2005, Eswar *et al*., 2006; 2007). The role and the peripheral mechanism of action of central dopamine on basal pancreatic exocrine secretion in conscious rats revealed that central dopamine inhibited pancreatic exocrine secretion *via* dopamine D_1 like receptors and that the inhibitory effect is mediated *via* sympathetic nerves, especially α-adrenoceptors (Blanca *et al*., 2005). Thus our studies suggest that dopamine D2 receptors are involved in the dopamine regulation of insulin secretion *via* Ca^{2+} release from pancreatic islets

Previous studies from our laboratory reported that addition of forskolin an activator of cAMP resulted in overcoming the effect of dopamine on insulin secretion (Abraham, 1998). The agonists of dopamine by acting through the neuroendocrine system improve peripheral energy metabolism and impaired islet function. 7-OH DPAT showed an inhibitory effect on glucose induced insulin secretion. Previous reports suggest that 7-OH DPAT induced hyperglycaemia decreased insulin secretion (Uvnäs-Moberg *et al.,* 1996).

It was found that dopamine D2 receptor antagonists effectively blocked the stimulatory and inhibitory effect of dopamine on Ca^{2+} release from pancreatic islets. Dopamine through dopamine D2 receptors differentially regulate the Ca^{2+} release from pancreatic islets and thereby insulin secretion during hypoglycaemia and diabetes. Thus our results suggest that dopamine acting through dopamine D_2 receptors regulate the glucose homeostasis. This has immense clinical significance.

Effect of glutamate on Ca^{2+} release from pancreatic islets *in vitro*

Glutamate acts as an intracellular messenger that couples glucose metabolism to insulin secretion (Maechler & Wollheim, 1999). Recent studies reported that insulin secretion is under the control of mGlu5 receptors (Storto *et al*., 2006). The role of NMDA receptor subunit of glutamate on glucose induced insulin secretion by pancreatic islets is poorly studied. In our *in vitro* Ca^{2+} release studies, glutamate significantly increased Ca^{2+} release from pancreatic islets in both hypoglycaemic and hyperglycaemic condition. Elevation of ATP is necessary for the membrane-dependant increase in cytosolic Ca^{2+}, the main trigger of insulin exocytosis (Maechler & Wollheim, 2000). It has been demonstrated by Cabrera *et al*., (2008) that glutamate acts on iGluRs, resulting in membrane depolarization, opening of voltage-gated Ca^{2+} channels, increase cytoplasmic free Ca^{2+} concentration. Intracellular Ca^{2+} measurements and electrophysiological recordings studies indicated that kainate, AMPA and NMDA all elicit increases of Ca^{2+} in single pancreatic β-cells and depolarize them (Inagaki *et al*., 1995). NMDA receptor antagonist, MK-801, blocked the stimulatory action of glutamate in hypoglycaemic and hyperglycaemic conditions. Previous studies reported that glutamate produced via α-ketoglutarate from glucose enhanced insulin secretion under conditions of clamped cytosolic Ca^{2+} and ATP at high levels (Macmullen *et al*., 2001). Our results suggest that glutamate regulation of insulin secretion is mediated through NMDA receptor which has therapeutic applications.

Our molecular and behavioural results showed that hypoglycaemic condition has more functional damage in brain than diabetes. The receptor mediated functional studies and *in vitro* studies using antagonists for the receptor subtypes confirmed the specific receptor mediated dopaminergic and glutamatergic brain damage in hypoglycaemia and diabetes. Thus it is suggested that the corrective measures for the

brain functional damage caused during diabetes and anti-diabetic treatment, through glutamatergic receptors, have clinical significance in the therapeutic management of hypoglycaemia and diabetes.

Summary

1. Insulin induced hypoglycaemia and streptozotocin induced diabetes in adult and old rats were used as models to study alterations in brain glutamatergic function and receptor subtype gene expression in hypoglycaemia and diabetes.

2. The body weight was analyzed to study the changes in body weight in hypoglycaemic and diabetic adult and old rats compared to control. Diabetes caused a reduction in the body weight in adult and old rats while hypoglycaemic adult and old rats did not show any significant change in the body weight.

3. Blood glucose level in the serum was measured to analyze the circulating glucose level changes due to hypoglycaemia and diabetes in adult and old rats compared to control. Diabetic adult and old rats showed increased blood glucose level. The D+IIH and C+IIH adult and old group showed significant reduction in blood glucose level. The blood glucose analysis also revealed that the D+IIH adult and old rats became hypoglycaemic at the 3rd hour and the C+IIH adult and old rats became hypoglycaemic at the 1st hour.

4. The circulating insulin level was analysed to study the changes insulin concentration in hypoglycaemic and diabetic adult and old rats compared to control. Diabetic adult and old rats showed a significant decrease in insulin level. The D+IIH and C+IIH adult and old group showed significant increase in the insulin concentration.

5. Serum T3 concentration was decreased in diabetic adult and old rats. The D+IIH and C+IIH adult and old group showed significant increase in the serum T3 content.

6. Glutamate content increased in the cerebral cortex, cerebellum, hippocampus and pancreas of adult and old diabetic, D+IIH and C+IIH rats.

7. Glutamatergic receptor functional status was analysed by Scatchard analysis using $[^3H]$glutamate. The total glutamate receptors in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old groups increased with decreased affinity in the cerebral cortex of old C+IIH rats. Thus an enhanced total glutamate function observed in different brain regions and pancreas, had a differential effect during hypoglycaemia and diabetes.

8. NMDA receptor functional status was analysed by Scatchard analysis using $[^3H]$MK801. The NMDA receptors in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old groups increased with no significant change the affinity.

9. NMDA mediates its action through its subunits –NMDAR1, NMDA2B, mGluR5. NMDA receptor binding parameters were confirmed by studying the mRNA status of the corresponding receptor using Real-Time PCR. NMDAR1, NMDA2B, mGluR5 receptors showed increased expression in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old rats. This shows a co-actvation of NMDA receptors subunits that affect

glutamate mediated functions. This enhanced activity of NMDA receptors produce intracellular signals through activation of signaling pathways.

10. To prevent glutamate mediated excitotoxic effects it should be cleared from the extracellular space by the glutamate transporters. The gene expression of GLAST glutamate transporter was studied in hypoglycaemic and diabetic adult and old rats compared to control. GLAST glutamate transporter showed decreased expression in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old rats. This shows less reuptake of extracellular glutamate formed in the experimental condition.

11. The IP3 levels increased significantly in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old rats compared to their respective controls.

12. The cGMP levels increased significantly in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old rats compared to their respective controls.

13. The cAMP levels increased significantly in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old rats compared to their respective controls.

14. Behavioural studies of the experimental groups of rats were carried out using rotarod test to assess the changes in the motor learing and motor in-coordination. The experiment demonstrated the impairment in the motor function and

coordination in the diabetic, D+IIH and C+IIH adult and old rats compared to their respective control rats by showing lower fall of time. The diabetic, D+IIH and C+IIH adult and old rats showed lower fall of time with increased rpm of the metallic rod compared to their respective control rats.

15. The increased expression of NMDAR1, NMDA2B and mGluR5 receptors in the diabetic, D+IIH and C+IIH rats obtained from the Real-time PCR was confirmed by confocal studies using receptor specific antibodies in the brain slices and pancreatic islets. Increased expression of IP3 receptors was also observed in the diabetic, D+IIH and C+IIH rats using receptor specific antibodies in the brain slices and pancreatic islets.

16. Calcium imaging results showed that dopamine at 10^{-5}M inhibited calcium release from the pancreatic islets in hypoglycaemic condition. Dopamine D2 receptor antagonist sulpiride at 10^{-5} M reversed the inhibition from the pancreatic islets in hypoglycaemic condition. In the normoglycaemic condition 10^{-5} M dopamine increased the calcium release. Dopamine D2 receptor antagonist sulpiride at 10^{-5}M inhibited the calcium release from the islet cells in the presence of 10^{-5} M dopamine. In the hyperglycaemic condition 10^{-5} M dopamine increased the calcium release. Dopamine D2 receptor antagonist sulpiride at 10^{-5} M inhibited the calcium release from the islet cells in the presence of 10^{-5} M dopamine.

17. Calcium imaging results showed that glutamate at 10^{-5}M increased calcium release from the pancreatic islets in the presence of 1 mM glucose. NMDA receptor antagonist MK801 at 10^{-5} M inhibited the release from the pancreatic islets in the presence of 1 mM glucose. In the normoglycaemic condition 10^{-5} M

glutamate increased the calcium release. NMDA receptor antagonist MK801 at 10^{-5} M inhibited the calcium release from the islet cells in the presence of 10^{-5} M glutamate and 4 mM glucose. In the hyperglycaemic condition 10^{-5} M glutamate increased the calcium release. NMDA receptor antagonist MK801 at 10^{-5} M inhibited the calcium release from the islet cells in the presence of 10^{-5} M glutamate and 20 mM glucose.

It is evident from our results that brain glutamate and NMDA receptor functional balance plays a major role in hypoglycaemia and diabetes management as a function age. Gene expression studies of NMDAR1, NMDA2B, mGluR5 receptor subunits and GLAST glutamate transporter showed a prominent glutamatergic functional disturbance in brain regions and pancreas of hypoglycaemic and diabetic adult and old rats. These findings have important implications for understanding the molecular mechanisms underlying memory and cognitive impairment by second messengers due to hypoglycaemia, diabetes and ageing. *In vitro* calcium release studies confirmed the regulatory role of dopamine, dopamine D2 receptor, glutamate and NMDA receptor subtypes in insulin secretion from pancreatic islets. A differential secretion of thyroid hormones in hypoglycaemia and diabetes was observed, which is an indicative of impairment in metabolic and neurological functions. The enhanced receptor activity and the second messenger cascades will lead to Ca^{2+} overload and thereby excitotoxic neurodegeneration. This affected the cognitive, memory and motor ability of the experimental rats.

Thus our studies showed hypoglycaemic and hyperglycaemic effect on brain function of glutamate through NMDA receptors, second messengers and *in vitro* studies confirming the receptor subtypes functional regulation. It is suggested that the

corrective measures for the brain functional damage caused during diabetes and anti-diabetic treatment, through glutamergic receptors, have therapeutic role in the management of hypoglycaemia and hyperglycaemia.

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Conclusion

Hypoglycaemia is the major obstacle to optimal blood glucose control in the treatment of diabetic patients. Severe hypoglycaemia triggers a cascade of events in vulnerable neurons that culminate in cell death even after glucose normalization. Our findings demonstrated that glutamatergic system is impaired during hypoglycaemia and diabetes. The evaluations of these damages have important implications in understanding the molecular mechanism underlying cognitive deficits due to intensive insulin treatment in diabetics. Glutamate content increased during hypoglycaemia and diabetes. We observed a prominent significant increase of glutamate content in the brain during hypoglycaemia compared to diabetes. This increased glutamate content caused an increase in glutamatergic function. NMDA receptor subtypes- NMDAR1, NMDA2B and mGluR5 have differential regulatory role in different brain regions and pancreas during hypoglycaemia and diabetes. Dopamine, through DA D2 receptors and glutamate through NMDA receptors regulates insulin secretion. *In vitro* studies on calcium release using specific antagonist of DA D2 and NMDA receptors have confirmed the role of these receptors in hypoglycaemic and diabetic conditions. The differential functional balance of these receptors control the glucose mediated insulin secretion. The binding parameters of glutamate and NMDA receptors and gene expression studies of NMDAR1, NMDA2B and mGluR5 receptors and GLAST glutamate transporter in diabetic, D+IIH and C+IIH showed a differential functional regulation during hypoglycaemia and diabetes. Hypoglycaemic brain showed an increased glutamate toxicity mediated through NMDA than in diabetic brain. The second messenger study confirmed that the changes in the receptor levels did enhance the IP3, cGMP and cAMP levels. These studies suggest that NMDA receptor potentiates Ca^{2+} release through IP3 receptor activation. Increased Ca^{2+} release is

suggested to trigger release of Cytochrome C thereby initiating the cell damage during hypoglycaemic stress. The behavioural studies by rotarod test show a decrease in motor activity in the hypoglycaemic and diabetic rats with more prominent decrease in hypoglycaemic rats. Thus our results showed that hypoglycaemic condition has more functional damage than diabetic in adult and old rats. It is suggested that the corrective measures for the brain functional damage caused during diabetes and anti-diabetic treatment, through glutamatergic receptors, have therapeutic role in the management of hypoglycaemia and diabetes.

References

Abbatecola AM, Maggi S, Paolisso G. (2008). New approaches to treating type 2 diabetes mellitus in the elderly: role of incretin therapies. *Drugs Aging*, 25: 913-925.

Abdelhafiz AH, Sinclair AJ. (2009). Hypoglycaemia in residential care homes. *Br J Gen Pract*., 59: 49-50.

Abdelmalik PA, Shannon P, Yiu A, Liang P, Adamchik Y, Weisspapir M, Samoilova M, Burnham WM, Carlen PL. (2007). Hypoglycemic seizures during transient hypoglycemia exacerbate hippocampal dysfunction. *Neurobiol Dis*., 26: 646-60.

Abdul-Ghani AS, Ghneim H, El-Lati S, Saca'an A. (1989). Changes in the activity of glutamate related enzymes in cerebral cortex, during insulin-induced seizures. *Int. J. Neurosci*., 44: 67-74.

Abdul-Ghani MA, Valiante TA, Carlen PL, Pennefather PS. (1996). Metabotropic glutamate receptors coupled to IP3 production mediate inhibition of IAHP in rat dentate granule neurons. *J Neurophysiol*., 76: 2691-2700.

Abraham A. (1998) Adrenergic receptors and monoamines in the brain and pancreatic islets of streptozotocin diabetic rats: role of insulin secretion as a function of age, 74–75 PhD Thesis.: Dept. of Biotechnology, Cochin University of Science and Technology.

Abraham A, Paulose CS (1999). Age-related alterations in noradrenergic function in the brain stem of streptozotocin-diabetic rats. *J Biochem Mol Bio Biophys.,* 3: 171-176.

Adeghate E, Schattner P, Dunn E. (2006). An update on the etiology and epidemiology of diabetes mellitus. *Ann. N. Y. Acad. Sci*.., 1084: 1-29.

Adler CM, Goldberg TE, Malhotra AK, Pickar D, Breier A. (1998). Effects of ketamine on thought disorder, working memory, and semantic memory in healthy volunteers. *Biol Psychiatry,* 43: 811-816.

Ahrén B, Lundquist I, Hedner P, Valdemarsson S, Scherstén B. (1985). Glucose tolerance and insulin and C-peptide responses after various insulin secretory stimuli in hyper- and hypothyroid subjects before and after treatment. *Diabetes Res.*, 2: 95-103.

Ahren. (2000). Autonomic regulation of islet hormone secretion—implications for health and disease. *Diabetologia*, 43: 393–410.

Akazawa C, Shigemoto R, Bessho Y, Nakanishi S, Mizuno N. (1994). Differential expression of five N-methyl-D-aspartate receptor subunit mRNAs in the cerebellum of developing and adult rats. *J. Comp. Neurol.*, 347: 150-160.

Akria T, Hirohisa I, Yasushi I, Eiko KI, Yujui M. (1994). Hypothalamic cholinergic and noradrenergic neurons in hyperglycemia induced by 2 deoxyglucose. *Brain Research*, 665: 13-17.

Albert SM, Sano M, Marder K, Jacobs DM, Brandt J, Albert M, Stern Y. (1997). Participation in clinical trials and long-term outcomes in Alzheimer's disease. *Neurology*, 49: 38-43.

Albus JS. (1971). A theory of cerebellar function. *Math. Biosci.*, 10: 25–61.

Alexandre FM DaSilva, Cristina G, Josh S, Nouchine H. (2007). Thickening in the somatosensory cortex of patients with migraine. *Neurology*, 69: 1990–1995.

Allen G, Buxton RB, Wong EC, Courchesne E. (1997). Attentional activation of the cerebellum independent of motor involvement. *Science,* 275: 1940-1943.

Amiel SA, Sherwin RS, Simonson DC, Tamborlane WV. (1988). Effects of intensive insulin therapy on glycemic thresholds for counterregulatory hormone release. *Diabetes*, 37: 901-907.

Amit DJ, Brunel N, Tsodyks MV. (1994). Correlations of cortical Hebbian reverberations: theory versus experiment. *J. Neurosci.*, 14: 6435-6445.

Amos AF, McCarty DJ, Zimmet P. (1997). The rising global burden of diabetes and its complications: Estimates and projections to the year 2010. *Diabetic Med.*, 14: S7-S85.

Ana RS, Susana CR, Paulo FS, Armando JC, Alistair JB, António FA. (2006). Elevated Glucose Changes the Expression of Ionotropic Glutamate Receptor Subunits and Impairs Calcium Homeostasis in Retinal Neural Cells. *Investigative Ophthalmology and Visual Science*, 47: 4130-4137.

Anderson CM, Swanson RA. (2000). Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia,* 32: 1-14.

Anderton. (1997). Changes in the ageing brain in health and disease. *Philos Trans R Soc Lond B Biol Sci*., 352: 1781-1792.

Ani VD, Remya R, Paulose CS. (2006a). Enhanced β- adrenergic receptors in the brain and pancreas during pancreatic regeneration in weanling rats. *Molecular and Cellular Biochemistry*, 272(1-2):165.

Ani VD, Savitha B, Paulose CS. (2006b). Decreased alpha1-adrenergic receptor binding in the cerebral cortex and brain stem during pancreatic regeneration in rats. *Neurochemical Research*, 31: 727-737.

Ani VD, Finla C, Paulose CS. (2006c). Decreased alpha 2- adrenergic receptor in the brainstem and pancreatic islets during pancreatic regeneration in weanling rats. *Life Sciences*, 79: 1507-1513.

Anno T, Uehara S, Katagiri H, Ohta Y, Ueda K, Mizuguchi H, Moriyama Y, Oka Y, Tanizawa Y. (2004). Overexpression of constitutively activated glutamate dehydrogenase induces insulin secretion through enhanced glutamate oxidation. *Am J Physiol Endocrinol Metab*., 286: E280-285.

Aral YZ, Gucuyener K, Atalay Y, Hasanoglu A, Turkyilmaz C, Sayal A, Biberoglu G. (1998). Role of excitatory aminoacids in neonatal hypoglycemia. *Acta Paediatr Jpn*., 40: 303–306.

Aramori I, Nakanishi S. (1992). Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells. *Neuron*, 8: 757-765.

Arison RN, Ciaccio EI, Glitzer MS, Cassaro JA, Pruss MP. (1967). Light and electron microscopy of lesions in rats rendered diabetic with streptozotocin. *Diabetes*, 16: 51–56.

Arkhammar P, Juntti BL, Larsson O, Welsh M, Nanberg E, Sjoholm A, Kohler M, Berggen PO. (1994). Protein kinase C modulates the insulin secretion by maintaining a proper function of b-cell voltage-activated Ca^{2+} channels. *J. Biol. Chem.*, 269: 2743-2749.

Arnáiz E, Almkvist O. (2003). Neuropsychological features of mild cognitive impairment and preclinical Alzheimer's disease. *Acta Neurol Scand Suppl.*, 179: 34-41.

Arzberger T, Krampfl K, Leimgruber S, Weindl A. (1997). Changes of NMDA receptor subunit (NR1, NR2B) and glutamate transporter (GTL1) mRNA expression in Huntington's disease: An in situ hybridization study. *Journal of Neuropathology and Experimental Neurology*, 56: 440-454.

Ashcroft FM, Proks P, Smith PA, Ammala C, Bokvist K, Rorsman P. (1994). Stimulus-secretion coupling in pancreatic beta cells. *J. Cell. Biochem.*, 55: 54-65.

Aswathy RN, Biju MP, Paulose CS. (1998). Effect of pyridoxine and insulin administration on brain glutamate dehydrogenase activity and blood glucose control in streptozotocin-induced diabetic rats. *Biochim Biophys Acta.*, 1381: 351–354.

Atlante A, Gagliardi S, Minervini GM, Ciotti MT, Marra E, Calissano P. (1997). Glutamate neurotoxicity in rat cerebellar granule cells: a major role for xanthine oxidase in oxygen radical formation. *J Neurochem.*, 68: 2038–2045.

Auer RN, Kalimo H, Olsson Y, Siesjö BK. (1985). The temporal evolution of hypoglycemic brain damage. II. Light- and electron-microscopic findings in the hippocampal gyrus and subiculum of the rat. *Acta Neuropathol.*, 67: 25-36.

Auer RN, Siesjo BK. (1988). Biological differences between ischemia, hypoglycaemia, and epilepsy. *Ann. Neural.*, 24: 699-707.

Auer RN, Hugh J, Cosgrove E, Curry B. (1989). Neuropathologic findings in three cases of profound hypoglycemia. *Clin Neuropathol.*, 8: 63-68.

Auer RN. (1991). Excitotoxic mechanisms, and age-related susceptibility to brain damage in ischemia, hypoglycemia and toxic mussel poisoning. *Neurotoxicology*, 12: 541-546.

Auer RN, Siesjo BK (1993) Hypoglycaemia: brain neurochemistry and neuropathology. Baillieres Clin Endocrinol Metab., 7: 611-625.

Auer RN. (2004). Hypoglycaemic brain damage. *Forensic Science International Forensic Neuropathology*, 146: 105-110.

Avenet P, Léonardon J, Besnard F, Graham D, Frost J, Depoortere H, Langer SZ, Scatton B. (1996). Antagonist properties of the stereoisomers of ifenprodil at NR1A/NR2A and NR1A/NR2B subtypes of the NMDA receptor expressed in Xenopus oocytes. *Eur J Pharmacol*., 296: 209-213.

Azmitia EC, Gannon P. (1986). Anatomy of the serotonergic system in the primate and sub-primate brain. *J. Adv. Neurol*., 43: 407–468.

Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P. (1990). Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature*, 347: 151-156.

Balarama KS, Gireesh G, Paulose CS. (2007). Decreased GABAA receptor function in the brainstem during pancreatic regeneration in rats. *Neurochem Res*., 32: 1813-1822.

Balarama KS, George KA, Paulose CS. (2008). Gamma-aminobutyric acid A receptor functional decrease in the hypothalamus during pancreatic regeneration in rats. *Pancreas*, 37: e20-30.

Baloh RW, Jacobson KM, Beykirch K, Honrubia V. (1998). Static and dynamic posturography in patients with vestibular and cerebellar lesions. *Arch. Neurol*., 55: 649-654.

Baltrons AM, Saadoun S, Agullo L, Garcia A. (1997). Regulation of calcium of the nitric oxide/cyclic GMP system in cerebellar granule cells and astroglia in culture. *J. Neurosci. Res*., 49: 333-341.

Barnes JM, Dev KK, Henley JM. (1994). Cyclothiazide unmask AMPA-evoked stimulation of [3H]-L-glutamate release from rat hippocampal synaptosomes. *Br. J. Pharmacol*., 113: 339-341.

Baskys A, Blaabjerg M. (2005). Understanding regulation of nerve cell death by mGluRs as a method for development of successful neuroprotective strategies. *J. Neurol. Sci*., 229-230: 201–209.

Basso AM, Gioino G, Molina VA, Cancela LM. (1999). Chronic amphetamine facilitates immunosuppression in response to a novel aversive stimulus: reversal by haloperidol pretreatment. *Pharmacol. Biochem. Behav*., 62: 307-314.

Bates B, Xie Y, Taylor N, Johnson J, Wu L, Kwak S, Blatcher M, Gulukota K, Paulsen JE. (2002). Characterization of mGluR5R, a novel, metabotropic glutamate receptor 5-related gene. *Brain Res. Mol. Brain Res*., 109: 18-33.

Bech K, Damsbo P, Eldrup E, Beck-Nielsen H, Røder ME, Hartling SG, Vølund A, Madsbad S. (1996). Beta-cell function and glucose and lipid oxidation in Graves' disease. *Clin Endocrinol (Oxf)*., 44: 59-66.

Ben-Ami H, Nagachandran P, Mendelson A, Edoute Y. (1999). Drug-induced hypoglycemic coma in 102 diabetic patients. *Arch Intern Med*., 159: 281-284.

Benke D, Wenzel A, Scheurer L, Fritschy JM, Mohler H. (1995). Immunobiochemical characterization of the NMDA-receptor subunit NR1 in the developing and adult rat brain. *J. Recept. Signal. Transduct. Res*., 15: 393-411.

Benquet P, Gee CE, Gerber U. (2002). Two distinct signaling pathways upregulate NMDA receptor responses via two distinct metabotropic glutamate receptor subtypes. *J. Neurosci*., 22: 9679-9686.

Benveniste H, Drejer J, Schousboe A, Diemer NH. (1984). Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J Neurochem*., 43: 1369-1374.

Bereiter DA, Rohner-Jeanrenaud F, Berthoud H, Jeanrenaud R. (1981). CNS modulation of pancreatic endocrine function: multiple mode of expression. *Diabetologia*, 20: 417–425.

Berg L, Hughes CP, Coben LA, Danziger WL, Martin RL, Knesevich J. (1982). Mild senile dementia of Alzheimer type: research diagnostic criteria, recruitment, and description of a study population. *J Neurol. Neurosurg. Psychiatry*., 45: 962-968.

Berman FW, Murray TF. (1996). Characterization of [3H]MK-801 binding to N-methyl-D-aspartate receptors in cultured rat cerebellar granule neurons and involvement in glutamate-mediated toxicity. *J Biochem. Toxicol*., 11: 217–226.

Berridge MJ. (1987). Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Annu Rev Biochem*., 56: 159-193.

Berridge MJ, Galione A. (1988). Cytosolic calcium oscillators. *FASEB J*., 2: 3074-3082.

Berridge M.J. (1991) Cytoplasmic calcium oscillations: a two pool model. *Cell Calcium*, 12: 63–72.

Berridge MJ. (1993). Inositol trisphosphate and calcium signalling. *Nature*, 361: 315–325.

Berridge MJ, Lipp P, Bootman MD. (2000). The versatility and universality of calcium signalling. *Nat Rev. Mol. Cell Biol*., 1: 11-21.

Berridge MJ, Bootman MD, Roderick HL. (2003). Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol*., 4: 517–529.

Bertrand G, Gross R, Puech R, Loubatieres-Mariani MM, Bockaert J. (1992). Evidence for a glutamate receptor of the AMPA subtype which mediates insulin release from rat perfused pancreas. *Br. J. Pharmacol*., 106: 354-359.

Bertrand G, Gross R, Puech R, Loubatières-Mariani MM, Bockaert J. (1993). Glutamate stimulates glucagon secretion via an excitatory amino acid receptor of the AMPA subtype in rat pancreas. *Eur. J. Pharmacol*., 237: 45-50.

Bertrand G, Puech R, Loubatières-Mariani MM, Bockaert J. (1995). Glutamate stimulates insulin secretion and improves glucose tolerance in rats. *Am. J. Physiol. Endocrinol. Metab*., 269: 551-556.

Bertrand G, Ishiyama N, Nenquin M, Ravier MA, Jean-Claude H. (2002). The Elevation of Glutamate Content and the Amplification of Insulin Secretion in Glucose-stimulated Pancreatic Islets Are Not Causally Related. *J. Biol. Chem.*, 277: 32883-32891.

Bettler B, Mulle C. (1995). Neurotransmitter receptors. II. AMPA and kainate receptors (Review). *Neuropharmacology,* 34: 123-139.

Bhardwaj SK, Sandhu SK, Sharma P, Kaur G. (1999). Impact of diabetes on CNS: role of signal transduction cascade. *Brain Res. Bull*., 49: 155-162.

Bhattacharya SK, Saraswathi M. (1991). Effect of intracerebroventricularly administered insulin on brain monoamines and acetylcholine in euglycemic and alloxan- induced hyperglycemic rats. *Indian J. Exp. Biol*., 29: 1095-1100.

Biessels GJ, Kamal A, Ramakers GM, Urban IJ, Spruijt BM, Erkelens DW, Gispen WH. (1996). Place learning and hippocampal synaptic plasticity in streptozotocin-induced diabetic rats. *Diabetes*, 45: 1259-1266.

Biggers DW, Myers SR, Neal D, Stinson R, Cooper NB, Jaspan JB, Williams PE, Cherrington AD, Frizzell RT. (1989). Role of brain in counterregulation of insulin-induced hypoglycemia in dogs. *Diabetes*, 38: 7-16.

Biggio G, Brodie BB, Costa E, Guidotti A. (1977). Mechanisms by which diazepam, muscimol, and other drugs change the content of cGMP in cerebellar cortex. *Proc. Natl. Acad. Sci. U S A*., 74: 3592-3596.

Biju MP, Paulose CS. (1998). Brain glutamate dehydrogenase changes in streptozotocin diabetic rats as a function of age. *Biochem Mol Biol Int*., 44: 1–7.

Biju MP, Pyroja S, Rajeshkumar NV, Paulose CS. (2002). Brain stem GABA receptor functional regulation during rat liver cell proliferation. *Neurochem Res*., 27: 905-910.

Bilotta F, Caramia R, Cernak I, Paoloni FP, Doronzio A, Cuzzone V, Santoro A, Rosa G. (2008). Intensive Insulin Therapy After Severe Traumatic Brain Injury: A Randomized Clinical Trial. *Neurocrit. Care*, 9: 159–166.

Bischof MG, Brehm A, Bernroider E, Krssák M, Mlynárik V, Krebs M, Roden M. (2006). Cerebral glutamate metabolism during hypoglycaemia in healthy and type 1 diabetic humans. *Eur J Clin Invest*., 36: 164-169.

Bittigau P, Ikonomidou C. (1997) Glutamate in neurological diseases. *J. Child Neurol.,* 12, 471–485.

Blanca R, Ljubicic S, Pournourmohammadi S, Carobbio S, Armanet M, Bartely C, Maechler P. (2005). Dopamine D2 like receptors are expressed in pancreatic beta cells and mediate inhibition of insulin secretion. *J. Biol. Chem*., 280: 36824–36832.

Blattner RJ. (1968). Central nervous system damage and hypoglycemia. *J. Pediatr*., 72: 904–906.

Bleakman D, Rusin KI, Chard PS, Glaum SR, Miller RJ. (1992). Metabotropic glutamate receptors potentiate ionotropic glutamate responses in the rat dorsal horn. *Mol. Pharmacol*., 42: 192-196.

Bode H-P, Himmen A, Goke B. (1996). Evidence for vacuolar-type proton pumps in nonmitochondrial and inositol 1,4,5-trisphosphate-sensitive calcium stores of insulin-secreting cells. *Pflügers Arch*., 432: 97–104.

Bode HP, Moormann B, Dabew R, Goke B. (1999). Glucagon-like peptide-1 elevates cytosolic calcium in pancreatic ß cells independently of protein kinase A. *Endocrinology*, 140: 3919–3927.

Boehning D, Patterson RL, Sedaghat L, Glebova NO, Kurosaki T, Snyder SH. (2003). Cytochrome c binds to inositol (1,4,5) trisphosphashate receptors, amplifying calcium-dependant apoptosis. *Nature Cell. Biol*., 5: 1051-1061.

Bondareff W, Geinisman Y. (1976). Loss of synapses in the dentate gyrus of the senescent rat. *Am J Anat*., 145: 129-136.

Bonde C, Noraberg J, Noer H, Zimmer J. (2005). Ionotropic glutamate receptors and glutamate transporters are involved in necrotic neuronal cell death induced by oxygen-glucose deprivation of hippocampal slice cultures. *Neuroscience,* 136: 779-794.

Bonsi P, Cuomo D, De Persis C, Centonze D, Bernardi G, Calabresi P, Pisani A. (2005). Modulatory action of metabotropic glutamate receptor (mGluR) 5 on mGluR1 function in striatal cholinergic interneurons. *Neuropharmacology*, 49: 104-113.

Bootman M, Niggli E, Berridge M, Lipp P. (1997). Imaging the hierarchical Ca2+ signalling system in HeLa cells. *J.Physiol.*, 499: 307-314.

Bordi F, Ugolini A. (1999). Group I metabotropic glutamate receptors: implications for brain diseases. *Prog.Neurobiol*., 59: 55-79.

Boyd AE. (1992). The role of ion channels in insulin secretion. *J. Cell. Biochem*., 48: 234-241.

Boyle, Nagy R, O' Connor A, Kempers S, yeo R, Qualis C. (1994). Adaption in brain glucose uptake following recurrent hypoglycaemia. *Proc Acad Sci USA*, 91: 9352- 69356.

Bredt DS, Snyder SH. (1990). Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc. Natl. Acad. Sci. USA*, 87: 682-685.

Breen MA, Ashcroft SJ. (1997). .A truncated isoform of Ca2+/calmodulin-dependent protein kinase II expressed in human islets of Langerhans may result from trans-splicing. *FEBS Lett*., 409: 375-379.

Broderick PA, Jacoby JH. (1989). Central monoamine dysfunction in diabetes: psychotherapeutic implications: electro analysis by voltammetry. *Acta Physiol. Pharmacol Latinoam*, 39: 211-225.

Bruno V, Copani A, Knöpfel T, Kuhn R, Casabona G, Dell'Albani P, Condorelli DF, Nicoletti F. (1995). Activation of metabotropic glutamate receptors coupled to inositol phospholipid hydrolysis amplifies NMDA-induced neuronal degeneration in cultured cortical cells. *Neuropharmacology,* 34: 1089–1098.

Budd SL, Nicholas DG. (1996). Mitochondria, calcium regulation, and acute glutamate excitotoxicity in cultured cerebellar granule cells. *J Neurochem*., 67:2282–2291.

Budson AE, Price BH. (2005). Memory dysfunction. *N. Engl. J Med*., 352: 692-699.

Burcelin R, Eddouks M, Maury J, Kande J, Assan R, Girard J. (1995). Excessive glucose production, rather than insulin resistance, accounts for hyperglycaemia in recent-onset streptozotocin-diabetic rats. *Diabetologia*, 38: 283-290.

Burke CW, Eastman CJ. (1974). Thyroid hormones. *Br Med Bull.,* 30: 93.

Burke MR, Barnes GR. (2006). Quantitative differences in smooth pursuit and saccadic eye movements. *Exp Brain Res*., 175: 596-608.

Burr IM, Slonim AE, Sharp R. (1976). Interaction of acetylcholine and epinephrine on the dynamics of insulin release in vitro. *J. Clin. Invest*., 58: 230–239.

Butcher SP, Sandberg M, Hagberg H, Hamberger A. (1987). Cellular origin of endogenous amino acids released into the extracellular fluid of the rat striatum during severe insulin-induced hypoglycaemia. *J. Neurochem*., 48: 722-728.

Butefisch CM, Khurana V, Kopylev L, Cohen LG. (2004). Enhancing encoding of a motor memory in the primary motor cortex by cortical stimulation. *J Neurophysiol*., 91: 2110–2116.

Butterworth RF. (1983). Metabolism of glutamate and related amino acids in insulin hypoglycaemia. Glutamine, Glutamate and GABA in the Central Nervous System. New York: Alan R. Liss, pp. 595 608.

Butterworth RF. (1999). Metabolic Encephalopathies. Basic neurochemistry Published by Lippincott Williams and Wilkins. 5:38.

Cabeza R, Anderson ND, Locantore JK, McIntosh AR (2002). Aging gracefully: compensatory brain activity in high-performing older adults. *Neuroimage*, 17: 1394-1402.

Cabrera O, Jacques-Silva MC, Speier S, Yang SN, Köhler M, Fachado A, Vieira E, Zierath JR, Kibbey R, Berman DM, Kenyon NS, Ricordi C, Caicedo A, Berggren PO. (2008). Glutamate is a positive autocrine signal for glucagon release. *Cell Metab*., 7: 545-554.

Caccamo D, Campisi A, Curró M, et al. (2004). Excitotoxic and postischemic neurodegeneration: involvement of transglutaminases. *Amino Acids*, 27: 373–379.

Callahan B, Yuan J, Stover G, Hatzidimitriou G, Ricaurte G. (1998). Effects of 2-deoxy-D-glucose on methamphetamine-induced dopamine and serotonin neurotoxicity. *J. Neurochem*., 70: 190-197.

Camacho A, Massieu L. (2006). Role of glutamate transporters in the clearance and release of glutamate during ischemia and its relation to neuronal death. *Arch Med Res*., 37: 11-18.

Campfield LA, Smith FJ. (1980). Modulation of insulin secretion by the autonomic nervous system. *Brain Res. Bull*., 4: 103–107.

Canaves JM, Taylor SS. (2002).Classification and phylogenetic analysis of the cAMP-dependent protein kinase regulatory subunit family. *J Mol Evol.*, 54: 17-29.

Cao WH, Morrison SF. (2001). Differential chemoreceptor reflex responses of adrenal preganglionic neurons. *Am J Physiol Regul Integr Comp Physiol*., 281: 1825-1832.

Caramanos Z, Shapiro ML. (1994). Spatial memory and N-methyl-D-aspartate receptor antagonists APV and MK-801: memory impairments depend on familiarity with the environment, drug dose, and training duration. *Behav Neurosci*., 108: 30-43.

Carpenter MK, Parker I, Miledi R. (1992). Messenger RNAs coding for receptors and channels in the cerebral cortex of adult and aged rats. *Molecular Brain Research*, 13: 1-5.

Cauli O, Mansouri MT, Agusti A, Felipo V. (2008). Hyperammonemia Increases GABAergic Tone in the Cerebellum but Decreases It in the Rat Cortex. *Gastroenterology*. 2008 Dec 31. [Epub ahead of print].

Cavaliere F, D'Ambrosi N, Sancesario G, Bernardi G, Volonte C. (2001). Hypoglycaemia-induced cell death: features of neuroprotection by the P2 receptor antagonist basilen blue. *Neurochem Int.,* 38:199-207.

Cavelier P, Attwell D. (2005). Tonic release of glutamate by a DIDS-sensitive mechanism in rat hippocampal slices. *J Physiol (Lond)*., 564: 397–410.

Cebers G, Cebere A, and Liljequist S. (1998). Metabolic inhibition potentiates AMPA-induced Ca^{2+} fluxes and neurotoxicity in rat cerebellar granule cells. *Brain Res*., 779: 194-204.

Cendelín J, Korelusová I, Vozeh F. (2008). The effect of repeated rotarod training on motor skills and spatial learning ability in Lurcher mutant mice. *Behav. Brain Res*., 189: 65-74.

Cerne R, Randic M. (1992). Modulation of AMPA and NMDA responses in rat spinal dorsal horn neurons by trans-1-aminocyclopentane- 1, 3-dicarboxylic acid. *Neurosci. Lett*., 144: 180-184.

Chabot RJ, Gugino LD, Aglio LS, Maddi R, Cote W. (1997). QEEG and neuropsychological profiles of patients after undergoing cardiopulmonary bypass surgical procedures. *Clin Electroencephalogr*, 28: 98-105.

Chalimoniuk M, Strosznajder JB. (1998). Aging modulates nitric oxide synthesis and cGMP levels in hippocampus and cerebellum. Effects of amyloid beta peptide. *Mol. Chem. Neuropathol*., 35: 77-95.

Chalmers M, Schell MJ, Thorn P. (2006). Agonist-evoked inositol trisphophate receptor (IP3R) clustering is not dependent on changes in the structure of the endoplasmic reticulum. *Biochem.J.*, 394: 57-66.

Charles RA, Goh SY. (2005). Occult hypoglycaemia in a diabetic patient on peritoneal dialysis. *Eur J Emerg Med*., 12: 322-323.

Chelliah A, Burge MR. (2004). Hypoglycaemia in elderly patients with diabetes mellitus: causes and strategies for prevention. *Drugs Aging,* 21: 511-530.

Chen S, Kobayashi M, Honda Y, Kakuta S, Sato F, Kishi K. (2007). Preferential neuron loss in rat piriform cortex following pilocarpine induced status epilepticus. *Epilepsy Res.*, 74: 1-18.

Cheng H, WJ Lederer, Cannell MB. (1993). Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science*, 262: 740-744.

Chetkovich DM, Sweatt JD. (1993). NMDA receptor activation increases cyclic AMP in area CA1 of the hippocampus via calcium/calmodulin stimulation of adenylyl cyclase. *J.Neurochem.*, 61: 1933-1942.

Choi DW. (1988). Glutamate neurotoxicity and diseases of nervous system. Neuron 1:623–634.

Choi DW. (1992). Excitotoxic cell death. *J Neurobiol*., 23: 1261–1276.

Choi DW. (1994). Calcium and excitotoxic neuronal injury. *Ann N Y Acad Sci*., 747: 162–171.

Chopra IJ, Williams DE, Orgiazzi J, et al. (1975). Opposite effects of dexamethasone on serum concentrations of 3.3',5'-triiodothyronine (reverse T3) and 3,3',5-triiodothyronine (173). *J Clin Endocrinol Metab*., 41: 911.

Chu PC, Lim MT, Shian LR, Liu SY. (1986). Alterations in physiologic functions and in monoamine content in streptozotocin - diabetic rats. *Diabetes*, 35: 481-485.

Chu Z, Hablitz JJ. (2000). Quisqualate induces an inward current via mGluR activation in neocortical pyramidal neurons. *Brain Res*., 879: 88-92.

Ciabarra AM, Sullivan JM, Gahn LG, Pecht G, Heinemann S, Sevarino KA. (1995). Cloning, characterization of chi-1: A developmentally regulated member of a novel class of the ionotropic glutamate receptor family. *J. Neurosci*., 15: 6498-6508.

Cohen SA, Müller WE. (1992). Age-related alterations of NMDA-receptor properties in the mouse forebrain: partial restoration by chronic phosphatidylserine treatment. *Brain Res*., 584: 174-180.

Coiro V, Volpi R, Marchesi C, Capretti L, Speroni G, Caffarri G, Chiodera P. (1997). Influence of residual C-peptide secretion on nocturnal serum TSH peak in well-controlled diabetic patients. *Clin Endocrinol (Oxf)*., 47: 305-310.

Collingridge GL, Bliss TVP. (1987). NMDA receptors-their role in long-term potentiation. *Trends Neurosci.*, 10: 288-293.

Collingridge GL, Singer W. (1990). Excitatory amino acid receptors and synaptic plasticity. *Trends Pharmacol. Sci*., 11: 290-296.

Conn PJ, Pin JP. (1997). Pharmacology and function of metabotropic glutamate receptors. *Annu.Rev.Pharmacol.Toxicol.*, 37: 205-237.

Coyle JT, Puttfarcken P. (1993). Oxidative stress, glutamate, and neurodegenerative disorders. *Science*, 262: 689–695.

Crandall EA, Gillis MA, Fernstrom JD. (1981). Reduction in brain serotonin synthesis rate in streptozotocin-diabetic rats. *Endocrinology*, 109: 310-312.

Croucher MJ, Morant R, Thomas LS, Jane DE. (1997). Evidence for a role of group I metabotropic glutamate receptors in the positive feedback control of synaptic glutamate release in vitro. *Pharmacologist*, 39: 215.

Cryer PE, Gerich J. (1985). Glucose counterregulation, hypoglycaemia, and intensive insulin therapy in diabetes mellitus. *N. Engl. J. Med*., 313: 232–241.

Cryer PE. (1994). Banting Lecture. Hypoglycemia: the limiting factor in the management of IDDM. *Diabetes,* 43: 1378-1389.

Cryer PE. (1997). Hierarchy of physiological responses to hypoglycaemia: relevance to clinical hypoglycaemia in type I (insulin dependent) diabetes mellitus. *Horm. Metab. Res.,* 29: 92-96.

Cryer PE. (1999). Symptoms of hypoglycemia, thresholds for their occurrence, and hypoglycemia unawareness. *Endocrinol Metab Clin North Am.,* 28: 495-500.

Cryer PE. (2001). The prevention and correction of hypoglycemia. In Handbook of Physiology. Section 7, The Endocrine System. Volume II, The Endocrine

Pancreas and Regulation of Metabolism. Jefferson LS, Cherrington AD, Eds. Oxford University Press, New York, p.1057–1092

Cryer PE. (2002a). Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes. *Diabetologia*, 45: 937-948.

Cryer PE. (2002b). The pathophysiology of hypoglycaemia in diabetes. *Diabetes Nutr Metab*., 15: 330-333.

Cryer PE. (2003). Hypoglycaemia in diabetes. *Diabetes Care*, 26:1902-1912.

Cryer PE, Davis SN, Shamoon H. (2003). Hypoglycemia in diabetes. *Diabetes Care*, 26: 1902–1912.

Cryer PE. (2007). Hypoglycemia, functional brain failure, and brain death. *J Clin Invest*., 117: 868–870.

Cryer PE. (2008). The barrier of hypoglycemia in diabetes. *Diabetes,* 57: 3169–3167.

Cryer PE. (2009). Hypoglycemia in Diabetes: Pathophysiology Prevalence and Prevention. Alexandria, VA, American Diabetes Association.

Cull-Candy S, Brickley S, Farrant M. (2001). "NMDA receptor subunits: diversity, development and disease". *Curr. Opin. Neurobiol*., 11: 327-335.

Dagogo-Jack SE, Craft S, Cryer PE. (1993). Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. *J Clin Invest.,* 91: 819–828.

Danbolt NC. (2001). Glutamate uptake. *Prog. Neurobiol*., 65:1–105.

Dang MT, Yokoi F, Yin HH, Lovinger DM, Wang Y, et al. (2006). Disrupted motor learning and long-term synaptic plasticity in mice lacking NMDAR1 in the striatum. *Proc Natl Acad Sci U S A*., 103: 15254–15259.

Danysz W, Parsons CG, Bresink I, Quack G. (1995). Glutamate in CNS disorders: A revived target for drug development. *Drug News Perspect*., 8: 261-277.

Darling BK, Abdel-Rahim M, Moores RR, Chang AS, Howard RS, O'Neill JT. (2001). Brain excitatory amino acid concentrations are lower in the neonatal pig: a buffer against excitotoxicity?. *Biol Neonate*., 80: 305-312.

Das S, Sasaki YF, Rothe T, Premkumar LS, Takasu M, Crandall JE, Dikkes P, Conner DA, Rayudu PV, Cheung W, Chen HS, Lipton SA, Nakanishi N. (1998). Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. *Nature (Lond.),* 393: 377-381.

Davis EA, Keating B, Byrne GC, Russell M, Jones TW. (1998). Impact of improved glycaemic control on rates of hypoglycaemia in insulin dependent diabetes mellitus. *Arch. Dis. Child,* 78: 111-115.

Davis MR, Mellman M, Shamoon H. (1992). Further defects in counterregulatory responses induced by recurrent hypoglycaemia in IDDM. *Diabetes*, 41: 1335–1340.

Davis MR, Shamoon H. (1991). Counterregulatory adaptation to recurrent hypoglycaemia in normal humans. *J. Clin. Endocrinol. Metab*., 73: 995–1001.

Davis SN, Shavers C, Mosqueda-Garcia R, Costa F. (1997). Effects of differing antecedent hypoglycaemia on subsequent counterregulation in normal humans. *Diabetes*, 46: 1328–1335.

De Camilli P, Miller PE, Levitt P, Walter U, Greengard P. (1984). Anatomy of cerebellar Purkinje cells in the rat determined by a specific immunohistochemical marker. *Neuroscience*, 11: 761-817.

De Vente J, Asan E, Gambaryan S, Markerink-van Ittersum M, Axer H, Gallatz K, Lohmann SM, Palkovits M. (2001). Localization of cGMP-dependent protein kinase type II in rat brain. *Neuroscience*, 108: 27-49.

Dejgaard A, Gade A, Larsson H, Balle V, Parving A, Parving HH. (1991). Evidence for diabetic encephalopathy. *Diabet Med*., 8: 162-167.

Denise T, Tom OV, Philip EC, William JP. (2004). Activation of human medial prefrontal cortex during autonomic responses to hypoglycaemia. *PNAS*, doi: 10.1073/pnas0307 0481 01.

Desai MA, Burnett JP, Schoepp DD. (1994). Cyclothiazide selectively potentiates AMPA and kainate-induced [3H] norepinephrine release from rat hippocampal slices. *J. Neurochem.*, 63: 231-237.

Deutch AY. (1993). Prefrontal cortical dopamine systems and the elaboration of functional corticostriatal circuits: implications for schizophrenia and Parkinson's disease. *J. Neural Transm. Gen. Sect.*, 91: 197-221.

Dey J, Misra A, Desai NG, Mahapatra AK, Padma MV. (1997). Cognitive function in younger type II diabetes. *Diabetes Care*, 20: 32 –35.

Di Mario U, Morano S, Valle E, Pozzessere G. (1995). Electrophysiological alterations of the central nervous system in diabetes mellitus. *Diabetes Metab. Rev.*, 11: 259-277.

Diederichs F. (2008). Ion homeostasis and the functional roles of SERCA reactions in stimulus-secretion coupling of the pancreatic beta-cell: A mathematical simulation. *Biophys Chem.*, 134: 119-143.

Diener H, Dichgans J, Guschlbauer B, Bacher M, Langenbach P. (1989). Disturbances of motor preparation in basal ganglia and cerebellar disorders. *Prog. Brain Res.*, 80: 481-488.

Dimitriadis G, Baker B, Marsh H, Mandarino L, Rizza R, Bergman R, Haymond M, Gerich J. (1985). Effect of thyroid hormone excess on action, secretion, and metabolism of insulin in humans. *Am. J. Physiol.*, 248: 593-601.

Dimitriadis G, Parry-Billings M, Bevan S, Leighton B, Krause U, Piva T, Tegos K, Challiss RA, Wegener G, Newsholme EA. (1997). The effects of insulin on transport and metabolism of glucose in skeletal muscle from hyperthyroid and hypothyroid rats. *Eur. J Clin. Invest.*, 27: 475-483.

Dimitriadis GD, Raptis SA. (2001). Thyroid hormone excess and glucose intolerance. *Exp Clin Endocrinol Diabetes.*, 109: 225-239.

Dingledine R, Borges K, Bowie D, Traynelis SF. (1999). The glutamate receptor ion channels. *Pharmacol Rev.*, 51: 7-51.

Doble A. (1999). The role of excitotoxicity in neurodegenerative disease: Implications for therapy. *Pharmacol Ther*., 81: 163–221.

Donda A, Reymond MJ, Zürich MG, Lemarchand-Béraud T. (1987). Influence of sex and age on T3 receptors and T3 concentration in the pituitary gland of the rat: consequences on TSH secretion. *Mol. Cell Endocrinol*., 54: 29-34.

Donnelly LA, Morris AD, Frier BM, Ellis JD, Donnan PT, Durrant R, Band MM, Reekie G, Leese GP. (2005). The DARTS/MEMO Collaboration: Frequency and predictors of hypoglycaemia in type 1 and insulin-treated type 2 diabetes: a population based study. *Diabet Med*., 22: 749–755.

Douglas WW. (1968). Stimulus-secretion coupling: the concept and clues from chromaffine and other cells. *Brit. J. Pharmac. Chemother*., 34: 451-474.

Dremier S, Pohl V, Poteet-Smith C, Roger PP, Corbin J, Doskeland SO, Dumont JE, Maenhaut C. (1997). Activation of cyclic AMP-dependent kinase is required but may not be sufficient to mimic cyclic AMP-dependent DNA synthesis and thyroglobulin expression in dog thyroid cells. *Mol. Cell Biol.*, 11: 6717-6726.

Duchen. (2000). Mitochondria and Ca(2+)in cell physiology and pathophysiology. *Cell Calcium*, 28: 339-348.

Dunham NW, Miya TS. (1957). A Note on a Simple Apparatus for Detecting Neurological Deficit in Rats & Mice. *J. Am. Pharmaceut. Assoc. Scientific Edit*, XLVI: No. 3.

Dunne MJ, Cosgrove KE, Shepherd RM, Aynsley G, Lindley KJ. (2004). Hyperinsulinism in infancy: From basic science to clinical disease. *Physiol. Rev.,* 84: 239-275.

Dunne MJ. (1991). Block of ATP-regulated potassium channels by phentolamine and other a-adrenoreceptor antagonists. *Br. J. Pharmacol*., 1071: 67-82.

Eichenbaum H. (2000). A cortical-hippocampal system for declarative memory. *Nat Rev Neurosci*., 1: 41–50.

Eisenstein Z, Hagg S, Vagenakis AG, et al: (1978). Effect of starvation on the production and peripheral metabolism of 3,3',5'-triiodothyronine in euthyroid obese subjects. *J Clin Endocrinol Metab*., 47: 889.

Eliasson L, Ma X, Renstrom E, Barg S, Berggren PO, Galvanovskis J, Gromada J, Jing X, Lundquist I, Salehi A, Sewing S, Rorsman P. (2003). SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic ß cells. J Gen Physiol., 121: 81–197.

Endoh T. (2004). Characterization of modulatory effects of postsynaptic metabotropic glutamate receptors on calcium currents in rat nucleus tractus solitarius. *Brain Res*., 1024: 212–224.

Enna SJ, Snyder SH. (1976). A simple, sensitive and specific radioreceptor assay for endogenous GABA in brain tissue. *J Neurochem*., 26:221–224.

Ennis K, Tran PV, Seaquist ER, Rao R. (2008). Postnatal age influences hypoglycemia-induced neuronal injury in the rat brain. *Brain Res*., 1224: 119-126.

Erceg S, Monfort P, Hernandez-Viadel M, Llansola M, Montoliu C, Felipo V. (2005). Restoration of learning ability in hyperammonemic rats by increasing extracellular cGMP in brain. *Brain Res*., 1036: 115-121.

Erecinska M, Silver IA. (1989). ATP and brain function. *J Cereb Blood Flow Metab*., 9: 2-19.

Erecinska. M. (1997). The neurotransmitter amino acid transport systems: A fresh outlook of an old problem. *Biochem. Pharmacol*., 36: 3547-3555.

Ertl AC, Davis SN. (2004). Evidence for a vicious cycle of exercise and hypoglycaemia in type 1 diabetes mellitus. *Diabetes Metab. Res. Rev*., 20: 124-1230.

Eswar SPN, Santhosh KT, Paulose CS. (2006). Dopaminergic regulation of glucose induced insulin secretion through Dopamine D2 receptors in the pancreatic islets in vitro. *IUBMB Life*, 58: 157-163.

Eswar Shankar, P. N., Anu Joseph, C. S. Paulose. (2007). Decreased [3H] YM-09151-2 binding to dopamine D2 receptors in the hypothalamus, brainstem and pancreatic islets of streptozotocin-induced diabetic rats. *European Journal of Pharmacology*, 557: 99-105.

Fernstrom JD, Wurtman RJ. (1971). Brain serotonin content: increase following ingestion of carbohydrate diet. *Science*, 174: 1023-1025.

Fernstrom JD, Wurtman RJ. (1972). Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science*, 178: 414-416.

Fibiger HC. (1995). Neurobiology of depression: focus on dopamine. *Adv. Biochem., Psychopharmacol*., 49: 1-17.

Fiez JA, Raife EA, Balota DA, Schwarz JP, Raichle ME, Petersen SE, Fox A. (1996). Positron emission tomography study of the short-term maintenance of verbal information. *J Neurosci*., 16: 808-822.

Filipponi P, Gregorio F, Ferrandina C, Nicoletti I, Mannarelli C, Pippi R, Santeusanio F. (1986). Alpha-adrenergic system in the modulation of pancreatic A and B cell function in normal rats. *Diabetes Res. Clin. Pract*., 2: 325-336.

Fiscus RR, Rapoport RM, Waldman SA, Murad F. (1985). Atriopeptin II elevates cyclic GMP, activates cyclic GMP-dependent protein kinase and causes relaxation in rat thoracic aorta. *Biochem.Biophys.Acta.*, 846: 179-184.

Flanagan DE, Evans ML, Monsod TP, Rife F, et al. (2003). The influence of insulin on circulating ghrelin. *Am J Physiol Endocrinol Metab*., 284: E313–E316.

Foskett JK, White C, Cheung KH, Mak DO. (2007). Inositol trisphosphate receptor Ca2+ release channels. *Physiol Rev*., 87: 593–658.

Francis SH, Corbin JD. (1999). Cyclic nucleotide-dependent protein kinases: intracellular receptors for cAMP and cGMP action. *Crit Rev Clin Lab Sci.,* 36: 275-328.

Franklin SO, Elliott K, Zhu YS, Wahlestedt C, Inturrisi CE. (1993). Quantitation of NMDA receptor (NMDAR1) messenger RNA levels in the adult and developing rat CNS. *Mol. Brain. Res*., 19: 93-100.

Frier BM. (2008). How hypoglycaemia can affect the life of a person with diabetes. *Diabetes Metab Res Rev.,* 24(2): 87-92.

Frizzell RT, Jones EM, Davis SN, Biggers DW, Myers SR, Connolly CC, Neal DW, Jaspan JB, Cherrington AD. (1993). Counterregulation during hypoglycemia is directed by widespread brain regions. *Diabetes*, 42: 1253-1261.

Fujii S, Sasaki H, Mikoshiba K, Kuroda Y, Yamazaki Y, Mostafa Taufiq A, Kato H. (2004). A chemical LTP induced by co-activation of metabotropic and N-methyl-D-aspartate glutamate receptors in hippocampal CA1 neurons. *Brain Res*., 999: 20-28.

Funahashi S, Bruce CJ, Goldman-Rakic PS. (1989). Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. *J. Neurophysiol*., 61: 331-349.

Gagne J, Milot M, Gelinas S, Lahsaini A, Trudeau F, Martinoli MG, Massicotte G. (1997). Binding properties of glutamate receptors in streptozotocin-induced diabetes in rats. *Diabetes*, 46: 841-846.

Galanopoulos E, Lellos V, Papadakis M, Philippidis H, Palaiologos G. (1988). Effects of fasting and diabetes on some enzymes and transport of glutamate in cortex slices or synaptosomes from rat brain. *Neurochem Res*., 13: 243-248.

Gallagher MJ, Huang H, Pritchett DB, Lynch DR. (1996). Interactions between ifenprodil and the NR2B subunit of the N-methyl-D-aspartate receptor. *J. Biol. Chem.*, 271: 9603-9611.

Ganel R, Rothstein JD. (1999). Chapter 15, Glutamate transporter dysfunction and neuronal death. in Monyer, Hannah; Gabriel A. Adelmann; Jonas, Peter. Ionotropic glutamate receptors in the CNS. Berlin: Springer. pp. 472-493.

Gao Z-Y, Li G, Najafi H, Wolf BA, Matschinsky FM. (1999). Glucose regulation of glutaminolysis and its role in insulin secretion. Diabetes 48: 1535–1542,

García O, Almeida A, Massieu L, Bolaños JP. (2005). Increased mitochondrial respiration maintains the mitochondrial membrane potential and promotes survival of cerebellar neurons in an endogenous model of glutamate receptor activation. *J Neurochem*., 92: 183-190.

Garris DR. (1990).Age and diabetes-associated alterations in regional brain norepinephrine concentrations and adrenergic receptor populations in C57BL/KsJ mice. *Brain Res. Dev. Brain* Res., 51:161-166.

Garris DR. (1995). Developmental and regional changes in brain norepinephrine levels in diabetic C57BL/KsJ mice: effects of estradiol and progesterone. *Brain Res. Dev. Brain. Res*., 89: 314-319.

Garthwaite J. (1991). Glutamate, nitric oxide and cell-cell signaling in the nervous system. *Trends Neurosci.*, 14: 60-67.

Garthwaite J. (2005). Dynamics of cellular NO-cGMP signaling. *Front. Biosci.*, 10: 1868-1880.

Gaskins HR, Baldeón ME, Selassie L, Beverly JL. (1995). Glucose modulates gamma-aminobutyric acid release from the pancreatic beta TC6 cell line. *J. Biol. Chem*., 270: 30286-30289.

Gasparova Z, Jariabka P, Stolc S. (2008). Effect of transient ischemia on long-term potentiation of synaptic transmission in rat hippocampal slices. *Neuro Endocrinol Lett*., 29: 702-705.

Gauthier C, Vranic M, Hetenyi G Jr. (1980). Importance of glucagon in regulatory rather than emergency responses to hypoglycemia. *Am J Physiol*., 23: 131-140.

Gazit V, Ben-Abraham R, Pick CG, Ben-Shlomo I, Katz Y. (2003). Long-term neurobehavioral and histological damage in brain of mice induced by L-cysteine. *Pharmacology biochemistry and behavior*, 75: 795-799.

Geesaman BJ. (2006).Genetics of aging: implications for drug discovery and development. *Am J Clin Nutr*., 83: 466S-469S.

Geinisman Y, de Toledo-Morrell L, Morrell F, Persina IS, Rossi M. (1992). Age-related loss of axospinous synapses formed by two afferent systems in the rat dentate gyrus as revealed by the unbiased stereological dissector technique. *Hippocampus,* 2: 437- 444.

Geinisman Y, de Toledo-Morrell L, Morrell F. (1986). Loss of perforated synapses in the dentate gyrus: morphological substrate of memory deficit in aged rats. *Proc Natl Acad Sci U S A*., 83: 3027-3031.

Geng MY, Saito H, Nishiyama N, Nishiyama N. (1997). Protective effects of pyridoxal phosphate against glucosedeprivation- induced damage in cultured hippocampal neurons. *Journal of Neurochemistry*, 68: 2500-2506.

George E, Harris N, Bedford C, Macdonald IA, Hardisty CA, Heller SR. (1995). Prolonged but partial impairment of the hypoglycaemic physiological response following short-term hypoglycaemia in normal subjects. *Diabetologia*, 38: 1183–1190.

German MS, Moss LG, Rutter WJ. (1990). Regulation of insulin gene expression by glucose and calcium in transfected primary islet cultures. *J. Biol. Chem*, 265: 22063-22066.

Gibson GE, Blass JP. (1976). Impaired synthesis of acetylcholine in brain accompanying hypoglycaemia and mild hypoxia *J. Neurochem*., 27: 37-42.

Gireesh G, Reas SK, Jobin M, Paulose CS. (2008a). Decreased muscarinic M1 receptor gene expression in the cerebral cortex of streptozotocin-induced diabetic rats and Aegle marmelose leaf extract's therapeutic function. *J Ethnopharmacol*., 116: 296-304.

Gireesh G, Kaimal SB, Kumar TP, Paulose CS. (2008b). Decreased muscarinic M1 receptor gene expression in the hypothalamus, brainstem, and pancreatic islets of streptozotocin-induced diabetic rats. *J Neurosci Res*., 86: 947-953.

Gispen WH, Biessels GJ. (2000). Cognition and synaptic plasticity in diabetes mellitus. *Trends Neurosci*., 23: 542-549.

Glowinski J, Iversen LL. (1966). Regional studies of catecholamines in the rat brain: The disposition of [3H] norepinephrine, [3H] dopamine and [3H] dopa in various regions of the brain. *J Neurochem*., 13: 655–669.

Gold AE, MacLeod KM, Deary IJ, Frier BM. (1995). Hypoglycaemia-induced cognitive dysfunction in diabetes mellitus: effect of hypoglycaemia unawareness. *Physiol Behav*., 58: 501-511.

Gómez-Isla T, Price JL, McKeel DW Jr, Morris JC, Growdon JH, Hyman BT (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J Neurosci.*, 16: 4491-4500.

González-Forero D, Portillo F, Gómez L, Montero F, Kasparov S, Moreno-López B. (2007). Inhibition of resting potassium conductances by long-term activation of the NO/cGMP/protein kinase G pathway: a new mechanism regulating neuronal excitability. *J Neurosci.*, 27: 6302-6312.

Gordon MJ. (2006). Hypoglycemia and seizures in large full-term neonates. *AAP Grand Rounds,* 16: 43–44.

Goulding EH, Ngai J, Kramer RH, Colicos S, Axel R, Siegelbaum SA, Chess, A. (1992). Molecular cloning and single-channel properties of the cyclic nucleotide-gated channel from catfish olfactory neurons. *Neuron,* 8: 45-58.

Gowen E, Miall C. (2005). Behavioural aspects of cerebellar function in adults with Asperger syndrome. *The Cerebellum*, 4: 1-11.

Greco D, Angileri G. (2004). Drug-induced severe hypoglycaemia in Type 2 diabetic patients aged 80 years or older. *Diabetes Nutr Metab.*, 17: 23-26.

Greenberg G, Pokol D. (1994). Neural modulation of glucose dependent insulinotropic peptide (GIP) and insulin secretion in conscious dogs. *Pancreas*, 9: 531-535.

Greene JG, Greenamyre JT. (1996). Bioenergetics and glutamate excitotoxicity. *Prog Neurobiol.*, 48: 613–634.

Gromada J, Dissing S, Bokvist K, Renstrom E, Frokjaer-Jensen J, Wulff BS, Rorsman P. (1995). Glucagon-like peptide-1 increases cytoplasmic Ca2+ in insulin-secreting ßTC3 cells by enhancement of intracellular Ca2+ mobilization. *Diabetes*, 44: 767–774.

Gromada J, Bokvist K, Ding WG, Holst JJ, Nielsen JH, Rorsman P. (1998a). Glucagon-like peptide 1 (7–36) amide stimulates exocytosis in human pancreatic ß cells by both proximal and distal regulatory steps in stimulus-secretion coupling. *Diabetes*, 47: 57–65.

Gromada J, Holst JJ, Rorsman P. (1998b). Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide-1. *Pflügers Arch*., 435: 583–594.

Gurcharan K, Sukwinder LS. (1994). Effect of alloxan-induced diabetes on Na+/K+-ATPase activity from descrete areas of rat brain. *Biochem. Mol. Biol. Int*., 34: 781-788.

Haber BA, Chin S, Chuang E, Buikhuisen W, Naji A, Taub R. (1995). High levels of glucose-6-phosphatase gene and protein expression reflect an adaptive response in proliferating liver and diabetes. *J. Clin. Invest*., 95:832-841.

Han BC, Koh SB, Lee EY, Seong YH. (2004). Regional difference of glutamate-induced swelling in cultured rat brain astrocytes. *Life Sci*., 76: 573-583.

Hannonen R, Tupola S, Ahonen T, Riikonen R. (2003). Neurocognitive functioning in children with type-1 diabetes with and without episodes of severe hypoglycaemia. *Dev. Med. Child Neurol*., 45: 262 –268.

Hansen AJ. (1985). Effect of anoxia on ion distribution in the brain. *Physiol Rev.,* 65: 101-148.

Hardingham GE, Fukunaga Y, and Bading H. (2002). Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nature Neuroscience*, 5: 405-414.

Harris TE, Persaud SJ, John PM. (1996). A typical isoform of protein kinase C and insulin secretion from pancreatic beta cell: evidence using GEO 6976 and RO 31-8220 as PK-C inhibitors. *Biochem. Biophysics. Res. Commun*., 227: 672-676.

Hawdon JM. (1999). Hypoglycaemia and the neonatal brain. *Eur. J. Pediatr*., 158: 9–12.

Hedden T, Gabrieli JD. (2004). Insights into the ageing mind: a view from cognitive neuroscience. *Nat Rev Neurosci*., 5: 87-96.

Heffner TG, Hartman JA, Seiden LS. (1980). A rapid method for the regional dissection of the rat brain. *Pharmacol. Biochem. Behav*., 13: 453-456.

Heller SR, Cryer PE. (1991). Reduced neuroendocrine and symptomatic responses to subsequent hypoglycaemia after 1 episode of hypoglycaemia in nondiabetic humans. *Diabetes*, 40: 223–226.

Hellman B, Sehlin J, Täljedal IB. (1976). Calcium and secretion: distinction between two pools of glucose-sensitive calcium in pancreatic islets. *Science*, 194: 1421-1423.

Hepburn DA, Patrick AW, Brash HM, Thomson I, Frier BM. (1991) Hypoglycaemia unawareness in type 1 diabetes: a lower plasma glucose is required to stimulate sympatho-adrenal activation. *Diabet Med*., 8: 934-945.

Hepler DJ, Olton DS, Wenk GL, Coyle JT. (1985). Lesions in nucleus basalis magnocellularis and medial septal area of rats produce qualitatively similar memory impairments. *J Neurosci*., 5: 866-873.

Hermann B, Seidenberg M, Sears L, Hansen R, Bayless K, Rutecki P, Dow C. (2004). Cerebellar atrophy in temporal lobe epilepsy affects procedural memory. *Neurology*, 63: 2129- 2131.

Hernández-Fonseca K, Cárdenas-Rodríguez N, Pedraza-Chaverri J, Massieu L. (2008). Calcium-dependent production of reactive oxygen species is involved in neuronal damage induced during glycolysis inhibition in cultured hippocampal neurons. *J Neurosci Res*., 86: 1768-1780.

Herrero I, Miras-Portugal MT, Sánchez-Prieto J. (1994). Rapid desensitization of the metabotropic glutamate receptor facilitates glutamate release in rat cerebrocortical nerve terminals. *Eur J Neurosci*., 6: 115–120.

Hershey T, Bhargava N, Sadler M, White NH, Craft S. (1999). Conventional versus intensive diabetes therapy in children with type 1 diabetes: effects on memory and motor speed. *Diabetes Care*, 22: 1318 –1324.

Hertz L, Dringen R, Schousboe A, Robinson SR. (1999). Astrocytes: glutamate producers for neurons. *J. Neurosci. Res*., 57: 417-428.

Hinoi E, Ogita K, Takeuchi Y, Ohashi H, Maruyama T, Yoneda Y. (2001). Characterization with [3H]quisqualate of group I metabotropic glutamate receptor subtype in rat central and peripheral excitable tissues. *Neurochem. Int*., 38: 277-285.

Hiroyuki U, Yoko Y, Yusuke S, Akihisa I. (2006). Microdialysis measurement of acetylcholine in rat hippocampus during severe insulin-induced hypoglycaemia. *Neuro Endocrinol Lett*., 29: 27.

Ho BC, Mola C, Andreasen NC. (2004). Cerebellar dysfunction in neuroleptic naive schizophrenia patients: Clinical, cognitive, and neuroanatomic correlates of cerebellar neurologic signs. *Biol. Psychiatry*, 55: 1146-1153.

Hof PR, Glannakopoulos P, Bouras C. (1996). The neuropathological changes associated with normal brain aging. *Histol Histopathol*., 11: 1075-1088.

Hoffman DJ, Zanelli SA, Kubin JM, Om P, Delivoria PM. (1996). The in vivo effect of bilirubin on the N-methyl-D-aspartate receptor/ion channel complex in the brains of newborn piglets. *Pediatr Res*., 40: 804–808.

Hofmann F, Ammendola A, Schlossmann J. (2000). Rising behind NO: cGMP-dependent protein kinases. *J Cell Sci*., 113: 1671-1676.

Hohenegger M, Rudas B. (1971). Kidney function in experimental diabetic ketosis. *Diabetologia*, 7: 334–338.

Hollmann M, Heinemann S. (1994). Cloned glutamate receptors. *Annu. Rev. Neurosci*., 17: 31-108.

Holness MJ, Sugden MC. (1987).Continued glucose output after re-feeding contributes to glucose intolerance in hyperthyroidism. *Biochem J*., 247: 801-804.

Holopainen I, Louve M, Enkvist MO, Akerman KE. (1989). Rubidium release from cultured primary astrocytes: effects of excitatory and inhibitory amino acids. *Neuroscience.* 30: 223-229.

Holopainen I, Louve M, Enkvist MOK, Akerman KEO. (1990). Coupling of glutamatergic receptors to changes in intracellular Ca2+ in rat cerebellar granule cells in primary culture. *J. Neurosci. Res.*, 25: 187-193.

Holz GG, Kuhtreiber WM, Habener JF. (1993). Pancreatic ß cells are rendered glucose competent by the insulinotropic hormone glucagon-like peptide-1-(7–37). *Nature,* 361: 362–365.

Holz GG, Leech CA, Habener JF. (1995). Activation of a cAMP-regulated Ca2+-signaling pathway in pancreatic ß cells by the insulinotropic hormone glucagon-like peptide-1. *J Biol Chem*., 270: 17749–17757.

Holz GG, Leech CA, Heller RS, Castonguay M, Habener JF. (1999). cAMP-dependent mobilization of intracellular Ca2+ stores by activation of ryanodine receptors in pancreatic ß cells. A Ca2+ signaling system stimulated by the insulinotropic hormone glucagon-like peptide-1-(7–37). *J Biol Chem*., 274: 14147–14156.

Horiuchi J, Saitoe M. (2005). Can flies shed light on our own age-related memory impairment? *Ageing Res. Rev*., 4: 83-101.

Howell SL, Taylor, KW. (1968). Pottassium ions and the secretions of insulin by islets of langerhans incubated *in vitro. Biochem. J*., 108: 17-24.

Hu BR, Kurihara J, Wieloch T. (1995). Persistent translocation and inhibition of Ca2+/calmodulin-dependent protein kinase II in the crude synaptosomal fraction of the vulnerable hippocampus following hypoglycaemia *J. Neurochem*., 64: 1361-1369.

Hutton JC, Peshavaria M. (1982). Proton-translocating Mg2+-dependent ATPase activity in insulin-secretory granules. *Biochem J*., 204: 161–170.

Hutton JC. (1989). The insulin secretory granule. *Diabetologia*, 32: 271–281.

Hvidberg A, Fanelli CG, Hershey T, Terkamp C, Craft S, Cryer PE. (1996). Impact of recent antecedent hypoglycaemia on hypoglycaemic cognitive dysfunction in nondiabetic humans. *Diabetes*, 46: 1031–1036.

Imazumi H, Miyauchi S, Tamada T, Sasaki Y, Takino R, Futz B, Yoshioka T, Kawato M. (2004). Human cerebellar activity reflecting an acquired internal model of a new tool. *Nature*, 3: 192-195.

Inagaki N, Kuromi H, Gonoi T, Okamoto Y, Ishida H, Seino Y, Kaneko T, Iwanaga T, Seino S. (1995). Expression and role of ionotropic glutamate receptors in pancreatic islet cells. *FASEB J.*, 9: 686-691.

InterPro. InterPro: IPR001786 Metabotropic glutamate receptor 4. (2008).

Isaacowitz DM, Wadlinger HA, Goren D, Wilson HR (2006). Selective preference in visual fixation away from negative images in old age? An eye tracking study. *Psychol Aging*., 21: 40-48.

Ivry R, Keele SW, Diener HC. (1988). Dissociation of the lateral and medial cerebellum in movement timing and movement execution. *Exp. Brain Res.*, 73: 167-180.

Jackson J, Pius SP, Thomas P, Paulose CS. (1997). Platelet mooamine changes in diabetic patients and streptozotocin induced diabetic rats. *Current Science*, 72: 137-139.

Jackson J, Paulose CS. (1999). Enhancement of [m-methoxy [3 H] MDL 100907 binding to 5-HT 2A receptors in the cerebral cortex and brain stem of streptozotocin induced diabetic rats. *Mol. Cell. Biochem.*, 199: 81-85.

Jackson J, Paulose CS. (2000). Brain 5HT2A receptor regulation by tryptophan supplementation in streptozotocin diabetic rats. *J. Biochem. Mol. Biol. & Biophys.*, 5: 1-7.

Jakobsen J, Knudsen GM, Juhler M. (1987). Cation permeability of the blood brain barrier in streptozotocin-diabetic rats. *Diabetologia*, 30: 409-413.

Jancke L, Loose R, Lutz K, Specht K, Shah NJ. (2000). Cortical activations during paced finger-tapping applying visual and auditory pacing stimuli. *Brain Res. Cogn. Brain Res.*, 10: 51-66.

Jane E M. (1999). Neonatal Hypoglycaemia. *Pediatrics in Review*, 20: 6-15.

Javitt DC, Steinschneider M, Schroeder CE, Arezzo JC. (1996). Role of cortical N-methyl-D-aspartate receptors in auditory sensory memory and mismatch negativity generation: implications for schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.*, 1593: 11962-11967.

Jiao J, Nakajima A, Janssen WGM, Bindokas VP, Xiong X, et al (2008). Expression of NR2B in Cerebellar Granule Cells Specifically Facilitates Effect of Motor Training on Motor Learning. *PLoS ONE* 3: e1684.

Jo J, Heon S, Kim MJ, Son GH, Park Y, Henley JM, Weiss JL, Sheng M, Collingridge GL, Cho K. (2008). Metabotropic glutamate receptor-mediated LTD involves two interacting Ca(2+) sensors, NCS-1 and PICK1. *Neuron*, 60: 1095-1111.

Johnson KA, Kijewski MF, Becker JA, Garada B, Satlin A, Holman BL. (1993). Quantitative brain SPECT in Alzheimer's disease and normal aging. *J Nucl Med*., 34: 2044-2048.

Johnson DA, Zambon NJ, Gibbs RB. (2002). Selective lesion of cholinergic neurons in the medial septum by 192 IgG-saporin impairs learning in a delayed matching to position T-maze paradigm. *Brain Res.,* 943: 132-141.

Johnston MV. (2005). Excitotoxicity in perinatal brain injury. *Brain Pathol*., 15: 234-240.

Joly C, Gomeza J, Brabet I, Curry K, Bockaert J, Pin JP. (1995). Molecular, functional, and pharmacological characterization of the metabotropic glutamate receptor type 5 splice variants: comparison with mGluR1. *J Neurosci*., 15: 3970-3981.

Joseph A, Remya R, Paulose CS. (2007). Enhanced [^{3}H] Glutamate Binding in the Cerebellum of Insulin Induced Hypoglycaemic and Streptozotocin Induced Diabetic Rats. *Cellular and Molecular Neurobiology,* 27: 1085-1095.

Joseph A, Sherin B, Paulose CS. (2008). Increased glutamate receptor gene expression in the cerebral cortex of insulin induced hypoglycaemic and streptozotocin induced diabetic rats. *Neuroscience*, 156: 298-304.

Jouvert P, Revel MO, Lazaris A, Aunis D, Langley K, Zwiller J. (2004). Activation of the GMP Pathway in Dopaminergic Structures Reduces Cocaine-Induced

EGR-1 Expression and Locomotor Activity. *J. Neuroscience*, 24: 10716-10725.

Julius D, Livelli T, Jessell TM, Axel, R. (1989). Ectopic expression of the serotonin 1c receptor and the triggering of malignant transformation. *Science*, 244: 1057-1062.

Junod A, Lambert AE, Staufferacher W, Renold AE. (1969). Diabetogenic action of Streptozotocin: Relationship of dose to metabolic response. *J. Clin. Invest*., 48: 2129-2139.

Kale AY, Paranjape SA, Briski KP. (2006). Type II glucocorticoid receptor involvement in habituated activation of lateral hypothalamic area orexin-A-immunopositive neurons during recurring insulin-induced hypoglycemia. *Neurosci Res*., 56: 309-313.

Kalimo H, Olsson Y. (1980). Effects of severe hypoglycemia on the human brain. Neuropathological case reports. *Acta Neurol Scand*., 62: 345-356.

Kang G, Chepurny OG, Rindler MJ, Collis L, Chepurny Z, Li WH, Harbeck M, Roe MW, Holz GG. (2005). A cAMP and Ca2+ coincidence detector in support of Ca2+-induced Ca2+ release in mouse pancreatic beta cells. *J Physiol*., 566: 173-188.

Karp MM. (1989). Hypoglycemia in diabetes among children and adolescents. *Indian J Pediatr*., 56: 93-98.

Katagiri H, Tanaka K, Manabe T. (2001). Requirement of appropriate glutamate concentrations in the synaptic cleft for hippocampal LTP induction. *Eur J Neurosci*., 14: 547–553.

Katherine LN, Roger PW, Paul MT, Philip S, Delbert R, Teodora D, Mala G, Arthur WT, Robert MB. (2007). Relationships between IQ and Regional Cortical Grey Matter Thickness in Healthy Adults. *Cerebral Cortex,* 17: 2163–2171.

Kaufman FR. (1998). Diabetes in children and adolescents. Areas of controversy. *Med Clin North Am.*, 82: 721-738.

Kaupp UB, Niidome T, Tanabe T, Terada S, Bönigk W, Stühmer W, Cook NJ, Kangawa K, Matsuo H, Hirose T. (1989). Primary structure and functional expression from complementary DNA of the rod photoreceptor cyclic GMP-gated channel. *Nature*, 342: 762-766.

Kawaai K, Hisatsune C, Kuroda Y, Mizutani A, Tashiro T, Mikoshiba K. (2009). 80K-H interacts with inositol 1,4,5-trisphosphate (IP3) receptors and regulates IP3-induced calcium release activity. *J Biol Chem*., 284: 372-380.

Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Matsuda M, Housman DE, Graybiel AM. (1998). A family of cAMP-binding proteins that directly activate Rap1. *Science*, 282: 2275-2259.

Kim AH, Kerchner GA, and Choi DW. Blocking Excitotoxicity. Chapter 1 in CNS Neuroproteciton. Marcoux FW and Choi DW, editors. Springer, New York. 2002. Pages 3-36.

Kim M, Zhao-Xue Yu, Bertil BF, Scott AR. (2005). Susceptibility of the developing brain to acute hypoglycemia involving A1 adenosine receptor activation. *Am J Physiol Endocrinol Metab*., 289:E562–E569.

Kito S, Miyoshi R, Nomoto T. (1990). Influence of age on NMDA receptor complex in rat brain studied by in vitro autoradiography. *J Histochem Cytochem*., 38: 1725.

Kleihues P, Kiessling M, Thilmann R, Xie Y, Uozumi A, Volk B. (1986). Resistance to hypoglycemia of cerebellar transplants in the rat forebrain. *Acta Neurol Pathol*., 72: 23–28.

Kleim JA, Jones TA, Schallert T. (2003). Motor enrichment and the induction of plasticity before or after brain injury. *Neurochem Res*., 28: 1757–1769.

Klein DC, Coon SL, Roseboom PH, Weller JL, Bernard M, Gastel JA, Zazt M, Iuvone PM, Rodriguez IR, Begay V, Falcon J, Cahill GM, Cassone VM, Baler R. (1997). The melatonin rhythm-generating enzyme: molecular regulation of serotonin N-acetyltransferase in the pineal gland. *Recent Prog. Horm. Res*., 52: 307-358.

Kotecha SA, Jackson MF, Al-Mahrouki A, Roder JC, Orser BA, MacDonald JF. (2003). Co-stimulation of mGluR5 and N-methyl- D-aspartate receptors is required for potentiation of excitatory synaptic transmission in hippocampal neurons. *J. Biol. Chem*., 278: 27742-27749.

Krakauer JW, Shadmehr R. (2006). Consolidation of motor memory. *PLoS Biol.,* 4: e316.

Krinsley JS, Grover A. (2007). Severe hypoglycemia in critically ill patients: risk factors and outcomes. *Crit Care Med*., 35(10): 2262–2267.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD. (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans: psychotomimetic, perceptional, cognitive and neuroendocrine response. *Arch. Gen. Psychiatry*, 51: 199-214.

Kumar PJ, Clark M. (2002). Diabetes mellitus and other disorders of metabolism. *In. Clinical Medicine, Saunders (London),* 1069-1121.

Kuo MF, Song D, Murphy S, Papadopoulos MD, Wilson DF, Pastuszko A. (1998). Excitatory amino acid receptor antagonists decrease hypoxia induced increase in extracellular dopamine in striatum of newborn piglets. *Neurochem Int*., 32: 281-289.

Kwok RP, Juorio AV. (1987). Facilitating effect of insulin on brain 5 hydroxytryptamine metabolism. *Neuroendocrinology*, 45:267-273.

Lacey RJ, Cable HC, James RF, London NJ, Scarpello JH, Morgan NG. (1993). Concentration-dependent effects of adrenaline on the profile of insulin secretion from isolated human islets of Langerhans. *J Endocrinol*., 138: 555-563.

Lackovic Z, Salkovic M, Kuci Z, Relja M. (1990). Effect of long lasting diabetes mellitus on rat and human brain monoamines. *J. Neurochem*., 54: 143-147.

Lahiri DK. (2005). Advances of Alzheimer's disease research: crossroad of basic and translational studies. *Curr Alzheimer Res*., 2: 495-496.

Lang CH. (1995). Inhibition of central GABAA receptors enhances hepatic glucose production and peripheral glucose uptake. *Brain Res. Bull*., 37: 611- 613.

Langan SJ, Deary IJ, Hepburn DA, Frier BM. (1991). Cumulative cognitive impairment following recurrent severe hypoglycaemia in adult patients with insulin-treated diabetes mellitus. *Diabetologia*, 34: 337 –344.

Larsen PR. (1972). Direct immunoassay of triiodothyronine in human serum. *J Clin invest.,* 51: 1939.

Larsen GA, Skjellegrind HK, Berg-Johnsen J, Moe MC, Vinje ML. (2006). Depolarization of mitochondria in isolated CA1 neurons during hypoxia, glucose deprivation and glutamate excitotoxicity. *Brain Res*., 1077: 153-160.

Latifpour J, McNeill JH. (1984). Cardiac autonomic receptors: effect of long-term experimental diabetes. *J Pharmacol. Exp. Ther*., 230: 242-249.

Latifpour J, Gousse, Yoshida M, Weiss RM. (1991). Muscarinic receptors in diabetic rat prostate. *Biochem. Pharmacol*., 42: 113-119.

Laurie DJ, Seeburg PH. (1994). Ligand affinities at recombinant N-methyl-D-aspartate receptors depend on subunit composition. *Eur J Pharmacol*., 268: 335-345.

Lea PM, Custer SJ, Vicini S, Faden AI. (2002). Neuronal and glial mGluR5 modulation prevents stretch-induced enhancement of NMDA receptor current. *Pharmacol. Biochem. Behav*., 73: 287-298.

Lees GJ. (1991). Inhibition of sodium-potassium-ATPase: a potentially ubiquitous mechanism contributing to central nervous system neuropathology. *Brain Res Rev*., 16: 283-300.

Leeson PD, Iversen LL. (1994). The glycine site on the NMDA receptor: structure-activity relationships and therapeutic potential. *J Med Chem*., 37: 4053-4067.

Lewis PA, Miall RC. (2003). Distinct systems for automatic and cognitively controlled time measurement: Evidence from neuroimaging. *Curr. Opin. Neurobiol*., 13: 250-255.

Li L, Fan M, Icton CD, Chen N, Leavitt BR, Hayden MR, Murphy TH, Raymond LA. (2003). Role of NR2B-type NMDA receptors in selective neurodegeneration in Huntington disease. *Neurobiol Aging,* 24:1113-1121.

Li C, Matter A, Kelly A, Petty TJ, Najafi H, MacMullen C, Daikhin Y, Nissim I, Lazarow A, Kwagh J, Collins HW, Hsu BY, Nissim I, Yudkoff M, Matschinsky FM, Stanley CA. (2006). Effects of a GTP-insensitive mutation of glutamate dehydrogenase on insulin secretion in transgenic mice. *J Biol Chem*., 281: 15064-15072.

Like AA, Rossini AA. (1976). Streptozotocin–induced pancreatic insulities: new model of diabetes mellitus. *Science*, 193: 415-417.

Lindström P, Ohlsson L. (1992). Effect of N-methyl-D,L-aspartate on isolated rat somatotrophs. *Endocrinology*, 131: 1903-1907.

Linn RT, Wolf PA, Bachman DL, Knoefel JE, Cobb JL, Belanger AJ, Kaplan EF, D'Agostino RB. (1995). The 'preclinical phase' of probable Alzheimer's disease. A 13-year prospective study of the Framingham cohort. *Arch Neurol*., 52: 485-490.

Lipton, Nicotera. (1998). Calcium, free radicals and excitotoxins in neuronal apoptosis. *Cell Calcium*. 23: 165-171.

Liu P, Scott J, Smith PM. (1998). Intracellular calcium signalling in rat parotid acinar cells that lack secretory vesicles*. Biochem J*., 330: 847-852.

Liu Y, Zhang J. (2000). "Recent development in NMDA receptors". *Chin. Med. J*., 113: 948-956.

Liu YQ, Montanya E, Leahy JL. (2001). Increased islet DNA synthesis and glucose derived lipid and amino acid production in association with beta cell hyperproliferation in normoglycaemic 60% pancreatectomy rats. *Diabetologia*, 44: 1026–1033.

Llansola M, Sanchez-Perez A, Cauli O, Felipo V. (2005). Modulation of NMDA receptors in the cerebellum. 1. Properties of the NMDA receptor that modulate its function. *Cerebellum*, 4: 154–161.

Llinas R, Welsh JP. (1993). On the cerebellum and motor learning. *Curr Opin Neurobiol*., 3: 958–965.

Lowry OH, Roserbbrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with Folin phenol reagent. *J Biol Chem*., 193: 265–275.

Lu Y, Li Y, Herin GA, Aizenman E, Epstein PM, Rosenberg PA. (2004). Elevation of intracellular cAMP evokes activity-dependent release of adenosine in cultured rat forebrain neurons. *Eur J Neurosci.,* 19: 2669-2681.

Lyon L, Kew JN, Corti C, Harrison PJ, Burnet PW. (2008). Altered hippocampal expression of glutamate receptors and transporters in GRM2 and GRM3 knockout mice. *Synapse*, 62: 842-850.

MacDermott AB, Mayer ML, Westbrook GL, Smith SJ, Barker JL. (1986). NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature*, 321: 519-522.

MacDonald MJ, Fahien LA. (2000). Glutamate is not a messenger in insulin secretion. *J Biol Chem*., 275: 34025–34027.

Macmullen C, Fang J, Hsu BYL, Kelly A, De Lonlay-Debeney P, Saudubray J-M, Ganguly A, Smith TJ, Stanley CA. (2001). Hyperinsulinism/hyperammonemia syndrome in children with regulatory mutations in the inhibitory guanosine triphosphate-binding domain of glutamate dehydrogenase. *J Clin Endocrinol Metab.,* 86: 1782–1787.

Madras BK, Cohen EL, Messing R, Munro HN, Wurtman RJ. (1974). Relevance of free tryptophan in serum to tissue tryptophan concentrations. *Metabolism,* 23: 1107-1116.

Maechler P, Wollheim CB. (1999). Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis. *Nature,* 402: 685-689.

Maechler P, Wollheim CB. (2000). Mitochondrial signals in glucose-stimulated insulin secretion in the beta cell. *J Physiol*., 529: 49-56.

Magnusson KR. (2000). Declines in mRNA expression of different subunits may account for differential effects of aging on agonist and antagonist binding to the NMDA receptor. *Journal of Neuroscience*, 20: 1666-1674.

Magnusson AK, Ulfendahl M, Tham R. (2002). Early compensation of vestibulo-oculomotor symptoms after unilateral vestibular loss in rats is related to GABA(B) receptor function. *Neuroscience*, 111: 625-634.

Magnusson KR, Kresge D, Supon J. (2006). Differential effects of aging on NMDA receptors in the intermediate versus the dorsal hippocampus. *Neurobiol Aging*., 27: 324-333.

Majdi M, Ribeiro-da-Silva A, Cuello AC. (2007). Cognitive impairment and transmitter-specific pre- and postsynaptic changes in the rat cerebral cortex during ageing. *Eur J Neurosci*., 26: 3583-3596.

Malaisse WJ, Pipeleers DG, Levy J. (1974). The stimulus-secretion coupling of glucose-induced insulin release. XVI. A glucose-like and calcium-independent effect of cyclic AMP. *Biochim Biophys Acta*., 362: 121-128.

Malaisse WJ. (1972). Hormonal and environment modification of islet activity. In endocrine pancreas. Ed. Greep RO & Astwood EB.. Washington DC. Am. Physiol. Soc., 237-260.

Malaisse WJ. (1973). Effect of glibenclamide upon 45 calcium efflux from perifused islets. *Arch Int Pharmacodyn Ther*., 203: 403-406.

Malarkey EB, Parpura V. (2008). Mechanisms of glutamate release from astrocytes. *Neurochem Int*., 52:142–154.

Malenka RC, Nicoll RA. (1999). Long-term potentiation - a decade of progress? *Science*, 285: 1870-1874.

Malone JI, Hanna S, Saporta S, Mervis RF, Park CR, Chong L, Diamond DM. (2008). Hyperglycemia not hypoglycemia alters neuronal dendrites and impairs spatial memory. *Pediatr Diabetes*., 9: 531-539.

Manev H, Favaron M, Guidotti A, and Costa E. (1989). Delayed increase of Ca^{2+} influx elicited by glutamate: role in neuronal death. *Molecular Pharmacoloy,* 36: 106-112.

Mangano RM, Schwarcz R. (1983). Chronic infusion of endogenous excitatory amino acids into rat striatum and hippocampus. *Brain Res Bull*., 10: 47–51.

Maran A, Cranston I, Lomas J, Macdonald I, Amiel SA. (1994). Protection by lactate of cerebral function during hypoglycaemia. *Lancet*, 343: 16–20.

Maran A, Lomas J, Macdonald IA, Amiel SA. (1995). Lack of preservation of higher brain function during hypoglycaemia in patients with intensively-treated IDDM. *Diabetologia*, 38: 1412-1418.

Marinelli S, Federici M, Giacomini P, Bernardi G, Mercuri NB. (2001). Hypoglycemia enhances ionotropic but reduces metabotropic glutamate responses in substantia nigra dopaminergic neurons. *J Neurophysiol*., 85: 1159-1166.

Marr D. (1969). A theory of cerebellar cortex. *J Physiol*., 437–470.

Martha CS, Abigail B, Leticia O, Lora MG. (2009). Alterations in Glutamate Uptake in NT2-Derived Neurons and Astrocytes after Exposure to Gamma Radiation. *Radiation Research,* 171: 41-52.

Martin RL, Lloyd HGE, Cowan AI. (1994). The early events of oxygen and glucose deprivation: setting the scene for neuronal death? *Trends Neurosci*., 17: 251-257.

Martin SJ, Grimwood PD, Morris RG. (2000). Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci*., 23: 649-711.

Marvel CL, Schwartz BL, Rosse RB. (2004). A quantitative measure of postural sway deficits in schizophrenia. *Schizophr. Res*., 68: 363-372.

Mather M, Carstensen LL. (2005). Aging and motivated cognition: the positivity effect in attention and memory. *Trends Cogn Sci*., 9: 496-502.

Matsuoka H, Ishii M, Sugimoto T, Hirata Y, Sugimoto T, Kangawa K, Matsuo H. (1985). Inhibition of aldosterone production by alpha-human atrial natriuretic polypeptide is associated with an increase in cGMP production. *Biochem. Biophys.Res.Commun.*, 127: 1052-1056.

Mattes JA. (1980). The role of frontal lobe dysfunction in childhood hyperkinetics. *Comp. Psychiatry*., 21: 358-369.

Matthews JL. (1970). Ultrastructure of calcifying tissues. *Am J Anat*., 129: 451-457.

Mavrothalassitis G, Tzimagiorgis G, Mitsialis A, Zannis V, Plaitakis A, Papamatheakis J, Moschonas N. (1988). Isolation and characterization of cDNA clones encoding human liver glutamate dehydrogenase: evidence for a small gene family. *Proc Natl Acad Sci*., 85: 3494-3498.

Mazure CM. (1995). Does stress cause psychiatric illness? In: Progress in Psychiatry 270-298. Washington, DC: American Psychiatric.

McAulay V, Deary IJ, Frier BM. (2001). Symptoms of hypoglycaemia in people with diabetes. *Diabet Med*., 18: 690-705.

McBain CJ, Mayer ML. (1994). N-Methyl-D-aspartic acid receptor structure and function. *Physiol. Rev*., 74: 723-760.

McCall, Millington Wr, Wurtman RJ. (1982). Metabolic fuel and amino acid transport into the brain in experimental diabetes mellitus. *Proc. Nadl. Acad. Sci. USA*, 97: 2881-2885.

McCrimmon RJ, Deary IJ, Frier BM (1997). Auditory information processing during acute insulin-induced hypoglycaemia in nondiabetic human subjects. *Neuropsychologia*, 35: 1547–1553.

McDonald JW, Johnston MV. (1990). Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Res Rev*., 15: 41-70.

McGeer PL, McGeer EG. (1982). Kainic acid: The neurotoxic breakthrough. *Crit Rev Toxicol*., 10: 1-26.

McGeer PL, Eccles JC, McGeer EG. (1987). Putative excitatory neurons: Glutamate and aspartate. In, Molecular Neurobiology of the Mammalian Brain, McGeer PL, Eccles JC, and McGeer EG (Eds.). New York: Plenum Press, 175-196.

McGowan JE, Haynes-Laing AG, Mishra OP, Delivoria-Papadopoulos M. (1995). The effect of acute hypoglycemia on the cerebral NMDA receptor in newborn piglets. *Brain Res*., 670: 283–288.

McNay EC, Fries TM, Gold PE. (2000). Decreases in rat extracellular hippocampal glucose concentration associated with cognitive demand during a spatial task. *Proc Natl Acad Sci U S A*., 97: 2881-2885.

McNay EC. (2005). The impact of recurrent hypoglycemia on cognitive function in aging. *Neurobiol Aging*. 26: 76-79.

McNay EC, Williamson A, McCrimmon RJ, Sherwin RS. (2006). Cognitive and neural hippocampal effects of long-term moderate recurrent hypoglycemia. *Diabetes*, 55: 1088-1095.

Milner RD, Hales CN. (1970). The mechanism of insulin secretion studied through the effects of electrolytes and inhibitors. *Adv Metab Disord*., 1: 59-65.

Minna LK, Pasi IS, Juhani AKE, Juha ST, Aimo R, Timo HM, Heikki VH. (2005). Effects of Sustained Insulin-Induced Hypoglycaemia on Cardiovascular Autonomic Regulation in Type 1 Diabetes. *Diabetes,* 54: 744-750.

Miriam K, Mark AD, Elena B, de Rodriguez T, Nicholas GB. (1996). Synergy by secretory phospholipase A2 and glutamate on inducing cell death and sustained arachidonic acid metabolic changes in primary cortical neuronal cultures. *J Biol Chem*., 271: 32722–32728.

Mishra OP, Delivoria-Papadopoulos M. (2004). Nuclear mechanisms of hypoxic cerebral injury in the newborn. *Clin Perinatol.*, 31: 91-105.

Mitchell KJ, Johnson MK, Raye CL, Mather M, D'Esposito M. (2000). Aging and reflective processes of working memory: binding and test load deficits. *Psychol Aging*., 15: 527-541.

Mitosek-Szewczyk K, Sulkowski G, Stelmasiak Z, Struzyńska L. (2008). Expression of glutamate transporters GLT-1 and GLAST in different regions of rat brain during the course of experimental autoimmune encephalomyelitis. *Neuroscience*, 155: 45-52.

Mohanan VV, Balarama KS, Paulose CS. (2005a). Decreased 5-HT1A receptor gene expression and 5-HT1A receptor protein in the cerebral cortex and brain stem during pancreatic regeneration in rats. *Neurochemical Research*, 30: 25-32.

Mohanan VV, Finla C, Paulose CS. (2005b). Decreased 5-HT2C receptor binding in the cerebral cortex and brain stem during pancreatic regeneration in rats. *Molecular and Cellular Biochemistry,* 272: 165-170.

Mohanan VV, Reas K, Paulose CS. (2006). Hypothalamic 5-HT functional regulation through 5-HT1A and 5-HT2C receptors during pancreatic regeneration. *Life Sciences*, 78: 1603-1609.

Mokuno H, Yamada N, Sugimoto T, Kobayashi T, Ishibashi S, Shimano H, Takizawa M, Kawakami M, Takaku F, Murase T. (1990). Cholesterol-free diet with a high ratio of polyunsaturated to saturated fatty acids in heterozygous familial hypercholesterolemia: significant lowering effect on plasma cholesterol. *Horm. Metab. Res*., 22: 246-251.

Molnár E, Váradi A, McIlhinney RA, Ashcroft SJ. (1995). Identification of functional ionotropic glutamate receptor proteins in pancreatic beta-cells and in islets of Langerhans. *FEBS Lett*., 371: 253-257.

Monkawa T, Miyawaki A, Sugiyama T, Yoneshima H, Yamamoto-Hino M, Furuichi T, Saruta T, Hasegawa M, Mikoshiba K. (1995). Heterotetrameric complex formation of inositol 1,4,5-trisphosphate receptor subunits. *J.Biol.Chem.*, 270: 14700-14704.

Montague PR, Gancayco CD, Winn MJ, Marchase RB, Friedlander MJ. (1994). Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex. *Science,* 263: 973-977.

Monyer H, Goldberg MP, Choi DW. (1989). Glucose deprivation neuronal injury in cortical culture. *Brain Res*., 483: 347-354.

Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, Seeburg PH. (1992). Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. *Science*, 256: 1217-1221.

Morita T, Tanimura A, Nexu A, Tojyo Y. (2002). Visualization of inositol 1,4,5-trisphosphate receptor type III with green fluorescent protein in living cells. *Cell Calcium*, 31: 59-64.

Morita T, Tanimura A, Nezu A, Kurosaki T, Tojyo Y. (2004). Functional analysis of the green fluorescent protein-tagged inositol 1, 4, 5-trisphosphate receptor type 3 in Ca2+ release and entry in DT40 B lymphocytes. *Biochem.J.*, 382: 793-801.

Moriyoshi K, Masu M, Ishii T, Shigemoto R, Mizuno N, Nakanishi S. (1991). Molecular cloning and characterization of the rat NMDA receptor. *Nature, (Lond.)* 354: 31-37.

Morley JE, Perry HM 3rd. (1991). The management of diabetes mellitus in older individuals. *Drugs*, 41: 548-565.

Morris JC, McKeel DW Jr, Storandt M, Rubin EH, Price JL, Grant EA, Ball MJ, Berg L. (1991). Very mild Alzheimer's disease: informant-based clinical, psychometric, and pathologic distinction from normal aging. *Neurology*, 41: 469-478.

Motta M, Bennati E, Ferlito L, Passamonte M, Cardillo E, Malaguarnera M. (2008). A review on the actual trends of insulin treatment in elderly with diabetes. *Arch Gerontol Geriatr.,* 47: 151-161.

Movsesyan VA, O'Leary DM, Fan L, Bao W, Mullins PG, Knoblach SM, Faden AI. (2001). mGluR5 antagonists 2-methyl-6-(phenylethynyl)- pyridine and (E)-2-methyl-6-(2-phenylethenyl)-pyridine reduce traumatic neuronal injury in vitro and in vivo by antagonizing N-methyl-D-aspartate receptors. *J. Pharmacol. Exp. Ther.*, 296: 41-47.

MRC (Medical Research Council), Glutamate receptors: Structures and functions., University of Bristol Centre for Synaptic Plasticity (2003).

Murphy SN, Miller RJ. (1988). A glutamate receptor regulates Ca2+ mobilization in hippocampal neurons. *Proc. Natl. Acad. Sci. USA*, 85: 8737-8741.

Mussa-Ivaldi FA, Bizzi E. (2000). Motor learning through the combination of primitives. *Philos Trans R Soc Lond B Biol Sci*., 355: 1755-1769.

Nakanishi N, Axel R, Shneider NA. (1992a). Alternative splicing generates functionally distinct N-methyl-D-aspartate receptors. *Proc. Natl. Acad. Sci. USA*., 89: 8552-8556.

Nakanishi S. (1992b). Molecular diversity of glutamate receptors and implications for brain function. *Science*, 258: 597–603.

Nakazaki M, Crane A, Hu M, Seghers V, Ullrich S, Aguilar-Bryan L, Bryan J. (2002). cAMP-activated protein kinase-independent potentiation of insulin secretion by cAMP is impaired in SUR1 null islets. *Diabetes*, 51: 3440–3449.

Nellgård B, Wieloch T. (1992). Cerebral protection by AMPA- and NMDA-receptor antagonists administered after severe insulin-induced hypoglycemia. *Exp Brain Res*., 92: 259-266.

Nelson MT, Cheng H, Rubart M, Santana LF, Bonev AD, Knot HJ, Lederer WJ. (1995). Relaxation of arterial smooth muscle by calcium sparks. *Science,* 270: 633- 637.

Nelson N, Harvey WR. (1999). Vacuolar and plasma membrane proton-adenosinetriphosphatases. *Physiol Rev*., 79: 361–385.

Ng YK, Zeng XX, Ling EA. (2004). Expression of glutamate receptors and calcium-binding proteins in the retina of streptozotocin-induced diabetic rats. *Brain Res*., 1018: 66–72.

Nicholls D, Attwell D. (1990). The release and uptake of excitatory amino acids. *Trends Pharmacol. Sci.*, 11: 462-468.

Nicoletti F, Bruno V, Catania MV, Battaglia G, Copani A, Barbagallo G, Cena V, Sanchez-Prieto J, Spano PF, Pizzi M. (1999). Group-I metabotropic glutamate receptors: hypotheses to explain their dual role in neurotoxicity and neuroprotection. *Neuropharmacology*, 38: 1477-1484.

Nilsson LG. (2003). Memory function in normal aging. *Acta Neurol Scand Suppl*., 179: 7-13.

Nogueira CR, Machado UF, Curi R, Carpinelli AR. (1994). Modulation of insulin secretion and 45Ca2 + efflux by dopamine in glucose-stimulated pancreatic islets. Gen. Pharmacol., 25: 909–916.

Nonogaki K. (2000). New insights into sympathetic regulation of glucose and fat metabolism. *Diabetologia*, 43: 533-49.

Novelli A, Reilly JA, Lysko PG. Henneberry, RC. (1988). Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Res*., 451: 205-212.

Nowicka D, Kaczmarek L. (1996). Spatio-temporal pattern of N-methyl-D-aspartate receptor NR1 mRNA expression during postnatal development of visual structures of the rat brain. *J Neurosci Res*., 44: 471-477.

O'Leary DM, Movsesyan V, Vicini S, Faden AI. (2000). Selective mGluR5 antagonists MPEP and SIB-1893 decrease NMDA or glutamate-mediated neuronal toxicity through actions that reflect NMDA receptor antagonism. *Br. J. Pharmacol*., 131: 1429-1437.

Oh S, Hoshi K, Ho IK. (1997). Role of NMDA receptors in pentobarbital tolerance/ dependence. *Neurochem. Res*., 22: 767-774.

Ohashi H, Maruyama T, Higashi-Matsumoto H, Nomoto T, Nishimura S, Takeuchi Y. (2002). A novel binding assay for metabotropic glutamate receptors using [3H L-quisqualic acid and recombinant receptors. *Z. Naturforsch., C, J. Biosci.*, 57: 348-355.

Ohata H, Tanaka KI, Maeyama N, Ikeuchi T, Kamada A, Yamamoto M, Momose K. (2001a). Physiological and pharmacological role of lysophosphatidic acid as modulator in mechanotransduction. *Jpn J Pharmacol*., 87:171-176.

Ohata H, Ikeuchi T, Kamada A, Yamamoto M, Momose K. (2001b). Lysophosphatidic acid positively regulates the fluid flow-induced local Ca(2+) influx in bovine aortic endothelial cells. *Circ Res*., 88: 925-932.

Ohata H, Tanaka K, Maeyama N, Yamamoto M, Momose K. (2001c). Visualization of elementary mechanosensitive Ca2+-influx events, Ca2+ spots, in bovine lens epithelial cells. *J Physiol*., 532: 31-42.

Ohtani N, Ohta M, Sugano T. (1997). Microdialysis study of modification of hypothalamic neurotransmitters in streptozotocin-diabetic rats. *J. Neurochem*., 69: 1622-1628.

Oiknine R, Mooradian AD. (2003). Drug therapy of diabetes in the elderly. *Biomed Pharmacother*., 57: 231-239.

Olney JW, Ho OL, Rhee V. (1973). Brain-damaging potential of protein hydrolysates. *N Engl J Med*., 289: 391-395.

Olney JW. (1989a). Excitatory amino acids and neuropsychiatric disorders. *Biol Psychiatry*., 26: 505–525.

Olney JW. (1989b). Excitotoxicity and N-methyl-D-aspartate receptors. *Drug Dev. Res.*, 17: 299-319.

Oppenheimer JH, Schwartz HL, Surks MI. (1972). Propylthiouracil inhibits the conversion of L-thyroxine to Ltriiodothyronine. An explanation of the antithyroxine effect of propyithiouracil and evidence supporting the concept that triiodothyronine is the active thyroid hormone. *J Clin Invest*., 51: 2493.

Ottersen OP, Storm-mathisen J. (1984). Neurons containing or accumulating transmitter amino acids. In Handbook of Chemical Neuroanatomy. Vol. 3: Classical Transmitters and Transmitter Receptors in the CNS, Part II. ed. Amsterdam, New York, Oxford: Elsevier Science Publishers B.V, :191-198.

Ouardouz M, Coderre E, Basak A, Chen A, Zamponi GW, Hameed S, Rehak R, Yin X, Trapp BD, Stys PK. (2009). Glutamate receptors on myelinated spinal cord axons: I. GluR6 kainate receptors. *Ann Neurol*., 2009 Feb 17. [Epub ahead of print].

Owen C, Wanling Z, Yuyan D, Rory JM, Robert SS. (2006). Blockade of GABAA Receptors in the Ventromedial Hypothalamus Further Stimulates Glucagon and Sympathoadrenal but Not the Hypothalamo-Pituitary-Adrenal Response to Hypoglycaemia. *Diabetes*, 55: 1080-1087.

Ozawa S, Kamiya H, Tsuzuki K. (1998). Glutamate receptors in the mammalian central nervous system. *Prog Neurobiol*., 54: 581.

Padayatti PS, Paulose CS. (1999). Alpha2 adrenergic and high affinity serotonergic receptor changes in the brain stem of streptozotocin-induced diabetic rats. *Life Sci*., 65: 403-414.

Paik SG, Fleischer N, Shin, SI. (1980). Insulin-dependent diabetes mellitus induced by subdiabetogenic doses of streptozotocin: obligatory role of cell –mediated autoimmune processes. *Proc. Natl. Acad. Sci*., 77: 6129-6133.

Paoletti P, Neyton J. (2007). "NMDA receptor subunits: function and pharmacology". *Curr Opin Pharmacol*., 7: 39-47.

Pardridge WM. (1983). Brain metabolism: a perspective from the blood-brain barrier. *Physiol. Rev*., 63: 1481-1535.

Pardridge WM, Boado RJ, Farrell CR. (1990). Brain-type glucose transporter (GLUT-1) is selectively localized to the blood-brain barrier. Studies with quantitative western blotting and in situ hybridization. *J Biol Chem.,* 265: 18035-18040.

Parsons CG, Danysz W, Quack G. (1998a). Glutamate in CNS disorders as a target for drug development: An update. *Drug News Perspect*., 11: 523-569.

Parsons CG, Danysz W, Hesselink M, Hartmann S, Lorenz B, Wollenburg C, Quack G. (1998b). Modulation of NMDA receptors by glycine--introduction to some basic aspects and recent developments. *Amino Acids*, 14: 207-216.

Parsons LM, Egan G, Liotti M, Brannan S, Denton D, Shade R, Robillard R, Madden L, Abplanalp B, Fox PT. (2001). Neuroimaging evidence implicating cerebellum in the experience of hypercapnea and hunger for air. *Proc Natl Acad Sci USA.,* 98: 2041-2046

Patel DR, Croucher MJ. (1997). Evidence for a role of presynaptic AMPA receptors in the control of neuronal glutamate release in the rat brain. *Eur J Pharmacol*., 332: 143–151.

Patrick AW, Campbell IW. (1990). Fatal hypoglycaemia in insulin-treated diabetes mellitus: clinical features and neuropathological changes. *Diabet Med.*, 7: 349-354.

Paulose CS, Padayatti PS, Sudha B. (1999). Neurotransmitter- Hormonal regulation of diabetes and liver regeneration. Comparative Endocrinology and Reproduction, Narosa Publishing House, NewDelhi, India, 559-568.

Paupard MC, Friedman LK, Zukin RS. (1997). Developmental regulation and cell-specific expression of N-methyl-D-aspartate receptor splice variants in rat hippocampus. *Neuroscience*, 79: 399-409.

Peinado MA, del Moral ML, Esteban FJ, Martínez-Lara E, Siles E, Jiménez A, Hernández-Cobo R, Blanco S, Rodrigo J, Pedrosa JA. (2000). Aging and neurodegeneration: molecular and cellular bases. *Rev Neurol*., 31: 1054-1065.

Pelligrino DA, Wang Q. (1998). Cyclic nucleotide crosstalk and the regulation of cerebral vasodilation. *Prog.Neurobiol.*, 56: 1-18.

Permutt MA, Kipnis DM. (1972). Insulin biosynthesis. II. Effect of glucose on ribonucleic acid synthesis in isolated rat islets. *J. Biol. Chem*., 247: 1200-1207.

Peters (2006). Golgi, Cajal, and the fine structure of the nervous system. *Brain Res Rev*., 55: 256-263.

Petralia RS, Yokotani N, Wenthold RJ. (1994). Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. *J. Neurosci*., 14: 667-696.

Piedrafita B, Cauli O, Montoliu C, Felipo V. (2007). The function of the glutamate-nitric oxide-cGMP pathway in brain in vivo and learning ability decrease in parallel in mature compared with young rats. *Learn Mem.,* 14: 254-258.

Pin JP, Duvoisin R. (1995). The metabotropic glutamate receptors: structure and functions. *Neuropharmacology*, 34:1–26.

Pipeleers DG, Veld IPA, Dewinkle VM, Maes E, Schuits FC, Gepts W. (1985). A new *in vitro* model for the study of pancreatic A and B cells. *Endocrinology*, **117:** 806-816.

Pittaluga A, Pattarini R, Severi P, Raiteri M (1996). Human brain N-methyl-D-aspartate receptors regulating noradrenaline release are positively modulated by HIV-1 coat protein gp120. *AIDS*, 10: 463-468.

Pittaluga A, Raiteri. M. (1992). N-Methyl-D-aspartic acid (NMDA) and non-NMDA receptors regulating hippocampal norepinephrine release. III. Changes in the NMDA receptor complex induced by their functional cooperation. *J. Pharmacol. Exp. Ther*., 263: 327-333.

Pius SP, Paulose CS, (1999). ALPHA2 adrenergic and high affinity serotonergic receptor changes in the brainstem of streptozotocin- induced diabetic rats. *Life Sciences*, 65: 403-414.

Platt SR. (2007). The role of glutamate in central nervous system health and disease--a review. *Vet. J.* 173: 278-286.

Ploghause A, Tracey I, Gati JS, Clare S, Menon RS, Matthews PM, Rawlins JNP. (1999). Dissociating pain from its anticipation in the human brain. *Science*, 284: 1979-81.

Porte D Jr. (1967). Beta adrenergic stimulation of insulin release in man. *Diabetes*, 16: 150-155.

Pow DV, Robinson SR. (1994). Glutamate in some retinal neurons is derived solely from glia. *Neuroscience*, 60: 355–366.

Pow DV, Barnett NL. (2000). Developmental expression of excitatory amino acid transporter 5: a photoreceptor and bipolar cell glutamate transporter in rat retina. *Neurosci Lett*., 280: 21-24.

Pramming S, Thorsteinsson B, Bendtson I, Binder C. (1991). Symptomatic hypoglycaemia in 411 type 1 diabetic patients. *Diabetic Med*., 8: 217–222.

Preetha N, Padayatti PS, Abraham A, Sudha B, Raghu KG, Paulose CS. (1996). Glutamate dehydrogenase induction in the brain of streptozotocin diabetic rats. *Indian J Biochem Biophys*., 33: 428–430.

Priestley T, Laughton P, Myers J, Le Bourdelles B, Kerby J, Whiting. (1995). Pharmacological properties of recombinant human N-methyl-D-aspartate receptors comprising NR1a/NR2A and NR1a/NR2B subunit assemblies expressed in permanently transfected mouse fibroblast cells. *Molecular Pharmacology*, 48: 841-848.

Pujic Z, Matsumoto I, Wilce PA. (1993). Expression of the gene coding for the NR1 subunit of the NMDA receptor during rat brain development. *Neurosci Lett*., 162: 67-70.

Pulsinelli WA, Cooper AJ. (1994). Metabolic encephalopathies and coma. Basic Neurochemistry: Molecular, Cellular and Medical Aspects, 5th ed. New York: Raven Press, 841–857.

Qing Li, Donald GP. (2002). Diabetes-Induced Dysfunction of the Glutamate Transporter in Retinal Müller Cells. *Investigative Ophthalmology and Visual Science*, 43: 3109-3116.

Quintana P, Alberi S, Hakkoum D, Muller D. (2006). Glutamate receptor changes associated with transient anoxia/hypoglycaemia in hippocampal slice cultures. *Eur J Neurosci*., 23: 975-983.

Ramakrishna R, Namasivayam A. (1995). Norepinephrine and Epinephrine levels in the brain of alloxan diabetic rats. *Neuarosci. Let*., 186: 200-202.

Rao SM, Harrington DL, Haaland KY, Bobholz JA, Cox RW, Binder JR. (1997). Distributed neural systems underlying the timing of movements. *J. Neurosci*., 17: 5528-5535.

Reetz A, Solimena M, Matteoli M, Folli F, Takei K, De Camilli P. (1991). GABA and pancreatic beta-cells: colocalization of glutamic acid decarboxylase (GAD) and GABA with synaptic-like microves icles suggests their role in GABA storage and secretion. *EMBO J*., 10: 1275-1284.

Renstrom E, Ding WG, Bokvist K, Rorsman P. (1996). Neurotransmitter induced inhibition of exocytosis in insulin secretory beta cells by activation of calcineurin. *Neuron*, 17: 513-522.

Renuka TR, Paulose CS. (2004). Alterations in the brain stem muscarinic M1 and M3 receptor gene expression during pancreatic regeneration and insulin secretion in weanling rats. *Life Science*, 75: 2269-2280.

Renuka TR, Savitha B, Paulose CS. (2005). Muscarinic M1 and M3 receptor binding alterations in pancreas during pancreatic regeneration of young rats. *Endocrine Research*, 31: 259-270.

Renuka TR, Remya R, Paulose CS. (2006). Increased insulin secretion by muscarinic M1 and M3 receptor function from rat pancreatic islets in vitro. *Neurochemical Research*, 31: 313-320.

Robinson R. (2007). Dopamine D1 and D2 receptor functional regulation: Glutamate receptor gene expression in the brain of hypoglycaemic and hyperglycaemic rats, Ph.D thesis pp- 120-122: Dept. of Biotechnology, Cochin University of Science and Technology, Cochin.

Robinson R, Krishnakumar A, Paulose CS. (2009). Enhanced Dopamine D1 and D2 Receptor Gene Expression in the Hippocampus of Hypoglycaemic and Diabetic Rats. *Cell Mol Neurobiol.* [Epub ahead of print]

Roettger V, Lipton P. (1996). Mechanism of glutamate release from rat hippocampal slices during in vitro ischemia. *Neuroscience,* 75: 677-685.

Rogawski MA. (1993) Therapeutic potential of excitatory amino acid antagonists: Channel blockers and 2,3-benzodiazepines. *Trends Pharmacol. Sci*., 14: 325-331.

Romano C, Sesma MA, McDonald CT, O'Malley K, Van den Pol AN, Olney JW. (1995). Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *J. Comp. Neurol.*, 335: 455-469.

Rose CR, Waxman SG, Ransom BR. (1998). Effects of glucose deprivation, chemical hypoxia, and simulated ischemia on Na^+ homeostasis in rat spinal cord astrocytes. *J Neurosci*., 18: 3554-3562.

Rosenberg PA, Aizenman E. (1989). Hundred-fold increase in neuronal vulnerability to glutamate toxicity in astrocyte-poor cultures of rat cerebral cortex. *Neurosci Lett*., 103: 162–168.

Rosenberg PA, Amin S, Leitner M. (1992). Glutamate uptake disguises neurotoxic potency of glutamate agonists in cerebral cortex in dissociated cell culture. *J Neurosci*., 12: 56–61.

Rosenmund C, Feltz A, Westbrook GL. (1995). Calcium-dependent inactivation of synaptic NMDA receptors in hippocampal neurons. *J. Neurophysiol.*, 73: 427-430.

Roszkowska K, Nauman J, Nauman A. (1974). Influence of propranolol on levels of thyroxine and triiodothyronine in hyperthyreotic patients. *Mater Med PcI.,* 6: 178.

Rothman SM, Thurston JH, Hauhart RE, Clark GD, Solomon JS. (1987). Ketamine protects hippocampal neurons from anoxia *in vitro. Neuroscience,* 21: 673-678.

Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang YF, Schielke JP, Welty DF. (1996). Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron*, 16: 675–686.

Rovet JF, Ehrlich RM. (1999). The effect of hypoglycaemic seizures on cognitive function in children with diabetes: a 7-year prospective study. *J. Pediatr.* 134: 503 –506.

Ryan C, Vega A, Drash A. (1985). Cognitive deficits in adolescents who developed diabetes early in life. *Pediatrics,* 75: 921-927.

Salkovic M, Lackovic Z. (1992). Brain D1 dopamine receptor in alloxan-induced diabetes. *Diabetes.* 41: 1119-1121.

Saller CF. (1984). Dopamenergic Activity is reduced in diabetic rats. *Neuroscience Letters*, 49: 301-306.

Sandberg M, Butcher PS, Hagberg H. (1986). Extracellular overflow of neuroactive amino acids during severe insulin-induced hypoglycaemia: in vivo dialysis of the rat hippocampus. *J. Neurochem*., 47: 178-184.

Sandrini M, Vitale, G, Vergoni AV, Beetolini A. (1997). Sreptozotocin induced diabetes provokes changes in serotonin concentration and on 5-HT1A and 5-HT 2A recap. *Int. J. Neurosci*., 69: 125-130.

Sang WS, Aoyama K, Chen Y, Garnier P, Matsumori Y, Gum E, Liu J, Swanson RA. (2003). Hypoglycemic neuronal death and cognitive impairment are prevented by poly (ADP-ribose) polymerase inhibitors administered after hypoglycemia. *J Neurosci*., 23: 10681–10690.

Sang WS, Garnier P, Aoyama K, Chen Y, Swanson RA. (2004). Zinc release contributes to hypoglycemiainduced neuronal death. *Neurobiol. Dis*., 16: 538–545.

Sang WS, Aoyama K, Matsumori Y, Liu J, Swanson RA. (2005a). Pyruvate administered after severe hypoglycemia reduces neuronal death and cognitive impairment. *Diabetes.,* 54: 1452–1458.

Sang WS, Yang F, Shwuhuey MH, Zhengyan Liu, Yasuhiko M, Philip RW, Raymond AS, Jialing L. (2005b). Hypoglycaemia Induces Transient Neurogenesis and Subsequent Progenitor Cell Loss in the Rat Hippocampus. *Diabetes*, 54: 1500-1509.

Sang SW, Gum ET, Hamby AM, Chan PH, Swanson RA (2007). Hypoglycaemic neuronal death is triggered by glucose reperfusion and activation of neuronal NADPH oxidase. *J Clin Invest.,* 117: 910-918.

Sara NB, Carol AB. (2006). Neural plasticity in the ageing brain. *Nature Reviews Neuroscience,* **7**: 30-40.

Satin LS, Kinard TA. (1998). Neurotransmitters and their receptors in the islets of Langerhans of the pancreas: what messages do acetylcholine, glutamate, and GABA transmit? *Endocrine*, 8: 213–223.

Sato T, Suzuki Y, Taketani T, et al. (1977). Enhanced peripheral conversion of thyroxine to triiodothyronine during hGH therapy in GH deficient children. *J Clin Endocrinol Metab*., 45: 324.

Scatchard G. (1949). The attractions of proteins for small molecules and ions. *Ann N Y Acad Sci*., 51: 660–672.

Schlienger JL, Anceau A, Chabrier G, North ML, Stephan F. (1982). Effect of diabetic control on the level of circulating thyroid hormones. *Diabetologia*, 24: 486-488.

Schmahmann JD, Sherman JC. (1998). The cerebellar cognitive affective syndrome. *Brain*, 121: 561-579.

Schoepp DD, Conn PJ. (1993). Metabotropic glutamate receptors in brain function and pathology. *Trends Pharmacol Sci*., 14: 13–20.

Schuit F. (1996). Factors determining the glucose sensitivity and glucose responsiveness of pancreatic β-cells. *Horm. Res*., 46: 99-106.

Schulz JB, Matthews R, Jenkins BG, Ferrante RJ, Siwek D, Henshaw DR, Cipollini PB, Mecocci P, Kowall NW, Rosen BR, Beal MF. (1995). Blockade of neuronal nitric oxide synthase protects against excitotoxicity in vivo. *J. Neurosci.*, 15: 8419-8429.

Schwab RS, Zieper I. (1965). Effects of mood, motivation, stress, and alertness on the performance in Parkinson's disease. *Psychiat Neurol*., 150: 345-357.

Seeburg PH. (1993). The TINS/TIPS lecture: The molecular biology of mammalian glutamate receptor channels. *Trends Neurosci*., 16: 359-365.

Serck-Hanssen G, Grønning M, Fladeby C, Skar R. (2002). Cholinergic activation of glucose transport in bovine chromaffin cells involves calmodulin and protein kinase Czeta signaling. *Ann N Y Acad Sci*., 971:117-126.

Serulle Y, Arancio O, Ziff EB. (2008). A role for cGMP-dependent protein kinase II in AMPA receptor trafficking and synaptic plasticity. *Channels (Austin),* 2: 230-232.

Shannon HE, Sawyer BD. (1989). Glutamate receptors of the N-methyl-D-aspartate subtype in the myenteric plexus of the guinea pig ileum. *J Pharmacol Exp Ther*., 251: 518-523.

Sheetz MJ, King GL. (2002). Molecular understanding of hyperglycaemia's adverse effects for diabetic complications. *JAMA*, 228: 2579-2588.

Shigemoto R, Kinoshita A, Wada E, Nomura S, Ohishi H, Takada M, Flor PJ, Neki A, Abe T, Nakanishi S, Mizuno N. (1997). Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. *J. Neurosci*., 17: 7503–7522.

Shigeri Y, Seal RP, Shimamoto K. (2004). Molecular pharmacology of glutamate transporters, EAATs and VGLUTs. *Brain Res. Brain Res. Rev*., 45: 250-265.

Shuai JW, Jung P. (2003). Optimal ion channel clustering for intracellular calcium signaling. *Proc.Natl.Acad.Sci.USA*, 100: 506-510.

Siesjo BK. (1978). Brain Energy Metabolism. New York: John Wiley & Sons, 380–446.

Siliprandi R, Lipartiti M, Fadda E, Sautter J, Manev H. (1992). Activation of the glutamate metabotropic receptor protects retina against N-methyl-D-aspartate toxicity. *Eur. J. Pharmacol*., 219: 173–174.

Simartirkis E, Miles PDG, Vranic M, Hunt R, Gougen-Rayburn R, Field CJ, Marliss EB. (1990). Glucoregulation during single and repeated bouts of intense exercise and recovery in man. *Clin. Invest. Med*., 13: 134.

Simon RP, Swan JH, Griffiths T, Meldrum BS.(1984). Blokade OF N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. *Science*, 226: 850-852.

Simonyi A, Ngomba RT, Storto M, Catania MV, Miller LA, Youngs B, DiGiorgi-Gerevini V, Nicoletti F, Sun GY. (2005). Expression of groups I and II metabotropic glutamate receptors in the rat brain during aging. *Brain Res*., 1043: 95-106.

Singh P, Jain A, Kaur G. (2004). Impact of hypoglycemia and diabetes on CNS: correlation of mitochondrial oxidative stress with DNA damage. *Mol Cell Biochem.*, 260: 153-159.

Skeberdis VA, Lan J, Opitz T, Zheng X, Bennett MV, Zukin RS. (2001). mGluR1-mediated potentiation of NMDA receptors involves a rise in intracellular calcium and activation of protein kinase C. *Neuropharmacology,* 40: 856-865.

Sladeczek F., Momiyama A.,Takahashi T. (1992). Presynaptic inhibitory action of metabotropic glutamate receptor agonist on excitatory transmission in visual cortical neurons. *Proc. Roy. Soc. Lond.*, B 253: 297-303.

Smith G. (1988). Animal models of Alzheimer's disease: experimental cholinergic denervation. *Brain Res.*, 472: 103-118.

Sokoloff. (1981). Relationships among local functional activity, energy metabolism, and blood flow in the central nervous system. *Fed. Proc.*, 40: 2311-2316.

Sorenson RL, Garry DG, Brelje TC. (1991). Structural and functional considerations of GABA in islets of Langerhans. Beta-cells and nerves. *Diabetes*, 40: 1365-1374.

Spooren WP, Gasparini F, Bergmann R, Kuhn R. (2000). Effects of the prototypical mGlu(5) receptor antagonist 2-methyl-6-(phenylethynyl)- pyridine on rotarod, locomotor activity and rotational responses in unilateral 6-OHDA-lesioned rats. *Eur. J. Pharmacol.*, 406: 403-410.

Stanley CA, Lieu YK, Hsu BYL, Burlina AB, Greenberg CR, Hopwood NJ, Perlman K, Rich BH, Zammarchi E, Poncz M. (1998). Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N Engl J Med.*, 338: 1352–1357.

Stanley CA, Fang J, Kutyna K, Hsu BYL, Ming JE, Glaser B, Poncz M. (2000). Molecular basis and characterization of the hyperinsulinism/hyperammonemia syndrome: predominance of mutations in exons 11 and 12 of the glutamate dehydrogenase gene. *Diabetes*, 49: 667–673.

Storto M, Capobianco L, Battaglia G, Molinaro G, Gradini R, Riozzi B, Di Mambro A, Mitchell KJ, Bruno V, Vairetti MP, Rutter GA, Nicoletti F. (2006). Insulin

secretion is controlled by mGlu5 metabotropic glutamate receptors. *Mol Pharmacol*., 69: 1234-1241.

Strachan MW, Deary IJ, Ewing FM, Frier BM. (2000). Recovery of cognitive function and mood after severe hypoglycaemia in adults with insulin-treated diabetes. *Diabetes Care*, 23: 305-312.

Sucher NJ, Akbarian S, Chi CL, Leclerc CL, Awobuluyi M, Deitcher DL, Wu MK, Yuan JP, Jones EG, Lipton SA. (1995). Developmental and regional expression pattern of a novel NMDA receptor-like subunit (NMDAR-L) in the rodent brain. *J. Neurosci*., 15: 6509-6520.

Sucher NJ, Awobuluyi M, Choi YB, Lipton SA. (1996). NMDA receptors: From genes to channels. *Trends Pharmacol. Sci*., 17: 348-355.

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

Sumiyoshi T, Ichikawa J, Meltzer HY. (1997). The effect of streptozotocin- induced diabetes on Dopamine D2, Serotonine 1A & serotonine 2A receptors in the rat brain. *Neuropscypharmacol*., 16: 183-190.

Sutherland EW. (1972). Studies on the mechanism of hormone action. *Science*, 177: 401-408.

Suvarna NU, O'Donnell JM. (2002). Hydrolysis of N-methyl-D-aspartate receptor-stimulated cAMP and cGMP by PDE4 and PDE2 phosphodiesterases in primary neuronal cultures of rat cerebral cortex and hippocampus. *J.Pharmacol.Exp.Ther.*, 302: 249-256.

Swillens S, Dupont G, Combettes L, Champeil P. (1999). From calcium blips to calcium puffs: Theoretical analysis of the requirements for interchannel communication. *Proc.Natl.Acad.Sci.USA*, 96: 13750-13755.

Szatkowski M, Attwell D. (1994). Triggering and execution of neuronal death in brain ischaemia: two phases of glutamate release by different mechanisms. *Trends Neurosci*., 17: 359.

Takehara K, Kawahara S, Munemoto Y, Kuriyama H, Mori H, et al. (2004). The N-methyl-D-aspartate (NMDA)-type glutamate receptor GluRepsilon2 is important for delay and trace eyeblink conditioning in mice. *Neurosci Lett* ., 364: 43-47.

Tamaru M, Yoneda Y, Ogita K, Shimizu J, Nagata Y. (1991). Age related decreases of the N-methyl-D-aspartate receptor complex in the rat cerebral cortex and hippocampus. *Brain Research*, 542: 83-90.

Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, Okuyama S, Kawashima N, Hori S, Takimoto M, Wada K. (1997). Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science*, 276: 1699–1702.

Tanaka H, Takamatsu T. (2001). Calcium spots: elementary signals in response to mechanical stress in vascular endothelial cells. *Circ. Res*., 88: 852-854.

Tanaka Y, Takata T, Satomi T, Sakurai T, Yokono K. (2008). The double-edged effect of insulin on the neuronal cell death associated with hypoglycemia on the hippocampal slice culture. *Kobe J Med Sci*., 54: E97-107.

Tasker RC, Coyle JT, Vornov JJ. (1992). The regional vulnerability to hypoglycaemia-induced neurotoxicity in organotypic hippocampal culture: protection by early tetrodotoxin or delayed MK-801. *J. Neurosci*., 12: 4298-4308.

Tassava TM, Okuda T, Romsos DR. (1992). Insulin secretion from ob/ob mouse pancreatic islets: Effects of neurotransmitters. *Am. J. Physiol*., 262: 338-343.

Tateishi Y, Hattori M, Nakayama T, Iwai M, Bannai H, Nakamura T, Michikawa T, Inoue T, Mikoshiba K. (2005). Cluster formation of inositol 1, 4, 5-trisphosphate receptor requires its transition to open state. *J. Biol. Chem.*, 280: 6816-6822.

Taylor CW, Genazzani AA, Morris SA. (1999). Expression of inositol trisphosphate receptors. *Cell Calcium*, 26: 237-251.

Temple MD, O'Leary DM, and Faden AI. The role of glutamate receptors in the pathophysiology of traumatic central nervous system injury. Chapter 4 in

Head Trauma: Basic, Preclinical, and Clinical Directions. Miller LP and Hayes RL, editors. Co-edited by Newcomb JK. John Wiley and Sons, Inc. New York. 2001. Pages 87-113.

Tevaarwerk GJM, Boyd D. (1977). Propranolol pretreatment and thyroid hoimone levels during subtotal thyroidectomy for Graves' disease (abstr). *Ann R Coll Physicians Surg Can.,* 10: 42.

Tevaarwerk GJM, Malik MH, Boyd D. (1978). Preliminary report on the use of propranolol in thyrotoxicosis: I. Effect on serum thyroxine, triiodothyronine and reverse triiodothyronine concentrations. *Can Med Assoc J*., 119: 350.

Thomas D, Lipp P, Berridge MJ, Bootman MD. (1998). Hormone-evoked elementary Ca2+ signals are not stereotypic, but reflect activation of different size channel clusters and variable recruitment of channels within a cluster. *J Biol Chem*. 273: 27130-27136.

Thomas LS, Jane DE, Harris JR, Croucher MJ. (2000). Metabotropic glumate autoreceptors of the mGlu5 subtype positively modulate neuronal glutamate release in the rat forebrain in vitro. *Neuropharmacology,* 39: 1554–1566.

Thomas G, Rojas MC, Epstein SK, Balk EM, Liangos O, Jaber BL. (2007). Insulin therapy and acute kidney injury in critically ill patients a systematic review. *Nephrol Dial Transplant*., 22(10): 2849–2855.

Thorens B. (1992). Expression cloning of the pancreatic ß-cell receptor for the gluco-incretin hormone glucagon-like peptide-1. *Proc Natl Acad Sci U S A.,* 89: 8641–8645.

Timothy JG, Young AB, Penny JB. (1984). Quantitative autoradiographic distribution of [3H] glutamate binding sites in the rat central nervous system. *J Neurosci*., 4: 2133–2144.

Tojyo Y, Morita T, Nezu A, Tanimura A. (2008). The clustering of inositol 1,4,5-trisphosphate (IP(3)) receptors is triggered by IP(3) binding and facilitated by depletion of the Ca(2+) store. *J.Pharmacol.Sci.*, 107: 138-150.

Tomiyama M, Furusawa K, Kamijo M, Kimura T, Matsunaga M, Baba M. (2005). Upregulation of mRNAs coding for AMPA and NMDA receptor subunits and

metabotropic glutamate receptors in the dorsal horn of the spinal cord in a rat model of diabetes mellitus. *Brain Res Mol Brain Res.,* 20: 2750–2781.

Tong G, Jahr CE. (1994). Block of glutamate transporters potentiates postsynaptic excitation. *Neuron*, 13, 1195-1203.

Towler DA, Havlin CE, Craft S, Cryer P. (1993). Department Mechanism of awareness of hypoglycaemia. Perception of neurogenic (predominantly cholinergic) rather than neuroglycopenic symptoms. *Diabetes*, 42: 1691-1693.

Trotti D, Danbolt NC, Volterra A. (1998). Glutamate transporters are oxidant-vulnerable: a molecular link between oxidative and excitotoxic neurodegeneration? *Trends Pharmacol., Sci*., 19: 328-334.

Trudeau F, Gagnon S, Massicotte G. (2004). Hippocampal synaptic plasticity and glutamate receptor regulation: influences of diabetes mellitus. *European Journal of Pharmacology*, 14: 177-186.

Tsintsadze TS, Lozovaya NA, Krishtal OA. (2001). Effects of long-term hypoxia/hypoglycemia on synaptic transmission between the CA3 and CA1 zones in rat hippocampal slices. *Neurophysiology*, 33: 365–371.

Tulving E, Markowitsch HJ. (1998). Episodic and declarative memory: role of the hippocampus. *Hippocampus* 8: 198–204.

Uvnäs-Moberg K, Ahlenius S, Alster P, Hillegaart V. (1996). Effects of selective serotonin and dopamine agonists on plasma levels of glucose, insulin and glucagon in the rat. *Neuroendocrinology*, 63: 269-274.

Vagenakis AG, Burger A, Portnay GI, et al: (1975). Diversion of peripheral thyroxine metabolism from activating to inactivating pathways during complete fasting. *J Clin Endocrinol Metab*., 41: 191.

Valenti O, Conn PJ, Marino MJ. (2002). Distinct physiological roles of the Gq-coupled metabotropic glutamate receptors Co-expressed in the same neuronal populations. *J. Cell. Physiol.*, 191: 125-137.

Vallano MK. (1998). Developmental aspects of NMDA receptor function. *Crit Rev Neurobiol*., 12: 177

Vallebuona F, Raiteri M. (1995). Age-related changes in the NMDA receptor/nitric oxide/cGMP pathway in the hippocampus and cerebellum of freely moving rats subjected to transcerebral microdialysis. *Eur. J. Neurosci*., 7: 694-701.

Van den Berghe G, Schoonheydt K, Becx P, et al. (2005). Insulin therapy protects the central and peripheral nervous system of intensive care patients. *Neurology*, 64: 1348–1353.

Van Donkelaar P, Lee RG. (1994). Interactions between the eye and hand motor systems: Disruptions due to cerebellar dysfunction. *J. Neurophysiol*., 72: 1674-1685.

Vannucci RC, Vannucci SJ. (2001). Hypoglycemic brain injury. *Semin. Neonatol*., 6: 147–155.

Veneman T, Mitrakou A, Mokan M, Cryer PE, Gerich J. (1993). Induction of hypoglycaemia unawareness by asymptomatic nocturnal hypoglycaemia. *Diabetes*, 42: 1233-1237.

Verhaeghen P. (2003). Aging and vocabulary scores: a meta-analysis. *Psychol Aging*, 18: 332-339.

Vizi. ES. (2000). Role of high-affinity receptors and membrane transporters in nonsynaptic communication and drug action in the central nervous system. *Pharmacol. Rev*., 52: 63- 89.

Walsh DA, Perkins JP, Krebs EG. (1968). n adenosine 3',5'-monophosphate-dependant protein kinase from rabbit skeletal muscle. *J. Biol. Chem.*, 10: 3763-3765.

Wang GJ, Chung HJ, Schnuer J, Lea E, Robinson MB, Potthoff WK, Aizenman E, Rosenberg PA. (1998). Dihydrokainate-sensitive neuronal glutamate transport is required for protection of rat cortical neurons in culture against synaptically released glutamate. *Eur J Neurosci*., 10: 2523–2531.

Wang J-Q, Brownell A-L. (2007). Development of metabotropic glutamate receptor ligands for neuroimaging. *Current Medical Imaging Reviews* 3: 186-205.

Wassmer E, Davies P, Whitehouse WP, Green SH. (2003). Clinical spectrum associated with cerebellar hypoplasia. *Pediatr. Neurol*., 28: 347-351.

Weaver CD, Yao TL, Powers AC, Verdoorn TA. (1996). Differential expression of glutamate receptor subtypes in rat pancreatic islets. *J. Biol. Chem*., 271: 12977-12984.

Weaver CD, Partridge JG, Yao TL, Moates JM, Magnuson MA, Verdoorn TA. (1999). Activation of glycine and glutamate receptors increases intracellular calcium in cells derived from the endocrine pancreas. *Br J Pharmacol*., 54: 639-646.

Weinberger DR, Berman KF, Zec RF (1986) Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia, I: regional cerebral blood flow evidence. *Arch. Gen. Psychiatry*, 43: 114-124.

Wenk GL, Walker LC, Price DL, Cork LC. (1991). Loss of NMDA, but not GABAA, binding in the brains of aged rats and monkeys. *Neurobiology of Aging*, 12: 93-98.

Widom B, Simonson DC. (1992). Intermittent hypoglycaemia impairs glucose counterregulation. *Diabetes*, 41:1597–1602.

Wieloch T, Harris RJ, Symon L, Siesjo BK. (1984). Influence of severe hypoglycaemia on brain extracellular calcium and potassium activities, energy-and phospholipid metabolism. *J Neurochem*., 43: 160-168.

Wieloch T, Engelsen B, Westerberg E, Auer R. (1985). Lesions of the glutamatergic cortico-striatal projections in the rat ameliorate hypoglycaemic brain damage in the striatum. *Neurosci Lett*., 58: 25-30.

Wieloch T. (1985). Hypoglycaemia-induced neuronal damage prevented by an N-methyl-D-aspartate antagonist. *Science*, 230: 68l-683.

Wilson BS, Pfeiffer JR, Smith AJ, Oliver JM, Oberdorf JA, Wojcikiewicz RJH. (1998). Calcium-dependent clustering of inositol 1,4,5-trisphosphate receptors. *Mol. Biol. Cell*, 9: 1465-1478.

Wojcikiewicz RJH. (1995). Type I, II, and III inositol 1,4,5-trisphosphate receptors are unequally susceptible to down-regulation and are expressed in markedly different proportions in different cell types. *J. Biol. Chem.*, 270: 11678-11683.

Wollheim CB. (2000). Beta-cell mitochondria in the regulation of insulin secretion: a new culprit in Type II diabetes. *Diabetologia*, 43: 265-277.

Wood PL, Rao TS, Iyengar S, Lanthorn T, Monahan J, Cordi A, Sun E, Vazquez M, Gray N, Contreras P. (1990). A review of the in vitro and in vivo neurochemical characterization of the NMDA/PCP/glycine/ion channel receptor macrocomplex. *Neurochem. Res.*, 15: 217-230.

Wredling R, Levander S, Adamson U, Lins PE. (1990). Permanent neuropsychological impairment after recurrent episodes of severe hypoglycaemia in man. *Diabetologia*, 33: 152-157.

Xing HQ, Hayakawa H, Gelpi E, Kubota R, Budka H, Izumo S. (2009). Reduced expression of excitatory amino acid transporter 2 and diffuse microglial activation in the cerebral cortex in AIDS cases with or without HIV encephalitis. *J Neuropathol Exp Neurol.*, 68: 199-209.

Yan H, Rivkees SA. (2006). Hypoglycemia influences oligodendrocyte development and myelin formation. *Neuroreport*, 17: 55-59.

Yoshikawa H, Honma T, Abe T. (2003). Persistent hyperinsulinemic hypoglycaemia followed as benign infantile convulsion. *Seizure*, 12: 186–187.

Yu SP, Sensi SL, Canzoniero LM, Buisson A, Choi DW. (1997). Membrane-delimited modulation of NMDA currents by metabotropic glutamate receptor subtypes 1/5 in cultured mouse cortical neurons. *J. Physiol.*, 499: 721-732.

Zammitt NN, Frier BM. (2005). Hypoglycemia in type 2 diabetes: pathophysiology, frequency, and effects of different treatment modalities. *Diabetes Care*, 28: 2948–2961.

Zhong J, Russell SL, Pritchett DB, Molinoff PB, Williams K. (1994). Expression of mRNAs encoding subunits of the N-methyl-D-aspartate receptor in cultured cortical neurons. *Mol. Pharmacol.*, 45: 846-853.

Zhu G, Okada M, Yoshida S, Mori F, Hirose S, Wakabayashi K, Kaneko S. (2006). Involvement of Ca(2+)-induced Ca2+ releasing system in interleukin-1beta-associated adenosine release. *Eur J Pharmacol.*, 532: 246-252.

Zimmet PZ. (1999) Diabetes epidemiology as a tool to trigger diabetes research and care. *Diabetologia,* 42: 499-518.

Zimmet P, Alberti KGMM, Shaw J. (2001) Global and societal implications of the diabetes epidemic. *Nature*, 414: 782-787.

Papers Published

1. **Anu Joseph**, Sherin Antony, C. S. Paulose. Increased glutamate receptor gene expression in the cerebral cortex of insulin induced hypoglycemic and streptozotocin-induced diabetic rats. *Neuroscience*, 156: 298-304 (2008).

2. **Anu Joseph**, Remya Robinson, C. S. Paulose. Enhanced [^{3}H] Glutamate Binding in the Cerebellum of Insulin Induced Hypoglycaemic and Streptozotocin Induced Diabetic Rats. *Cellular and Molecular Neurobiology,* 27: 1085-1095 (2007).

3. Eswar Shankar, P. N., **Anu Joseph**, C. S. Paulose Decreased [^{3}H] YM-09151-2 binding to dopamine D_2 receptors in the hypothalamus, brainstem and pancreatic islets of streptozotocin-induced diabetic rats. *European Journal of Pharmacology*, 557/2-3, 99-105, (2007).

Awards

1. **ISN CAEN Travel Award** for attending the workshop on Modern Neurochemical Techniques to Understand Brain Functions, APSN-SNCI sponsored workshop, Bangalore – 2008.
2. **ISN CAEN Travel Award** for attending the workshop on Neurochemical Techniques for the Neuroscientists and Kolkata Neuroscience meeting – 2007.
3. Cochin University Junior Research Fellowship, February 3, 2007 – July 1, 2007
4. ICMR, Govt of India JRF, July, 2007 - to date

Abstracts Presented

1. **Anu Joseph**, Sherin Antony, C. S. Paulose. Enhanced NMDA Receptors Gene Expression and Neurodegeneration in the Cerebellum of Insulin Induced Hypoglycaemic and Streptozotocin Induced Diabetic Old Rats. 21st Kerala science Congress –Young Scientist award contest paper, Kollam (2009).

2. Sherin Antony, **Anu Joseph,** Peeyush Kumar T, C. S. Paulose. Enhanced Muscarinic M3 receptor gene expression in the cerebral cortex of Diabetic and Insulin Induced Hypoglycemic rats. 77th Annual Meeting of the Society of Biological Chemists (India) IIT Madras, Chennai. (2008).

3. C. S. Paulose, **Anu Joseph**. Enhanced NMDA and metabotrophic GluR5 glutamate receptor subunits gene expression in the corpus striatum of insulin induced hypoglycaemic and streptozotocin induced diabetic rats. International Symposiumon Molecular aspects of brain aging and neurological disorders and annual meeting of society for neurochemistry, India held at Guru Nanak Dev University, Amritsar (2008)

4. Sherin Antony, **Anu Joseph**, C. S. Paulose. Decreased [^{3}H] QNB binding to muscarinic M1 receptors in the cerebral cortex of diabetic and insulin induced hypoglycemic rats. 8th Biennial meeting of Asia Pacific Society for Neurochemistry Symposium organized by International Society for Neurochemistry/Asia Pacific Society for Neurochemistry/International Brain Research Organization held in Shanghai, China (2008)

5. **Anu Joseph**, Sherin Antony, C. S. Paulose. Enhanced Glutamate and IP3 receptor gene expression in the Cerebral Cortex of Insulin Induced Hypoglycemic and Streptozotocin Induced Diabetic Rats. International Symposium on Regenerative Neuroscience and Annual Meeting of Society for Neurochemistry (India), NIMHANS, Bangalore, INDIA (2008).

6. **Anu Joseph**, Sherin Antony, C. S. Paulose. Decreased α-$_{2a}$ adrenergic receptor gene expression in the Hypothalamus and Brainstem of Insulin Induced Hypoglycaemic and Streptozotocin Induced Diabetic Rats. Annual meeting of Society for Biotechnologists, India and National Symposium on Current Trends in Stem Cell Biology, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram (2007)

7. Sherin Antony, **Anu Joseph**, C. S. Paulose. Kinetic parameters of acetylcholine esterase in the heart of Streptozotocin Induced Diabetic and Insulin Induced Hypoglycemic rats. Meeting of Society of Biological Chemists (India) conducted in Sri Venkiteswara University, Thirupathi (2007).

8. **Anu Joseph**, Finla Chathu, C. S. Paulose. Enhanced glutamate dehydrogenase activity in the cerebral cortex and liver of hypoxic rats: Effect of oxygen, epinephrine and glucose treatment. Abstract Memorabilia of XXV National Symposium on Reproductive Biology and Comparative Endocrinology -Translational Endocrinology and Reproductive Biology jointly organised by Society for Reproductive Biology and Comparative Endocrinology and Department of Zoology, University of Kerala (2007).

9. **Anu Joseph**, Remya Robinson, C. S. Paulose. Glutamate dehydrogenase activity in the cerebellum of streptozotocin induced diabetic and insulin induced hypoglycaemic rats. Abstracts of the 3rd International Syposium on Neurodegeneration and Neuroprotection jointly organised by Indian Institute of Chemical Biology and Society for Neurochemistry (India) (2007).

10. C. S. Paulose, Amee Krishnakumar, **Anu Joseph**. Neurotransmitter Functional Role in Neurodegenerative Diseases. Proceedings sent to National Conference on "Environment & Human Health" held by School of Environmental Studies, Dept. of Environmental Science, Cochin University of Science and Technology, Cochin, India (2006).

11. C. S. Paulose, Amee Krishnakumar and **Anu Joseph**. Neurotransmitter Functional Role in Neurodegenerative Disease Management: Recent Advances. *J. Science & Society*, 5(1) 23- 30, (2007).

12. **Anu Joseph** and C. S. Paulose. Kinetic parameters of Norepinephrine binding and its functional relationship with Epinephrine and Serotonin. Presented at the 18th Kerala Science Congress held at Centre for Earth Science Studies (CESS), Trivandrum, India (2006).

13. **Anu Joseph**, Ani Das. V and C. S Paulose. Enhanced EPI binding nuclear protein in the pancreatic islets of pancreatectomised rats. Presented in International Conference on Biotechnology and Neurosciences, Dec 29-31, Centre for Neuroscience, Cochin University of Science and Technology, Cochin, India (2004).

Figure Legends

Figure 70

Confocal image of NMDAR1 receptors in the cerebral cortex of control, Diabetic, D+IIH and C+IIH rats using immunofluorescent NMDAR1 receptor specific primary antibody and FITC as secondary antibody. There was an up regulation of NMDAR1 receptors in the cerebral cortex of experimental rats when compared to control rats. ⟶ in pink shows NMDAR1 receptors.

Figure 71

Confocal image of NMDA2B receptors in the cerebral cortex of control, Diabetic, D+IIH and C+IIH rats using immunofluorescent NMDA2B receptor specific primary antibody and FITC as secondary antibody. There was an up regulation of NMDA2B receptors in the cerebral cortex of experimental rats when compared to control rats. ⟶ in pink shows NMDA2B receptors.

Figure 72

Confocal image of mGluR5 receptors in the cerebral cortex of control, Diabetic, D+IIH and C+IIH rats using immunofluorescent mGluR5 receptor specific primary antibody and Rhodamine as secondary antibody. There was an up regulation of mGluR5 receptors in the cerebral cortex of experimental rats when compared to control rats. ⟶ in pink shows mGluR5 receptors.

Figure 73

Confocal image of IP3 receptors in the cerebral cortex of control, Diabetic, D+IIH and C+IIH rats using immunofluorescent IP3 receptor specific primary antibody and FITC as secondary antibody. There was an up regulation of IP3 receptors in the cerebral cortex of experimental rats when compared to control rats. The ⟶ in pink shows IP3 receptors.

Figure 74

Confocal image of NMDAR1 receptors in the cerebellum of control, Diabetic, D+IIH and C+IIH rats using immunofluorescent NMDAR1 receptor specific primary antibody and FITC as secondary antibody. There was an up regulation of NMDAR1 receptors in the cerebellum of experimental rats when compared to control rats. The ⟶ in pink shows NMDAR1 receptors.

Figure 75

Confocal image of NMDA2B receptors in the cerebellum of control, Diabetic, D+IIH and C+IIH rats using immunofluorescent NMDA2B receptor specific primary antibody and FITC as secondary antibody. There was an up regulation of NMDA2B receptors in the cerebellum of experimental rats when compared to control rats. The ⟶ in pink shows NMDA2B receptors.

Figure 76

Confocal image of mGluR5 receptors in the cerebellum of control, Diabetic, D+IIH and C+IIH rats using immunofluorescent mGluR5 receptor specific primary antibody and Rhodamine as secondary antibody. There was an up regulation of mGluR5 receptors in the cerebellum of experimental rats when compared to control rats. ⟶ in pink shows mGluR5 receptors.

Figure 77

Confocal image of IP3 receptors in the cerebellum of control, Diabetic, D+IIH and C+IIH rats using immunofluorescent IP3 receptor specific primary antibody and FITC as secondary antibody. There was an up regulation of IP3 receptors in the cerebellum of experimental rats when compared to control rats. ⟶ in pink shows IP3 receptors.

Figure 78

Confocal image of NMDAR1 receptors in the pancreas of control, Diabetic, D+IIH and C+IIH rats using immunofluorescent NMDAR1 receptor specific primary

antibody and FITC as secondary antibody. There was an up regulation of NMDAR1 receptors in the pancreas of experimental rats when compared to control rats. ⟶ in pink shows NMDAR1 receptors.

Figure 79

Confocal image of NMDA2B receptors in the pancreas of control, Diabetic, D+IIH and C+IIH rats using immunofluorescent NMDA2B receptor specific primary antibody and FITC as secondary antibody. There was an up regulation of NMDA2B receptors in the pancreas of experimental rats when compared to control rats. ⟶ in pink shows NMDA2B receptors.

Figure 80

Confocal image of mGluR5 receptors in the pancreas of control, Diabetic, D+IIH and C+IIH rats using immunofluorescent mGluR5 receptor specific primary antibody and Rhodamine as secondary antibody. There was an up regulation of mGluR5 receptors in the pancreas of experimental rats when compared to control rats. The ⟶ in pink shows mGluR5 receptors.

Figure 81

Confocal image of IP3 receptors in the pancreas of control, Diabetic, D+IIH and C+IIH rats using immunofluorescent IP3 receptor specific primary antibody and FITC as secondary antibody. There was an up regulation of IP3 receptors in the pancreas of experimental rats when compared to control rats. ⟶ in pink shows IP3 receptors.

Figure 82

Confocal image of calcium release from the pancreas in the presence of 1 mM Glucose, 10^{-5} M Dopamine, 10^{-5} M Dopamine + 10^{-5} M Sulpiride. Dopamine inhibited the calcium release from pancreatic islets. The presence of 10^{-5} M Dopamine and Sulpiride reversed the inhibition.

Figure 83

Confocal image of calcium release from the pancreas in the presence of 4 mM Glucose, 10^{-5} M Dopamine, 10^{-5} M Dopamine + 10^{-5} M Sulpiride. Dopamine increased the calcium release from pancreatic islets. The presence of 10^{-5} M Dopamine and Sulpiride reversed the calcium release.

Figure 84

Confocal image of calcium release from the pancreas in the presence of 20 mM Glucose, 10^{-5} M Dopamine, 10^{-5} M Dopamine + 10^{-5} M Sulpiride. Dopamine increased the calcium release from pancreatic islets. The presence of 10^{-5} M Dopamine and Sulpiride reversed the calcium release.

Figure 85

Confocal image of calcium release from the pancreas in the presence of 1 mM Glucose, 10^{-5}M Glutamate, 10^{-5}M Glutamate + MK801. Glutamate increased the calcium release from pancreatic islets. The presence of 10^{-5} M Glutamate and MK801 reversed the calcium release.

Figure 86

Confocal image of calcium release from the pancreas in the presence of 4 mM Glucose, 10^{-5}M Glutamate, 10^{-5}M Glutamate + MK801. Glutamate increased the calcium release from pancreatic islets. The presence of 10^{-5} M Glutamate and MK801 reversed the calcium release.

Figure 87

Confocal image of calcium release from the pancreas in the presence of 20 mM Glucose, 10^{-5}M Glutamate, 10^{-5}M Glutamate + MK801. Glutamate increased the calcium release from pancreatic islets. The presence of 10^{-5} M Glutamate and MK801 reversed the calcium release.

Figure-1

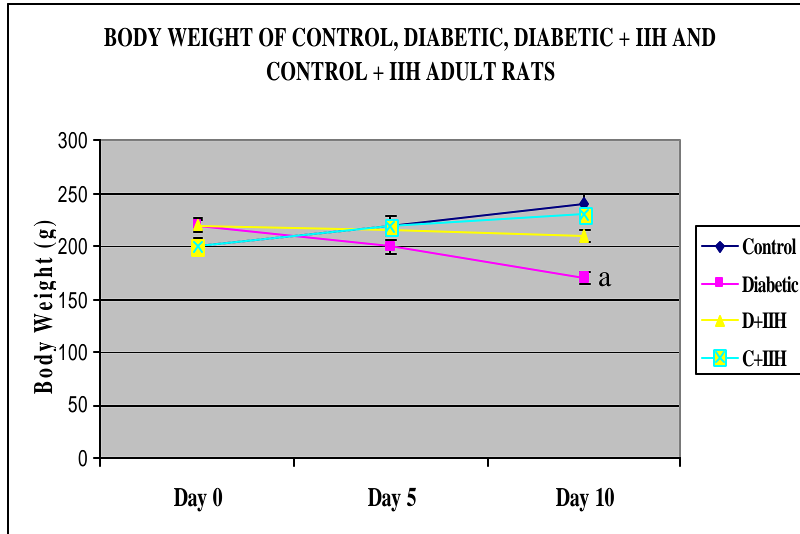


Table - 1
Body weight of Control, Diabetic, Diabetic + IIH and Control + IIH adult rats

Animal Status	Body Weight (g)		
	Day 0	**Day 5**	**Day 10**
Control	200 ± 20	235 ± 0	240 ± 15
Diabetic	220 ± 08	190 ± 08	170 ± 10[a]
D+IIH	220 ± 10	215 ± 10	210 ± 05
C+IIH	200 ± 12	220 ± 10	230 ± 16

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control. IIH - Insulin Induced Hypoglycaemia

Figure-2

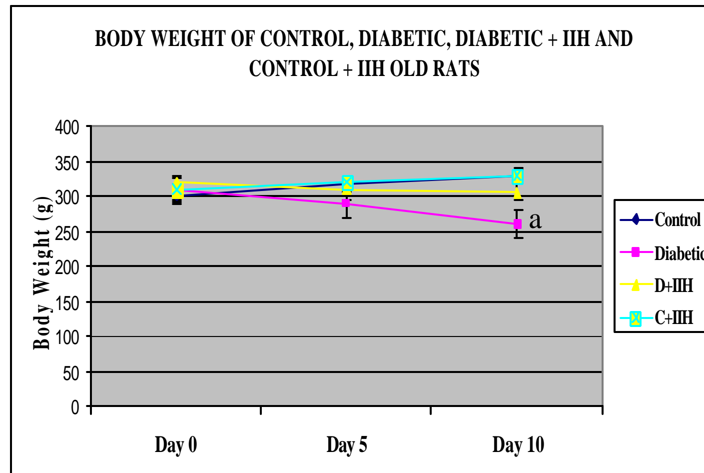


Table-2

Body weight of Control, Diabetic, Diabetic + IIH and Control + IIH old rats

Animal Status	Body Weight (g)		
	Day 0	Day 5	Day 10
Control	300 ± 20	315 ± 10	320 ± 05
Diabetic	310 ± 10	290 ± 05	260 ± 10[a]
D+IIH	320 ± 05	310 ± 12	305 ± 10
C+IIH	310 ± 10	320 ± 10	330 ± 05

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control. IIH - Insulin Induced Hypoglycaemia

Figure-3

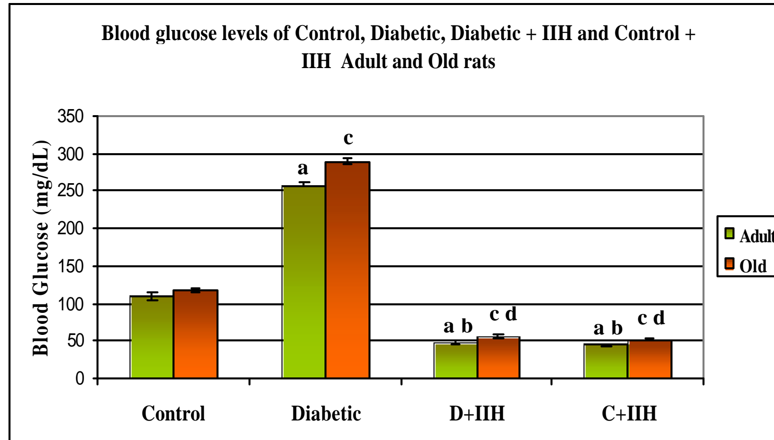


Table-3
Blood glucose levels of Control, Diabetic, Diabetic + IIH and Control + IIH Adult and Old rats

Animal Status	Blood Glucose (mg/dL)	
	Adult	Old
Control	108 ? 5.77	116 ? 3.24
Diabetic	257 ? 3.18 [a]	288 ? 4.68 [c]
D+IIH	47 ? 3.05 [a b]	55 ? 2.53 [c d]
C+IIH	44 ? 1.45 [a b]	50 ? 2.02 [c d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats. [c] p<0.001 when compared with control old rats, [d] p<0.001 when compared with diabetic old rats. IIH - Insulin Induced Hypoglycaemia

Figure-4

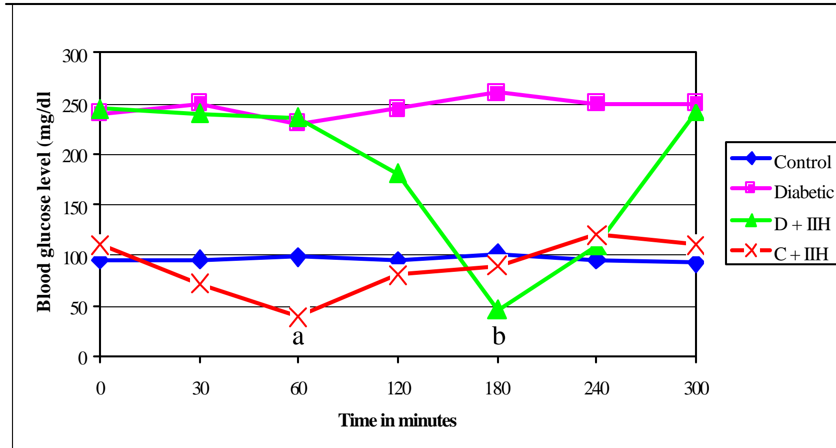
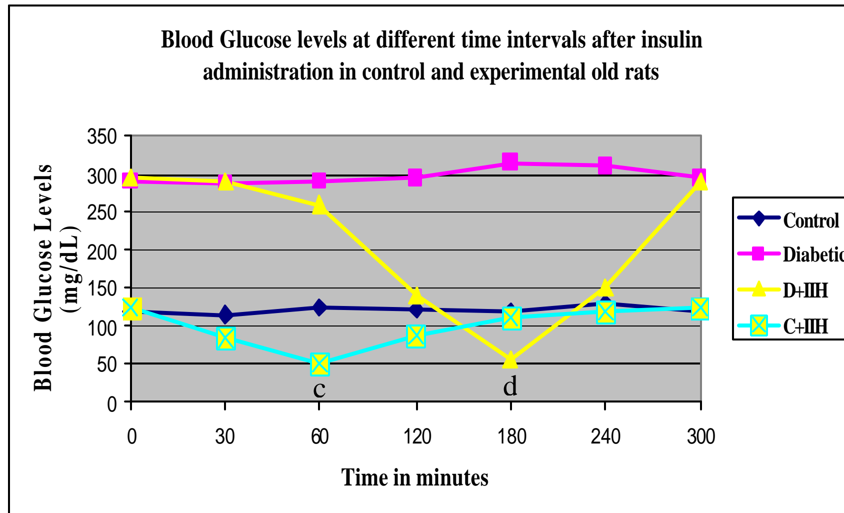


Figure-5



Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats. [c] p<0.001 when compared with control old rats, [d] p<0.001 when compared with diabetic old rats. IIH - Insulin Induced Hypoglycaemia

Figure-6

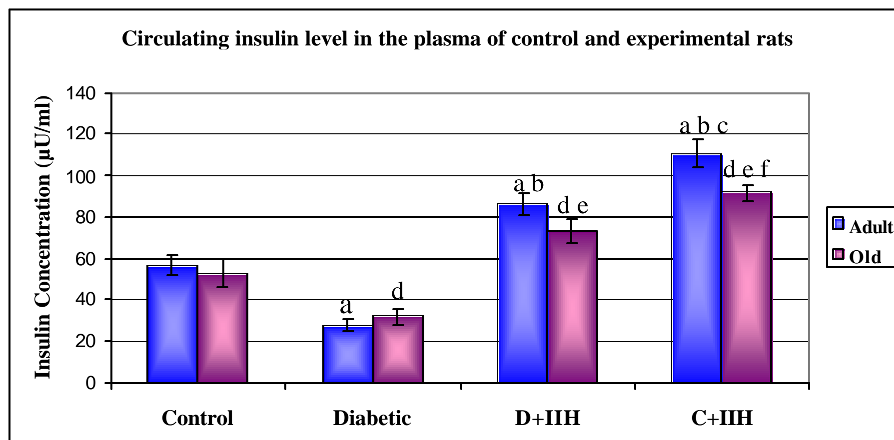


Table-4

Circulating insulin level in the plasma of control and experimental rats

Animal Status	Insulin Concentration (µU/ml)	
	Adult	**Old**
Control	56.4 ? 4.51	52.8 ? 6.94
Diabetic	27.5 ? 3.12 [a]	32.1 ? 3.72 [d]
D+IIH	86.2 ? 5.23 [a b]	73.4 ? 5.66 [d e]
C+IIH	110.8 ? 6.34 [a b c]	91.9 ? 3.91 [d e f]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats. [c] p<0.01 when compared with D+IIH adult rats, [d] p<0.001 when compared with control old rats, [e] p<0.001 when compared with diabetic old rats, [f] p<0.001 when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure-7

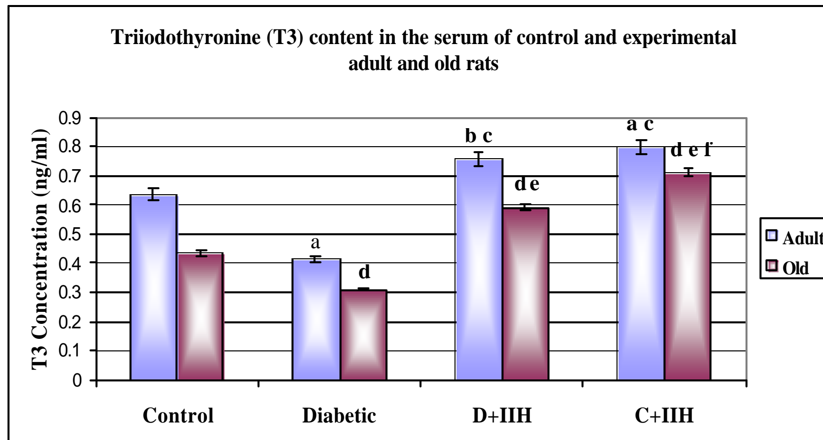


Table-5
Triiodothyronine (T3) content in the serum of control and experimental rats

Animal Status	Concentration (ng/ml)	
	Adult	**Old**
Control	0.64 ? 0.19	0.44 ? 0.13
Diabetic	0.41 ? 0.02 [a]	0.31 ? 0.11 [d]
D+IIH	0.76 ? 0.26 [b c]	0.60 ? 0.06 [d e]
C+IIH	0. 80 ? 0.08 [a c]	0.71 ? 0.17 [d e f]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [b] $p<0.01$, [a] $p<0.001$ when compared with control adult rats, [c] $p<0.001$ when compared with diabetic adult rats. [d] $p<0.001$ when compared with control old rats, [e] $p<0.001$ when compared with diabetic old rats, [f] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure-8

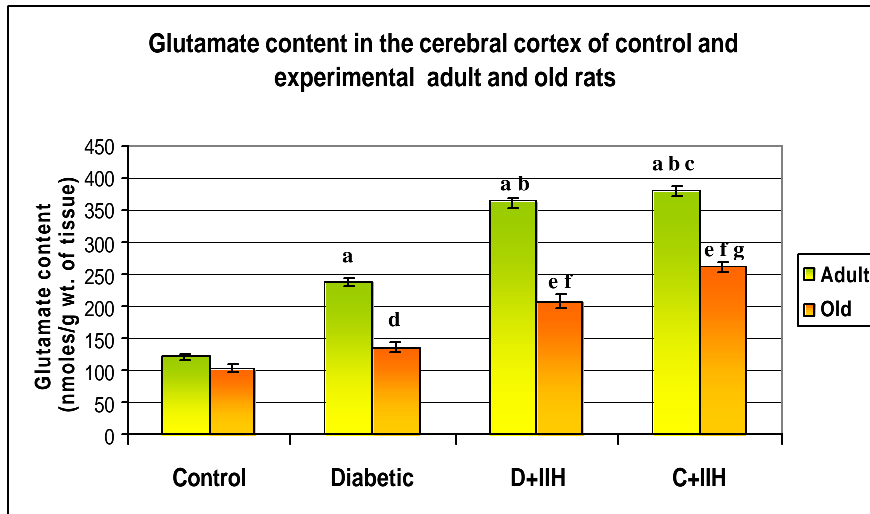


Table-6
Glutamate content in the cerebral cortex of control and experimental adult and old rats

Animal Status	Glutamate content (nmoles/g wt. of tissue)	
	Adult	**Old**
Control	122.4 ± 5.0	103.7 ± 6.0
Diabetic	239.0 ± 6.6 [a]	136.0 ± 7.9 [d]
D+IIH	364.8 ± 4.5 [a b]	206.7 ± 10.4 [e f]
C+IIH	380.7 ± 5.2 [a b c]	262.0 ± 6.6 [e f g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.001$ when compared with diabetic adult rats. [c] $p<0.001$ when compared with D+IIH adult rats. [d] $p<0.01$, [e] $p<0.001$ when compared with control old rats, [f] $p<0.001$ when compared with diabetic old rats, [g] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure-9

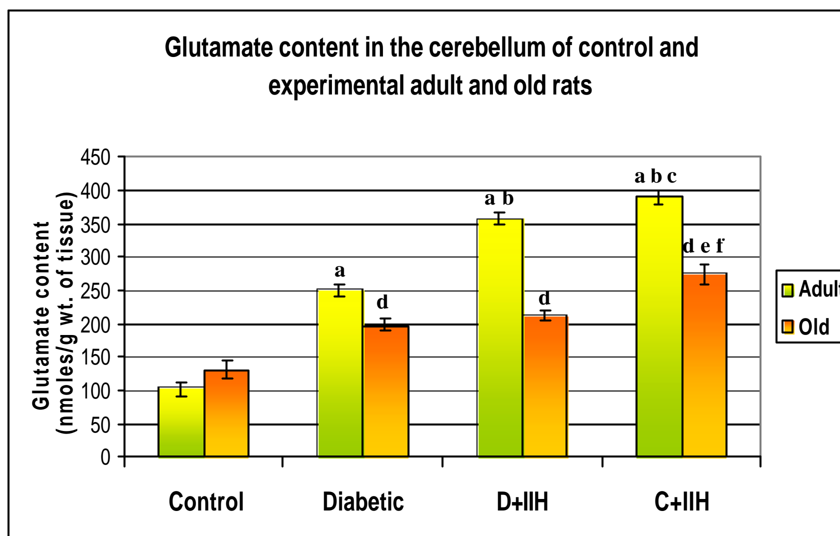


Table-7
Glutamate content in the cerebellum of control and experimental adult and old rats

Animal Status	Glutamate content (nmoles/g wt. of tissue)	
	Adult	**Old**
Control	105.6 ± 7.0	131.0 ± 12.8
Diabetic	251.4 ± 5.8 [a]	198.0 ± 9.0 [d]
D+IIH	358.3 ± 6.3 [a b]	212.7 ± 7.5 [d]
C+IIH	392.9 ± 7.1 [a b c]	275.3 ± 14.9 [d e f]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats. [c] p<0.001 when compared with D+IIH adult rats, [d] p<0.001 when compared with control old rats, [e] p<0.001 when compared with diabetic old rats, [f] p<0.001 when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-10

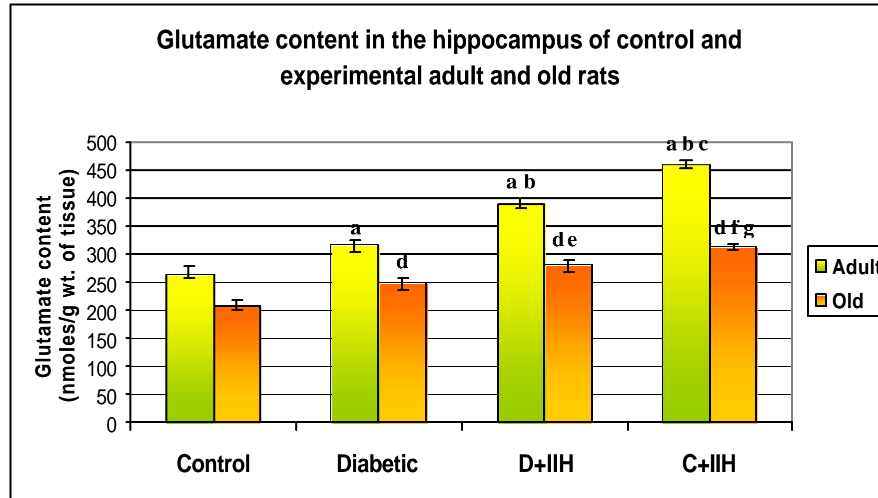


Table-8
Glutamate content in the hippocampus of control and experimental adult and old rats

Animal Status	Glutamate content (nmoles/g wt. of tissue)	
	Adult	Old
Control	263.7 ± 12.8	208.3 ± 7.6
Diabetic	315.0 ± 10.4 [a]	247.3 ± 9.7 [d]
D+IIH	389.3 ± 10.1 [a b]	279.5 ± 9.0 [d e]
C+IIH	459.7 ± 9.5 [a b c]	312.7 ± 6.4 [d f g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.001$ when compared with diabetic adult rats. [c] $p<0.001$ when compared with D+IIH adult rats, [d] $p<0.001$ when compared with control old rats, [e] $p<0.01$, [f] $p<0.001$ when compared with diabetic old rats, [g] $p<0.01$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-11

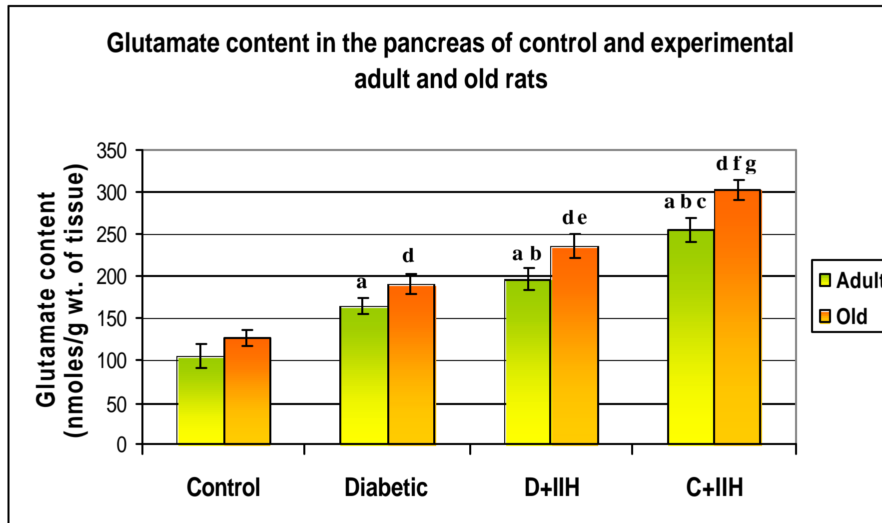


Table-9

Glutamate content in the pancreas of control and experimental adult and old rats

Animal Status	Glutamate content (nmoles/g wt. of tissue)	
	Adult	Old
Control	104.3 ± 14.7	126.5 ± 10.3
Diabetic	164.7 ± 8.3 [a]	191.0 ± 12.5 [d]
D+IIH	196.7 ± 11.7 [a b]	235.3 ± 13.6 [d e]
C+IIH	256.0 ± 14.4 [a b c]	302.3 ± 10.8 [d f g]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.001$ when compared with diabetic adult rats. [c] $p<0.001$ when compared with D+IIH adult rats, [d] $p<0.001$ when compared with control old rats, [e] $p<0.01$, [f] $p<0.001$ when compared with diabetic old rats, [g] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-12
Scatchard analysis of [^{3}H]Glutamate binding against glutamate in the cerebral cortex of control and experimental adult rats

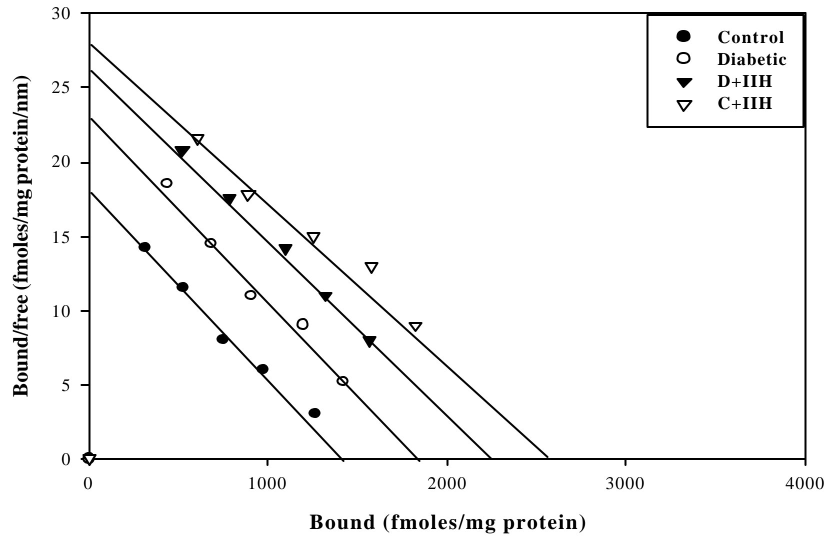


Table-10
Scatchard analysis of [^{3}H]Glutamate binding against glutamate in the cerebral cortex of control and experimental adult rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	1265.0 ± 17.7	104.2 ± 0.8
Diabetic	1736.3 ± 15.3 [a]	106.7 ± 0.7
D+IIH	2468.0 ± 14.4 [a b]	104.9 ± 1.0
C+IIH	2861.7 ± 17.0 [a b c]	105.6 ± 1.2

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.001$ when compared with diabetic adult rats. [c] $p<0.001$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-13

Scatchard analysis of [^{3}H]Glutamate binding against glutamate in the cerebral cortex of control and experimental old rats

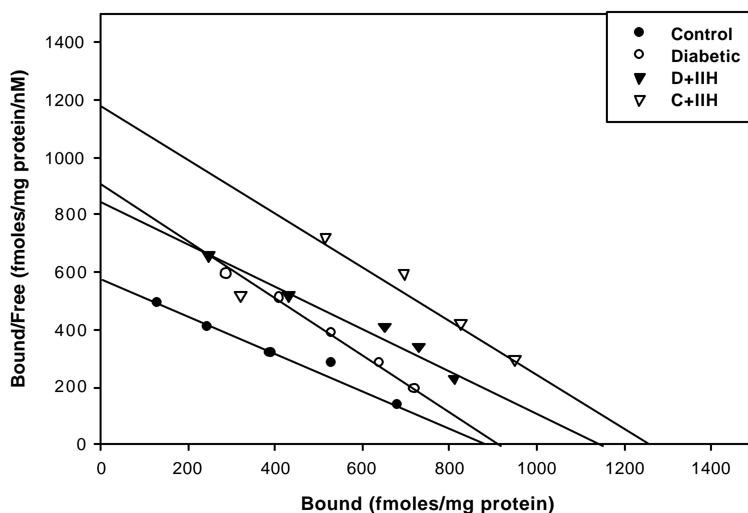


Table-11

Scatchard analysis of [^{3}H]Glutamate binding against glutamate in the cerebral cortex of control and experimental old rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	873.3 ± 7.6	1.5 ± 0.4
Diabetic	921.7 ± 7.6[a]	1.0 ± 0.8
D+IIH	1152.3 ± 6.8[a b]	1.4 ± 0.5
C+IIH	1238.9 ± 7.5[a b c]	1.0 ± 0.8

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control old rats, [b] p<0.001 when compared with diabetic old rats. [c] p<0.001 when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-14
Scatchard analysis of NMDA receptors using [^{3}H]MK801 binding against MK801 in the cerebral cortex of control and experimental adult rats

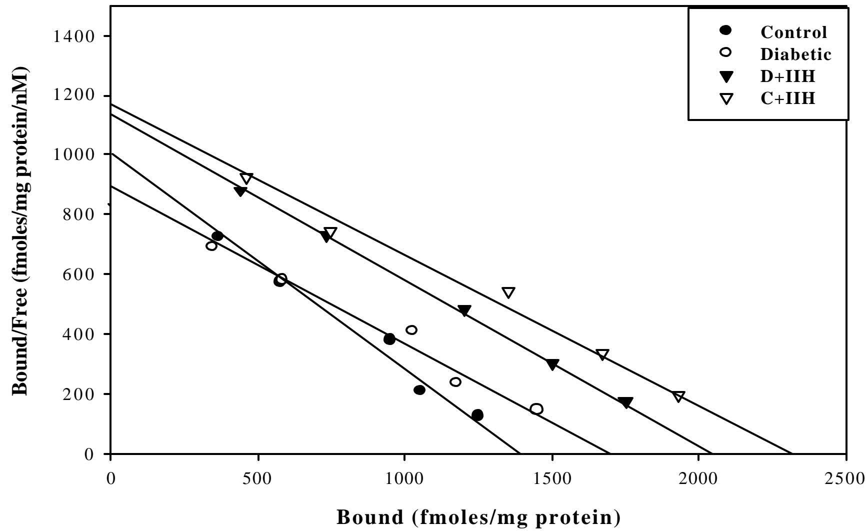


Table-12
Scatchard analysis of NMDA receptors using [^{3}H]MK801 binding against MK801 in the cerebral cortex of control and experimental adult rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	1240.3 ± 12.7	1.5 ± 0.8
Diabetic	1491.7 ± 17.0[a]	1.7 ± 0.7
D+IIH	1993.3 ± 14.4[b c]	1.5 ± 1.0
C+IIH	2351.7 ± 16.0[b c d]	1.6 ± 1.2

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.05$, [b] $p<0.001$ when compared with control adult rats, [c] $p<0.001$ when compared with diabetic adult rats. [d] $p<0.01$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia

Figure-15

Scatchard analysis of NMDA receptors using [^{3}H]MK801 binding against MK801 in the cerebral cortex of control and experimental old rats

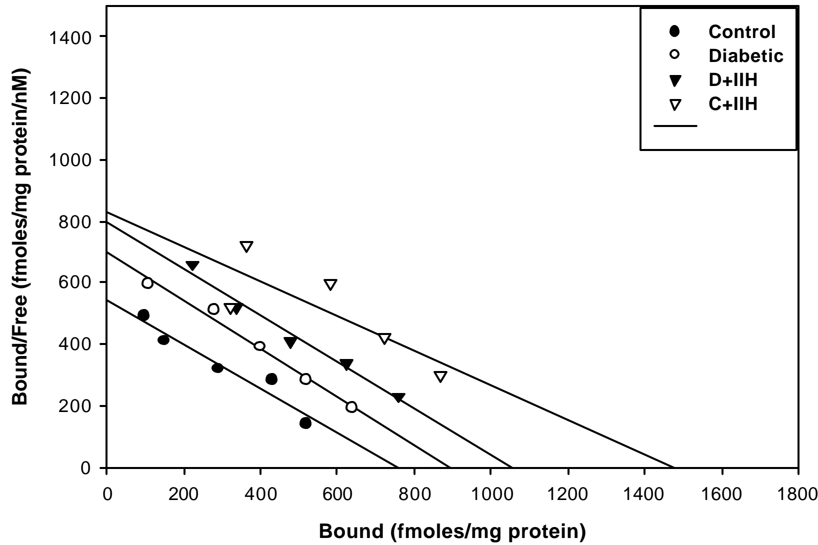


Table-13

Scatchard analysis of NMDA receptors using [^{3}H]MK801 binding against MK801 in the cerebral cortex of control and experimental old rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	753.6 ± 7.6	1.4 ± 0.2
Diabetic	894.7 ± 7.6[a]	1.3 ± 0.6
D+IIH	1037.2 ± 6.8[a b]	1.3 ± 0.5
C+IIH	1472.8 ± 7.5[a b c]	1.8 ± 0.3[a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control old rats, [b] p<0.001 when compared with diabetic old rats. [c] p<0.001 when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure-16

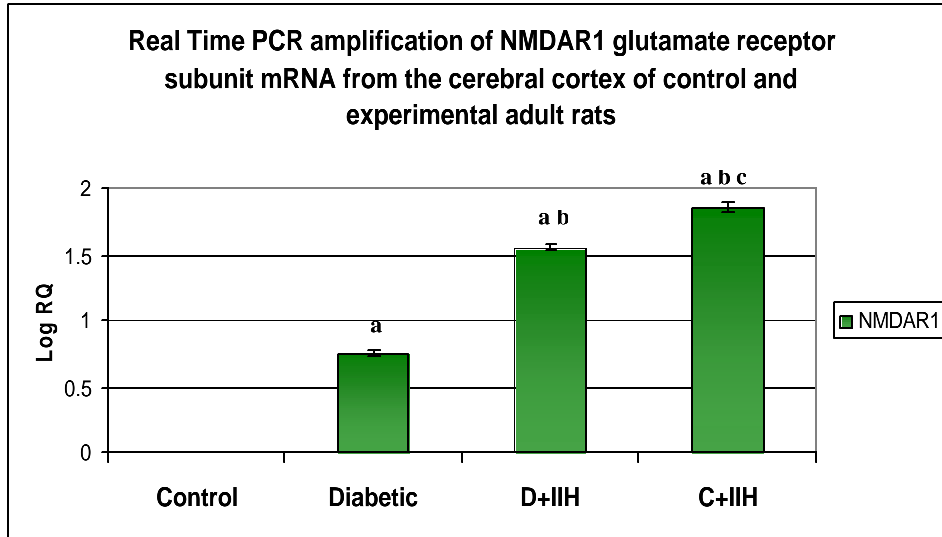


Table-14

Real Time PCR amplification of NMDAR1 glutamate receptor subunit mRNA from the cerebral cortex of control and experimental adult rats

Animal Status	NMDAR1 Log RQ
Control	0
Diabetic	0.75 ± 0.02 [a]
D + IIH	1.55 ± 0.02 [a b]
C + IIH	1.85 ± 0.04 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.001$ when compared with diabetic adult rats. [c] $p<0.001$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia

Figure-17

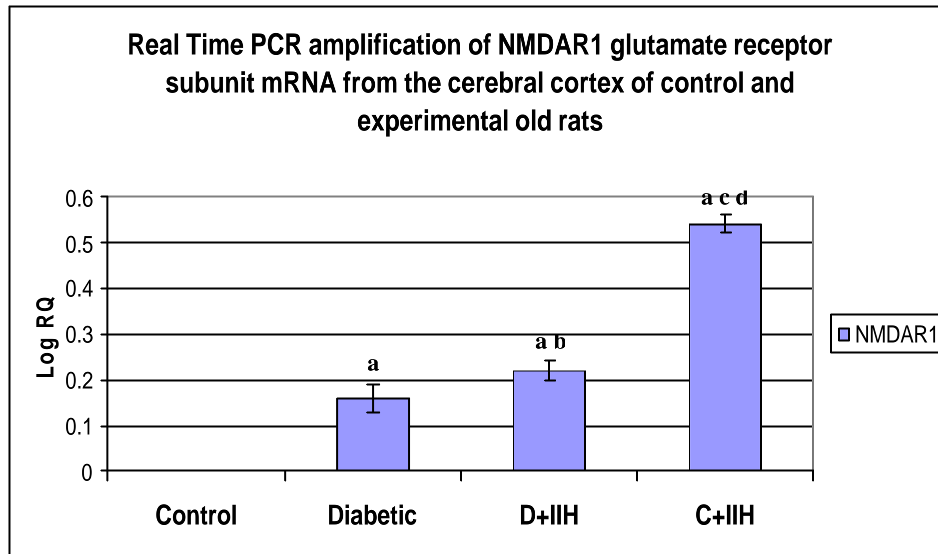


Table-15

Real Time PCR amplification of NMDAR1 glutamate receptor subunit mRNA from the cerebral cortex of control and experimental old rats

Animal Status	NMDAR1 Log RQ
Control	0
Diabetic	0.16 ± 0.03 [a]
D + IIH	0.22 ± 0.02 [a b]
C + IIH	0.54 ± 0.02 [a c d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.01$, [c] $p<0.001$ when compared with diabetic old rats. [d] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure-18

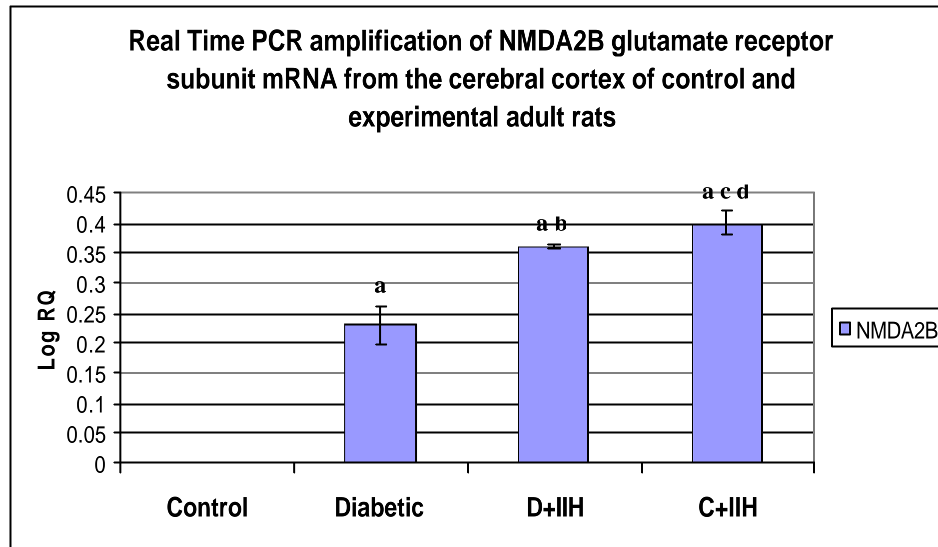


Table-16

Real Time PCR amplification of NMDA2B glutamate receptor subunit mRNA from the cerebral cortex of control and experimental adult rats

Animal Status	NMDA2B Log RQ
Control	0
Diabetic	0.23 ± 0.03 [a]
D + IIH	0.36 ± 0.01 [a b]
C + IIH	0.40 ± 0.02 [a c d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.01$, [c] $p<0.001$ when compared with diabetic adult rats. [d] $p<0.05$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia

Figure-19

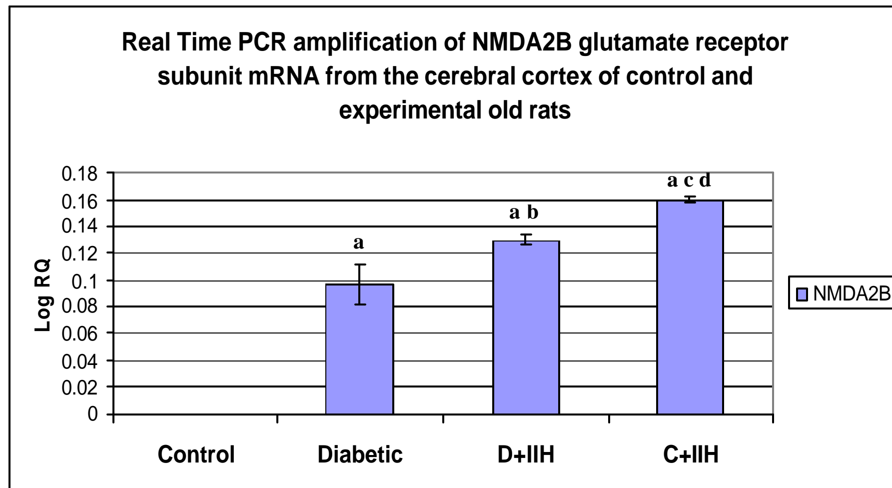


Table-17

Real Time PCR amplification of NMDA2B glutamate receptor subunit mRNA from the cerebral cortex of control and experimental old rats

Animal Status	NMDA2B Log RQ
Control	0
Diabetic	0.097 ± 0.02 [a]
D + IIH	0.130 ± 0.04 [a b]
C + IIH	0.160 ± 0.02 [a c d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.01$, [c] $p<0.001$ when compared with diabetic old rats. [d] $p<0.01$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure-20

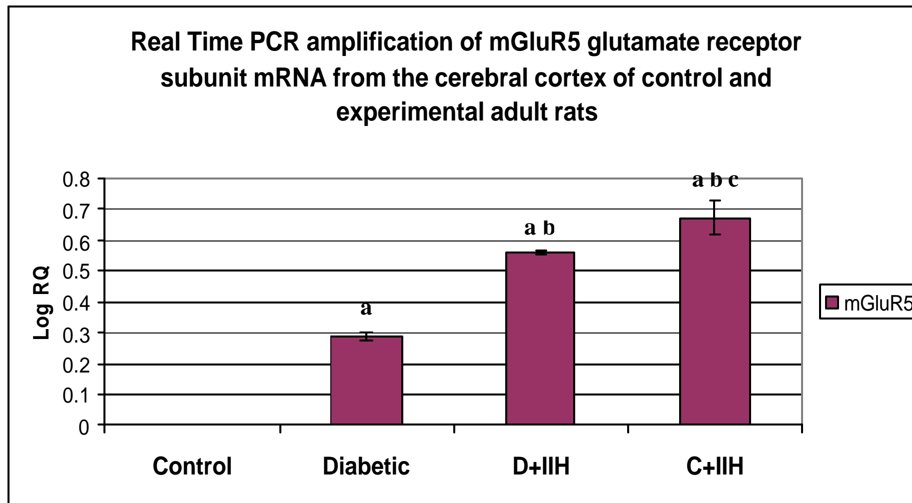


Table-18

Real Time PCR amplification of mGluR5 glutamate receptor subunit mRNA from the cerebral cortex of control and experimental adult rats

Animal Status	mGluR5 Log RQ
Control	0
Diabetic	0.29 ± 0.01^{a}
D + IIH	$0.56 \pm 0.04^{a\,b}$
C + IIH	$0.67 \pm 0.05^{a\,b\,c}$

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats. [c] p<0.001 when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia

Figure -21

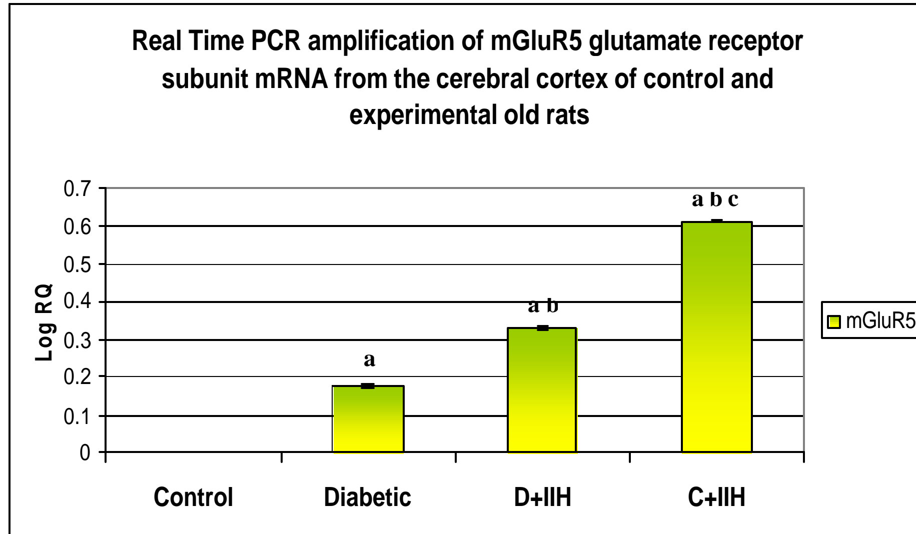


Table -19

Real Time PCR amplification of mGluR5 glutamate receptor subunit mRNA from the cerebral cortex of control and experimental old rats

Animal Status	mGluR5 Log RQ
Control	0
Diabetic	0.178 ± 0.04 [a]
D + IIH	0.330 ± 0.04 [a b]
C + IIH	0.613 ± 0.03 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control old rats, [b] p<0.001 when compared with diabetic old rats. [c] p<0.001 when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure-22

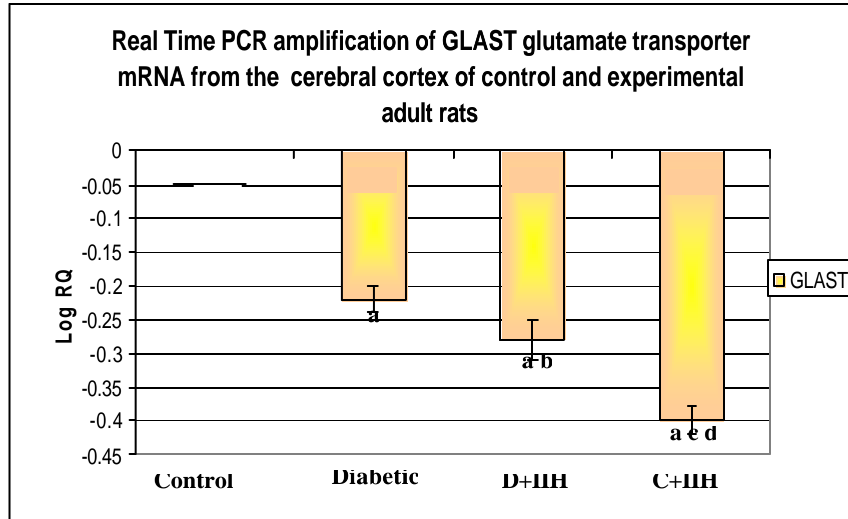


Table-20

Real Time PCR amplification of GLAST glutamate transporter mRNA from the cerebral cortex of control and experimental adult rats

Animal Status	GLAST Log RQ
Control	0
Diabetic	-0.22 ± 0.02^{a}
D + IIH	$-0.28 \pm 0.03^{a\,b}$
C + IIH	$-0.40 \pm 0.02^{a\,c\,d}$

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.01$, [c] $p<0.001$ when compared with diabetic adult rats. [d] $p<0.001$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-23

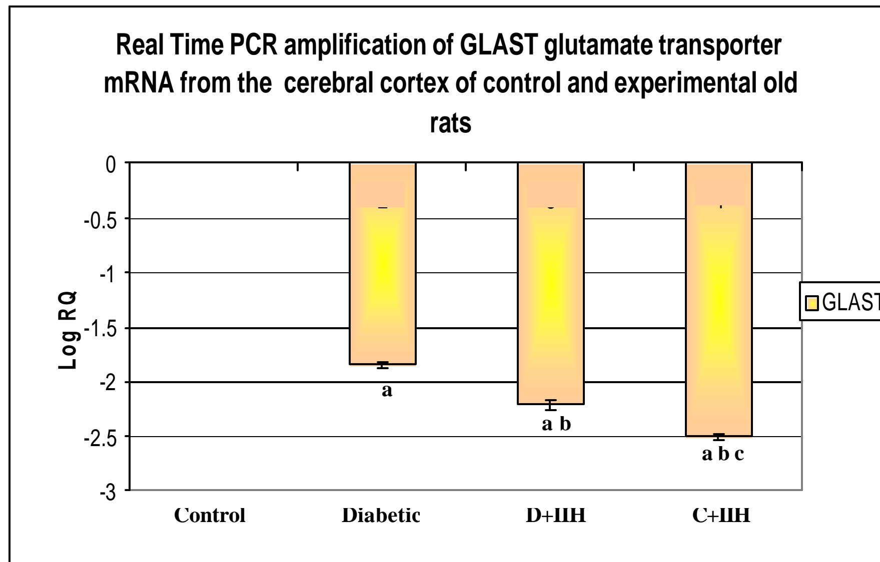


Table-21

Real Time PCR amplification of GLAST glutamate transporter mRNA from the cerebral cortex of control and experimental old rats

Animal Status	GLAST Log RQ
Control	0
Diabetic	-1.85 ± 0.03 [a]
D + IIH	-2.21 ± 0.04 [a b]
C + IIH	-2.51 ± 0.04 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats. [c] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-24

Scatchard analysis of [^{3}H]Glutamate against glutamate in the cerebellum of control and experimental adult rats

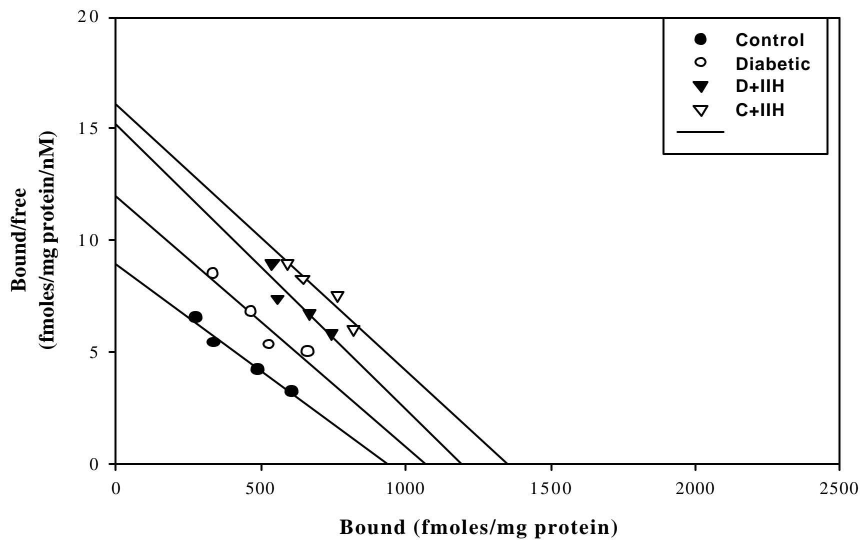


Table-22

Scatchard analysis of [^{3}H]Glutamate against glutamate in the cerebellum of control and experimental adult rats

Animal status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	1003.3 ± 3.3	147.7 ± 0.9
Diabetic	1493.3 ± 3.4 [a]	138.7 ± 1.9 [b]
D+IIH	1726.7 ± 6.7 [a c]	143.3 ± 0.9 [b c]
C+IIH	1826.4 ± 5.2 [a c d]	151.3 ± 1.9 [b c e]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [b] $p<0.01$, [a] $p<0.001$ when compared with control adult rats, [c] $p<0.001$ when compared with diabetic adult rats. [e] $p<0.01$, [d] $p<0.001$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia

Figure-25

Scatchard analysis of [^{3}H]Glutamate against glutamate in the cerebellum of control and experimental old rats

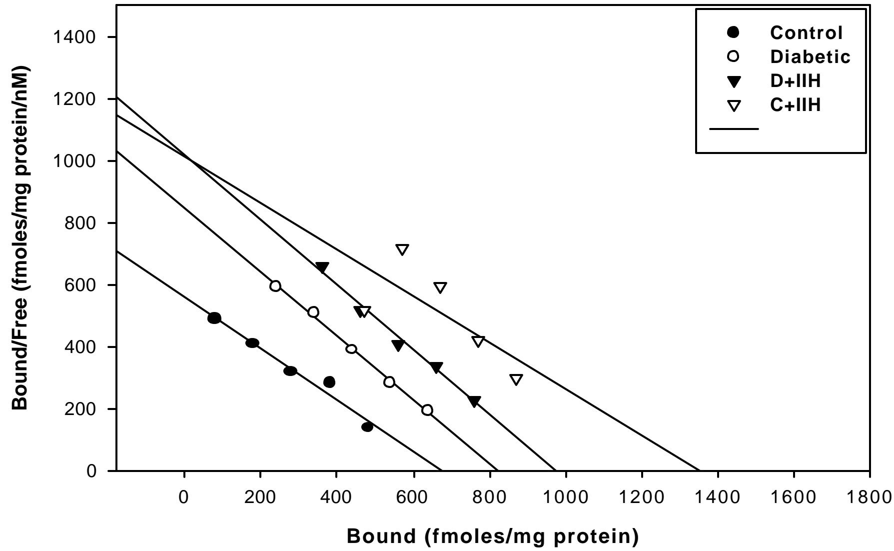


Table-23

Scatchard analysis of [^{3}H]Glutamate binding against glutamate in the cerebellum of control and experimental old rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	633.6 ± 11.9	0.9 ± 0.6
Diabetic	811.7 ± 7.6[a]	0.8 ± 0.8
D+IIH	976.8 ± 15.3[a b]	0.8 ± 0.5
C+IIH	1366.7 ± 12.5[a b c]	1.2 ± 0.2

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control old rats, [b] p<0.001 when compared with diabetic old rats. [c] p<0.001 when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure -26

Scartchard analysis of NMDA receptors using [^{3}H] MK 801 Binding to MK801 in cerebellum of control and experimental adult rats

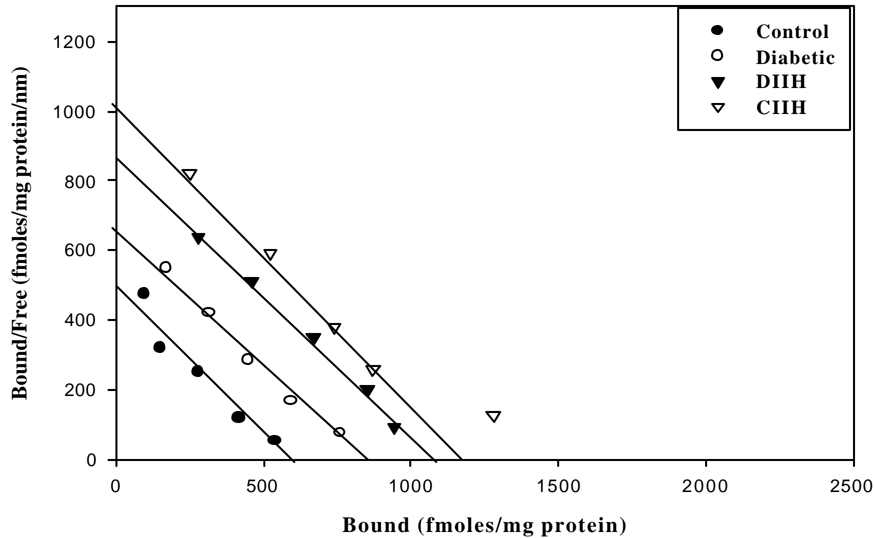


Table -24

Scatchard analysis of NMDA receptors using [^{3}H]MK801 binding to MK801 in cerebellum of control and experimental adult rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	594.6 ± 10.2	0.8 ± 1.8
Diabetic	850.2 ± 15.0 [a]	0.8 ± 0.9
D+IIH	1020.8 ± 20.2 [a b]	0.8 ± 1.4
C+IIH	1140.3 ± 12.0 [a b c]	0.9 ± 1.1

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.001$ when compared with diabetic adult rats. [c] $p<0.001$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia

Figure-27

Scatchard analysis of NMDA receptors using [^{3}H]MK801 binding to MK801 in cerebellum of control and experimental old rats

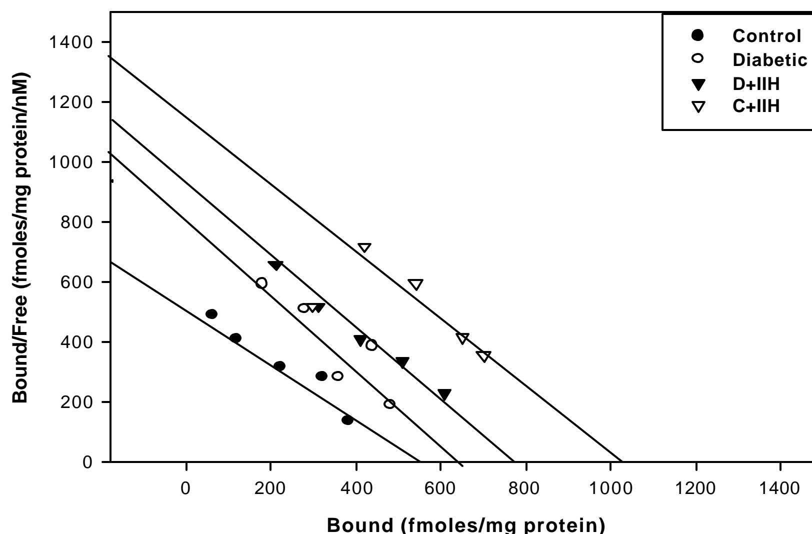


Table-25

Scatchard analysis of NMDA receptors using [^{3}H]MK801 binding to MK801 in cerebellum of control and experimental old rats

Animal Status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	543.7 ± 20.4	0.9 ± 0.3
Diabetic	627.3 ± 14.2 [a]	0.6 ± 0.6
D+IIH	780.6 ± 20.0 [a b]	0.7 ± 0.7
C+IIH	1029.0 ± 16.5 [a b c]	0.8 ± 0.5

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats. [c] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure -28

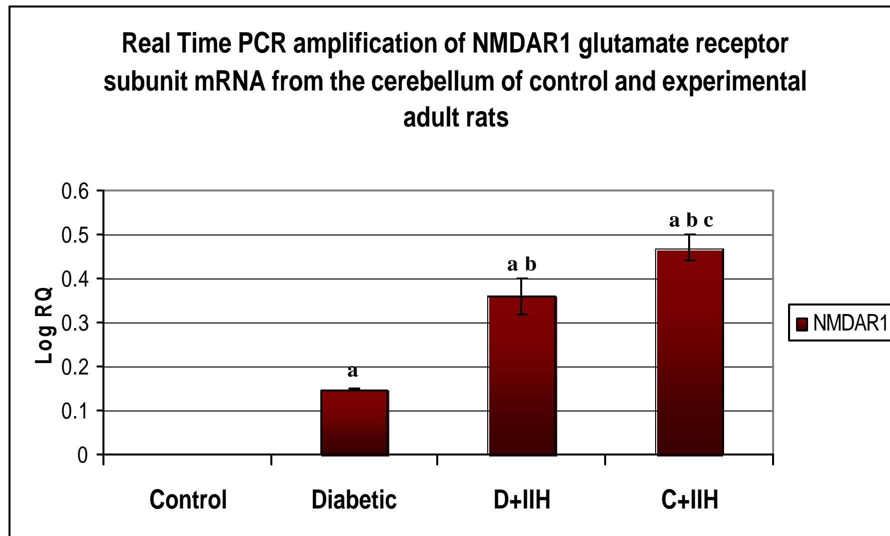


Table -26

Real Time PCR amplification of NMDAR1 glutamate receptor subunit mRNA from the cerebellum of control and experimental adult rats

Animal Status	NMDAR1 Log RQ
Control	0
Diabetic	0.15 ± 0.01 [a]
D + IIH	0.36 ± 0.04 [a b]
C + IIH	0.47 ± 0.03 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.001$ when compared with diabetic adult rats. [c] $p<0.001$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia

Figure -29

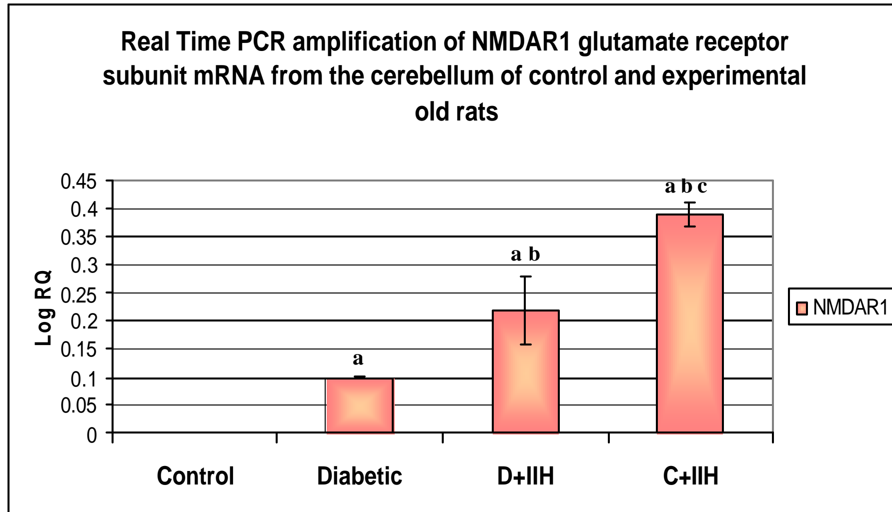


Table -27

Real Time PCR amplification of NMDAR1 glutamate receptor subunit mRNA from the cerebellum of control and experimental old rats

Animal Status	NMDAR1 Log RQ
Control	0
Diabetic	0.10 ± 0.02 [a]
D + IIH	0.22 ± 0.06 [a b]
C + IIH	0.39 ± 0.02 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats. [c] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure-30

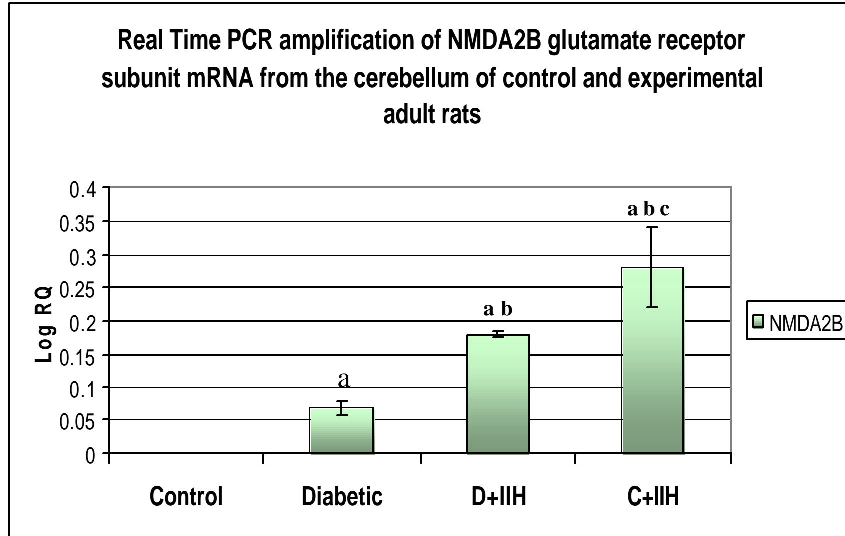


Table-28

Real Time PCR amplification of NMDA2B glutamate receptor subunit mRNA from the cerebellum of control and experimental adult rats

Animal Status	NMDA2B Log RQ
Control	0
Diabetic	0.07 ± 0.01 [a]
D + IIH	0.18 ± 0.00 [a b]
C + IIH	0.28 ± 0.06 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.01$ when compared with diabetic adult rats. [c] $p<0.01$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia

Figure-31

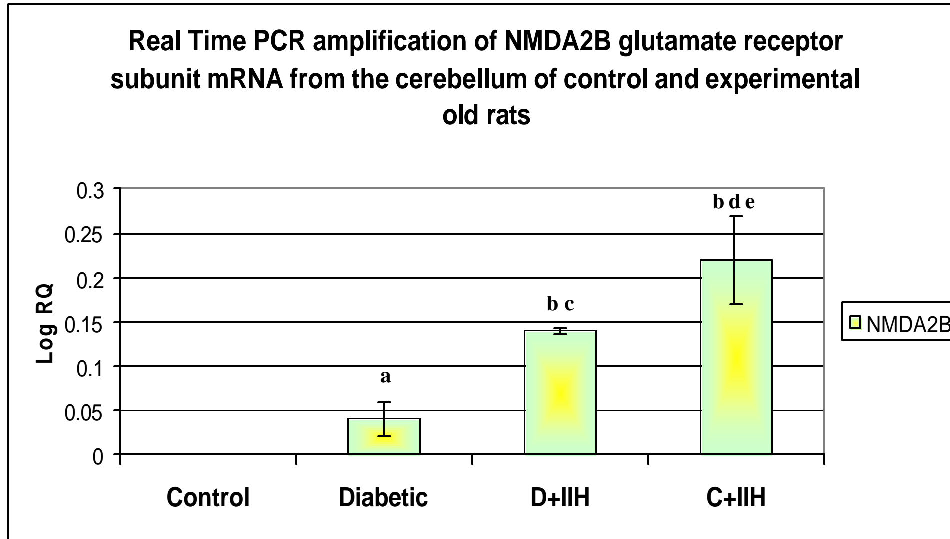


Table-29

Real Time PCR amplification of NMDA2B glutamate receptor subunit mRNA from the cerebellum of control and experimental old rats

Animal Status	NMDA2B Log RQ
Control	0
Diabetic	0.04 ± 0.02 [a]
D + IIH	0.14 ± 0.01 [b c]
C + IIH	0.22 ± 0.05 [b d e]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.05$, [b] $p<0.001$ when compared with control old rats, [c] $p<0.01$, [d] $p<0.001$ when compared with diabetic old rats. [e] $p<0.01$ when compared with D+IIH old rats.
IIH - Insulin Induced Hypoglycaemia

Figure-32

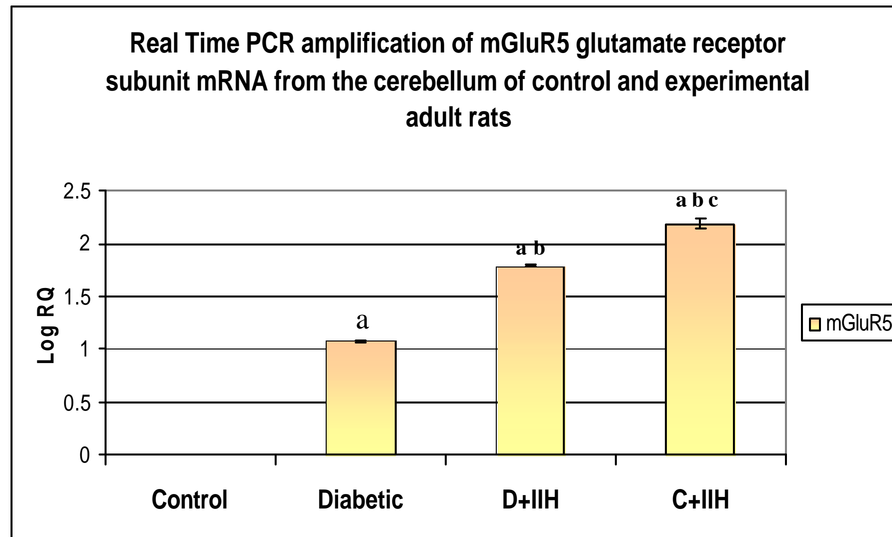


Table-30

Real Time PCR amplification of mGluR5 glutamate receptor subunit mRNA from the cerebellum of control and experimental adult rats

Animal Status	mGluR5 Log RQ
Control	0
Diabetic	1.08 ± 0.01 [a]
D + IIH	1.79 ± 0.01 [a b]
C + IIH	2.18 ± 0.05 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats. [c] p<0.001 when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia

Figure-33

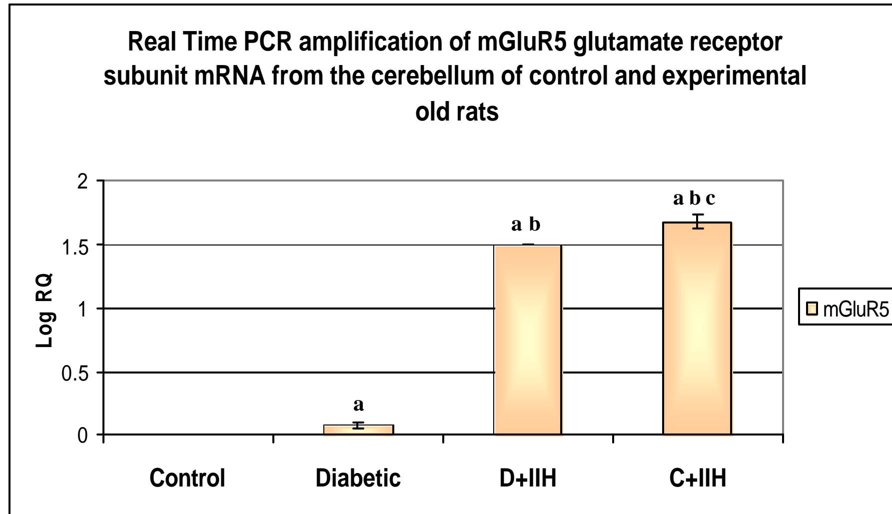


Table-31

Real Time PCR amplification of mGluR5 glutamate receptor subunit mRNA from the cerebellum of control and experimental old rats

Animal Status	mGluR5 Log RQ
Control	0
Diabetic	0.08 ± 0.02 [a]
D + IIH	1.50 ± 0.01 [a b]
C + IIH	1.68 ± 0.06 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats. [c] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure-34

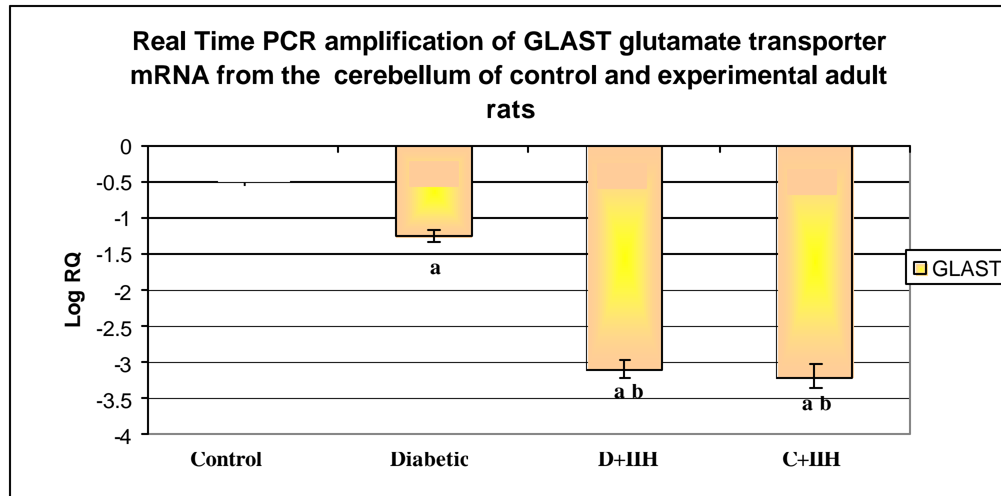


Table-32

Real Time PCR amplification of GLAST glutamate transporter mRNA from the cerebellum of control and experimental adult rats

Animal Status	GLAST Log RQ
Control	0
Diabetic	-1.26 ± 0.08 [a]
D + IIH	-3.09 ± 0.12 [a b]
C + IIH	-3.20 ± 0.17 [a b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.001$ when compared with diabetic adult rats. IIH - Insulin Induced Hypoglycaemia

Figure -35

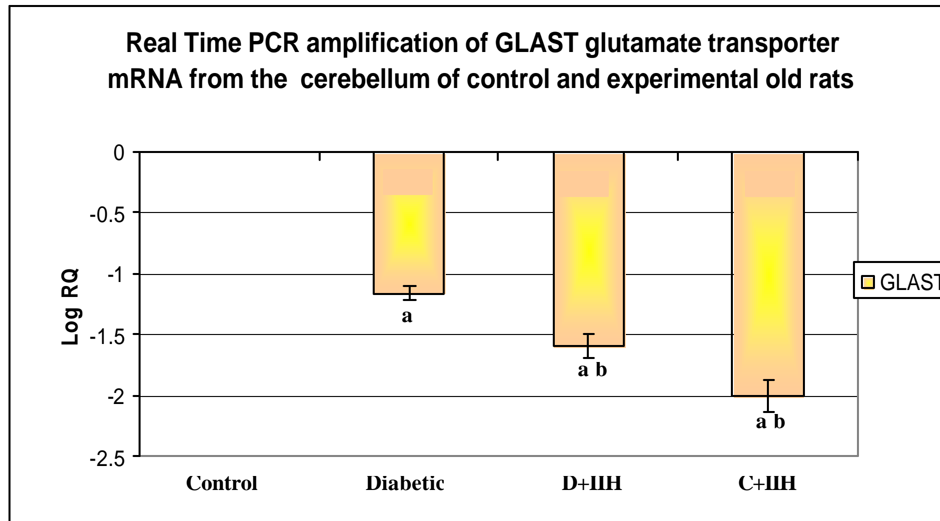


Table -33

Real Time PCR amplification of GLAST glutamate transporter mRNA from the cerebellum of control and experimental old rats

Animal Status	GLAST Log RQ
Control	0
Diabetic	-1.16 ± 0.06 [a]
D + IIH	-1.59 ± 0.10 [a b]
C + IIH	-2.00 ± 0.13 [a b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats. IIH - Insulin Induced Hypoglycaemia

Figure-36

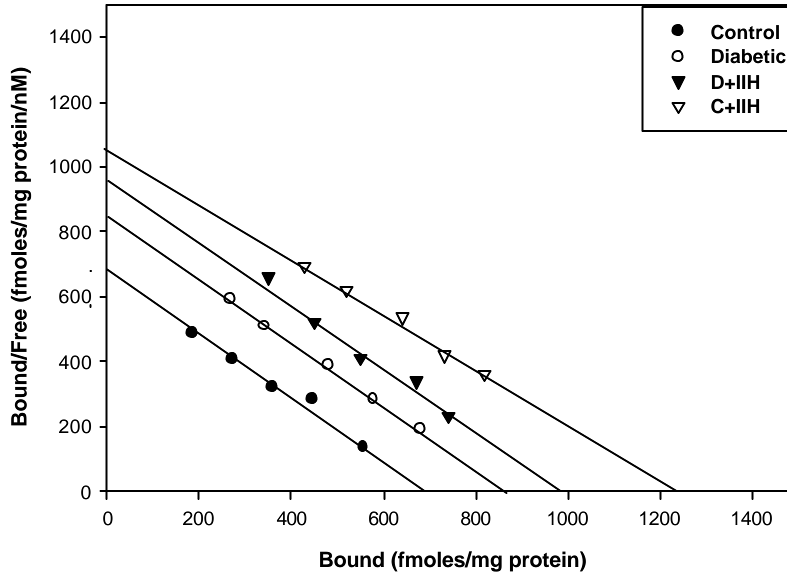


Table-34

Scatchard analysis of $[^3H]$Glutamate binding against glutamate in the hippocampus of control and experimental adult rats

Animal status	Bmax (fmoles/mg protein)	Kd (nM)
Control	660.0 ± 10.0	1.0 ± 0.3
Diabetic	881.7 ± 10.5 [a]	1.1 ± 0.2
D+IIH	1000.0 ± 9.9 [a b]	1.0 ± 0.2
C+IIH	1250.8 ± 11.2 [a b c]	1.2 ± 0.1

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats, [c] p<0.001 when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia

Figure-37

Scatchard analysis of [^{3}H]Glutamate binding against glutamate in the hippocampus of control and experimental old rats

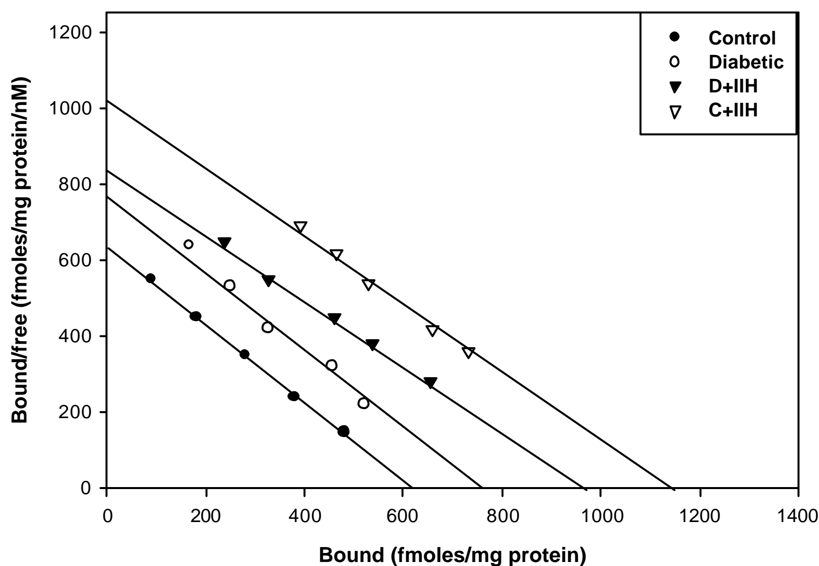


Table-35

Scatchard analysis of [^{3}H]Glutamate binding against glutamate in the hippocampus of control and experimental old rats

Animal status	Bmax (fmoles/mg protein)	Kd (nM)
Control	613.0 ± 9.5	1.1 ± 0.2
Diabetic	769.3 ± 3.1 [a]	1.0 ± 0.3
D+IIH	970.5 ± 6.8 [a b]	1.2 ± 0.2
C+IIH	1161.3 ± 7.6 [a b c]	1.2 ± 0.1

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats, [c] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure-38

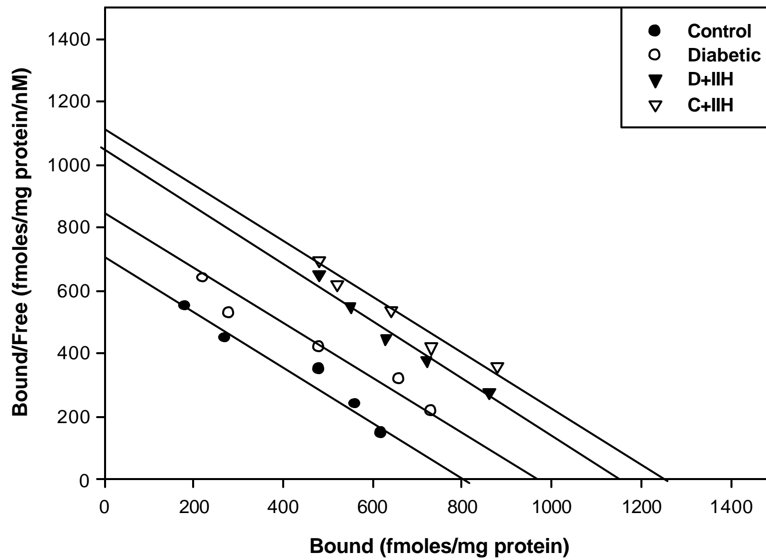


Table-36

Scatchard analysis of NMDA receptors using [^{3}H]MK801 binding against MK801 in the hippocampus of control and experimental adult rats

Animal status	Bmax (fmoles/mg protein)	Kd (nM)
Control	804.7 ± 9.0	1.2 ± 0.1
Diabetic	978.8 ± 8.1 [a]	1.2 ± 0.1
Diabetic+IIH	1154.3 ± 5.1 [a b]	1.1 ± 0.0
Control+IIH	1268.7 ± 9.3 [a b c]	1.0 ± 0.2

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats, [c] p<0.001 when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-39

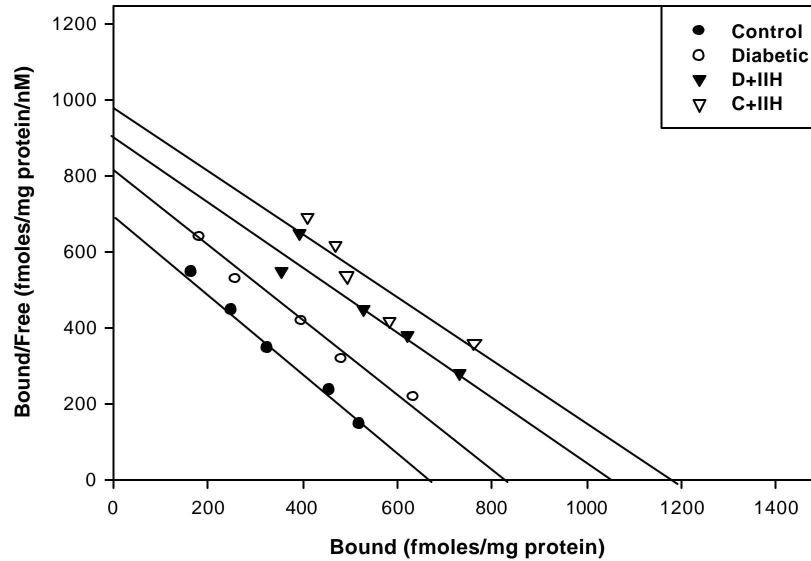


Table-37

Scatchard analysis of NMDA receptors using $[^3H]$MK801 binding against MK801 in the hippocampus of control and experimental old rats

Animal status	Bmax (fmoles/mg protein)	Kd (nM)
Control	660.0 ± 10.0	1.0 ± 0.2
Diabetic	812.7 ± 5.5 [a]	1.0 ± 0.3
Diabetic+IIH	1025.6 ± 5.1 [a b]	1.0 ± 0.1
Control+IIH	1198.3 ± 7.6 [a b c]	1.2 ± 0.1

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats, [c] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-40

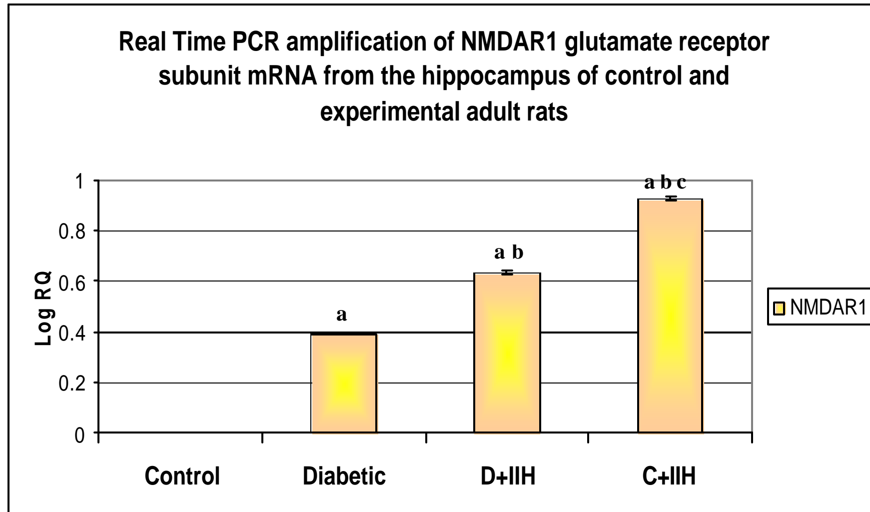


Table-38

Real Time PCR amplification of NMDAR1 glutamate receptor subunit mRNA from the hippocampus of control and experimental adult rats

Animal Status	NMDAR1 Log RQ
Control	0
Diabetic	0.39 ± 0.01 [a]
D + IIH	0.64 ± 0.01 [a b]
C + IIH	0.93 ± 0.01 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats. [c] p<0.001 when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-41

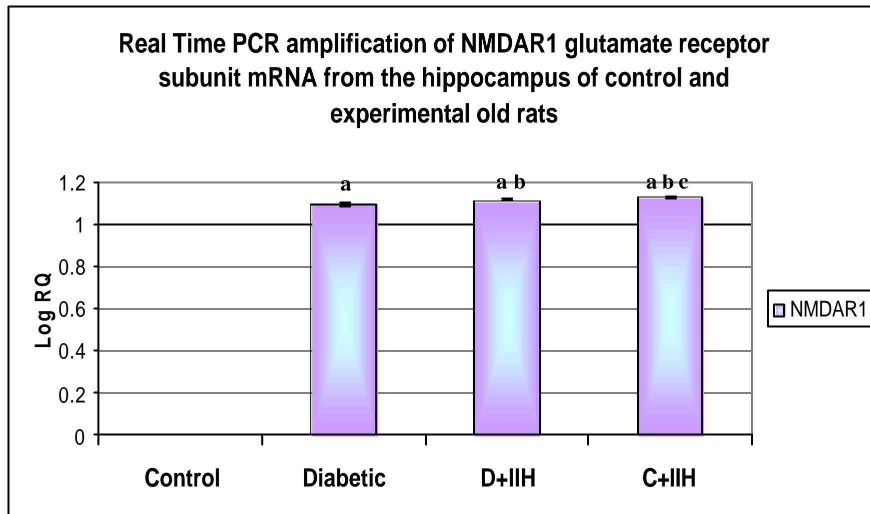


Table-39

Real Time PCR amplification of NMDAR1 glutamate receptor subunit mRNA from the hippocampus of control and experimental old rats

Animal Status	NMDAR1 Log RQ
Control	0
Diabetic	1.10 ± 0.01 [a]
D + IIH	1.12 ± 0.01 [a b]
C + IIH	1.15 ± 0.00 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats. [c] $p<0.05$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure -42

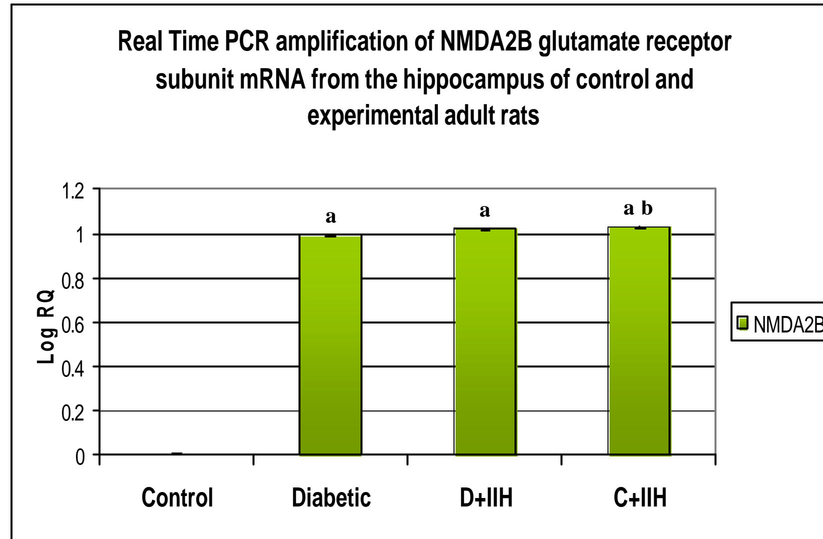


Table -40

Real Time PCR amplification of NMDA2B glutamate receptor subunit mRNA from the hippocampus of control and experimental adult rats

Animal Status	NMDA2B Log RQ
Control	0
Diabetic	0.99 ± 0.01 [a]
D + IIH	1.02 ± 0.01 [a]
C + IIH	1.03 ± 0.00 [a b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.001$ when compared with diabetic adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure -43

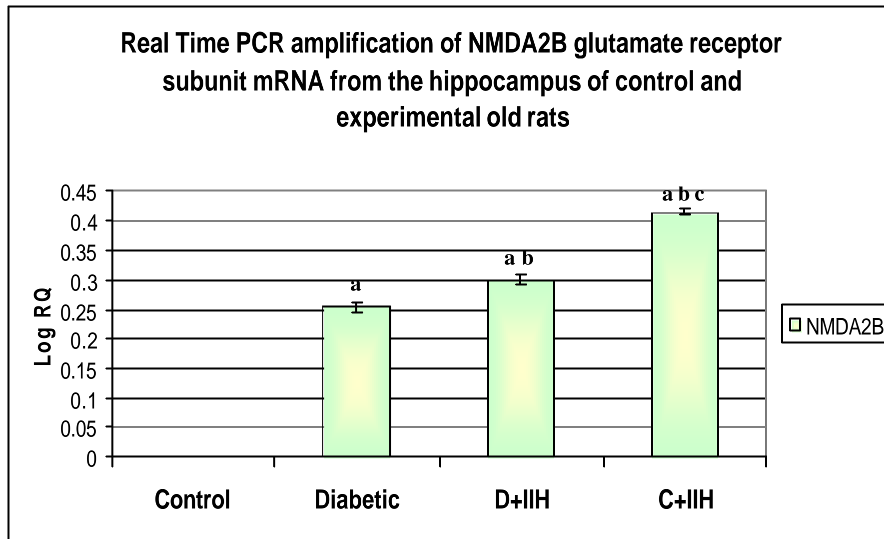


Table -41

Real Time PCR amplification of NMDA2B glutamate receptor subunit mRNA from the hippocampus of control and experimental old rats

Animal Status	NMDA2B Log RQ
Control	0
Diabetic	0.25 ± 0.01 [a]
D + IIH	0.30 ± 0.01 [a b]
C + IIH	0.42 ± 0.01 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats, [c] $p<0.001$ when compared with D+IIH old rats IIH - Insulin Induced Hypoglycaemia.

Figure -44

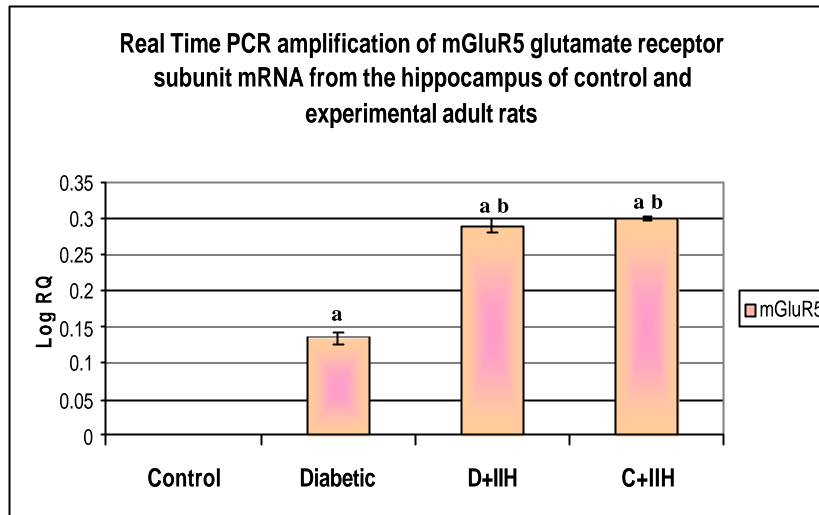


Table -42

Real Time PCR amplification of mGluR5 glutamate receptor subunit mRNA from the hippocampus of control and experimental adult rats

Animal Status	mGluR5 Log RQ
Control	0
Diabetic	0.14 ± 0.01 [a]
D + IIH	0.29 ± 0.01 [a b]
C + IIH	0.30 ± 0.00 [a b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-45

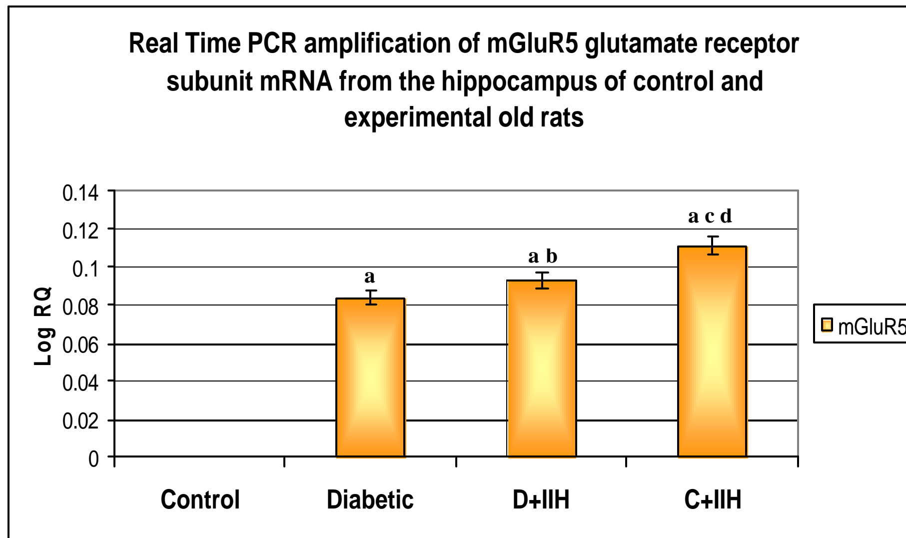


Table-43

Real Time PCR amplification of mGluR5 glutamate receptor subunit mRNA from the hippocampus of control and experimental old rats

Animal Status	mGluR5 Log RQ
Control	0
Diabetic	0.084 ± 0.004 [a]
D + IIH	0.093 ± 0.004 [a b]
C + IIH	0.111 ± 0.005 [a c d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control old rats, [b] p<0.05, [c] p<0.001 when compared with diabetic old rats, [d] p<0.001 when compared with D+IIH old rats.
IIH - Insulin Induced Hypoglycaemia.

Figure-46

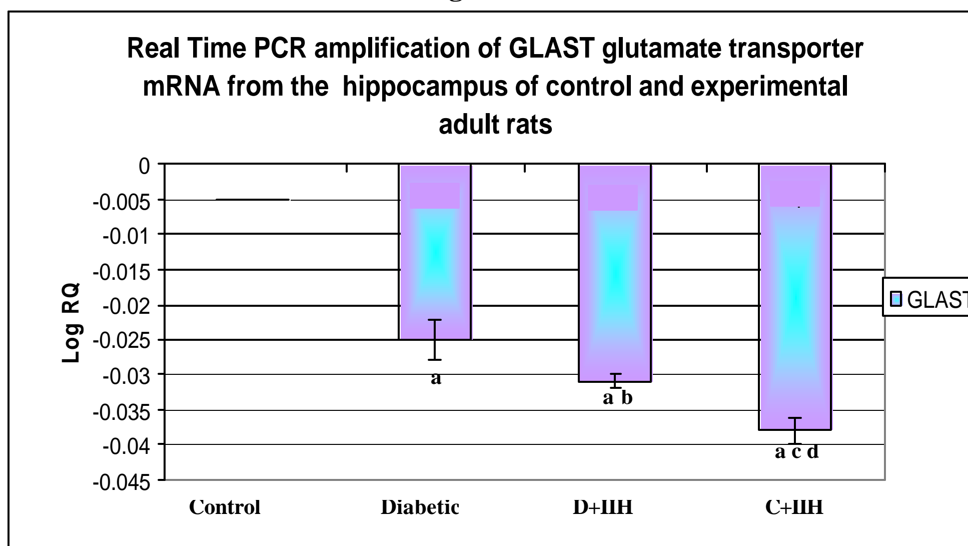


Table-44

Real Time PCR amplification of GLAST glutamate transporter mRNA from the hippocampus of control and experimental adult rats

Animal Status	GLAST Log RQ
Control	0
Diabetic	-0.025 ± 0.003 [a]
D + IIH	-0.031 ± 0.001 [a b]
C + IIH	-0.038 ± 0.002 [a c d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.01$, [c] $p<0.001$ when compared with diabetic adult rats, [d] $p<0.01$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-47

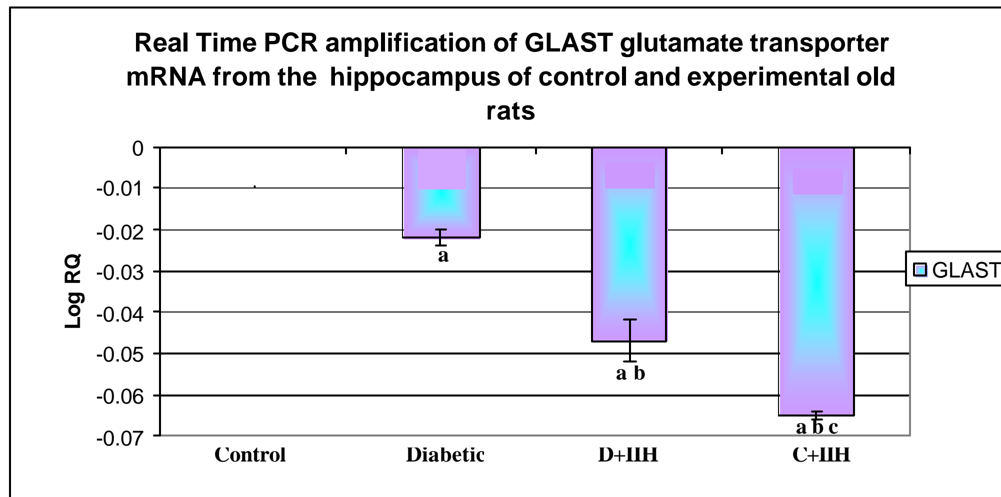


Table-45

Real Time PCR amplification of GLAST glutamate transporter mRNA from the hippocampus of control and experimental old rats

Animal Status	GLAST Log RQ
Control	0
Diabetic	-0.022 ± 0.002 [a]
D + IIH	-0.047 ± 0.005 [a b]
C + IIH	-0.065 ± 0.001 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats, [c] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-48

Scatchard analysis of [^{3}H]Glutamate binding against glutamate in the pancreas of control and experimental adult rats

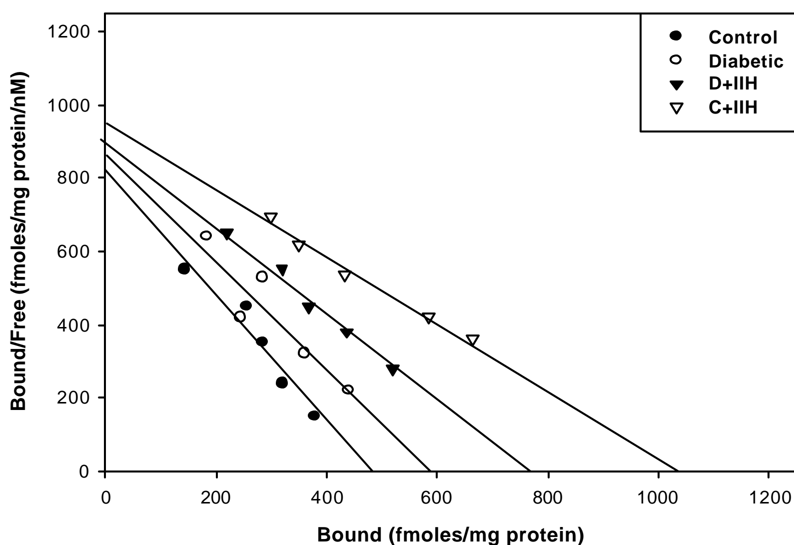


Table-46

Scatchard analysis of [^{3}H]Glutamate binding against glutamate in the pancreas of control and experimental adult rats

Animal status	Bmax (fmoles/mg protein)	Kd (nM)
Control	426.3 ± 5.5	0.5 ± 1.1
Diabetic	574.6 ± 9.0 ^{a}	0.7 ± 0.2
D+IIH	763.7 ± 7.5 $^{a\,b}$	0.9 ± 0.2
C+IIH	1018.0 ± 6.2 $^{a\,b\,c}$	1.1 ± 0.1

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. ^{a} $p<0.001$ when compared with control adult rats, ^{b} $p<0.001$ when compared with diabetic adult rats, ^{c} $p<0.001$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia

Figure-49

Scatchard analysis of [^{3}H]Glutamate binding against glutamate in the pancreas of control and experimental old rats

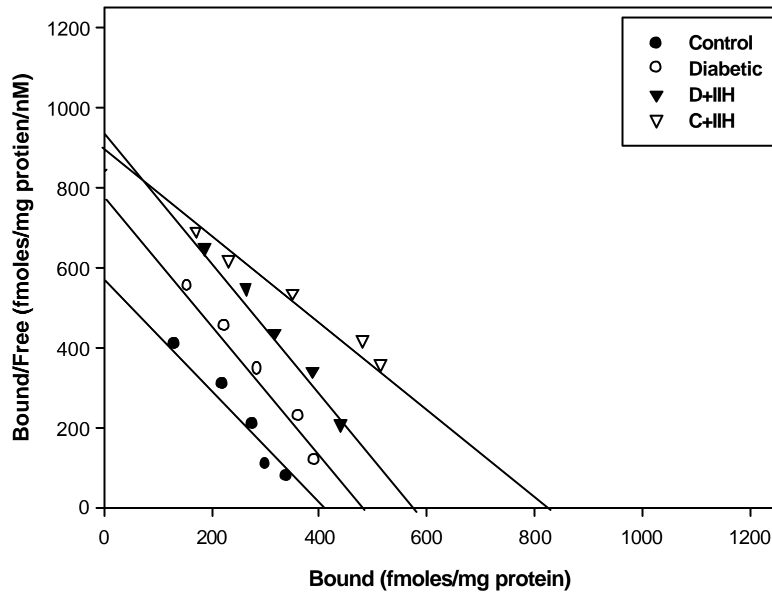


Table-47

Scatchard analysis of [^{3}H]Glutamate binding against glutamate in the pancreas of control and experimental old rats

Animal status	Bmax (fmoles/mg protein)	Kd (nM)
Control	408.3 ± 9.2	0.7 ± 0.2
Diabetic	514.6 ± 9.0 [a]	0.7 ± 0.2
D+IIH	584.7 ± 8.6 [a b]	0.6 ± 0.2
C+IIH	804.0 ± 7.1 [a b c]	0.9 ± 0.1

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats, [c] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-50

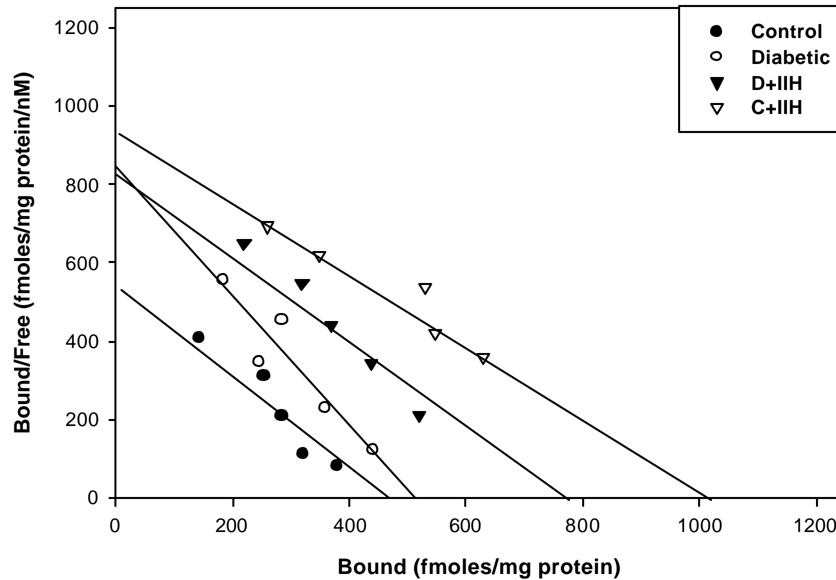


Table-48

Scatchard analysis of NMDA receptors using [^{3}H]MK801 binding against MK801 in the pancreas of control and experimental adult rats

Animal status	Bmax (fmoles/mg protein)	Kd (nM)
Control	477.0 ± 15.7	0.8 ± 0.2
Diabetic	524.7 ± 11.4 [a]	0.7 ± 0.2
D+IIH	740.3 ± 4.0 [a b]	0.9 ± 0.2
C+IIH	1005.8 ± 5.0 [a b c]	1.1 ± 0.1

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats, [c] p<0.001 when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-51

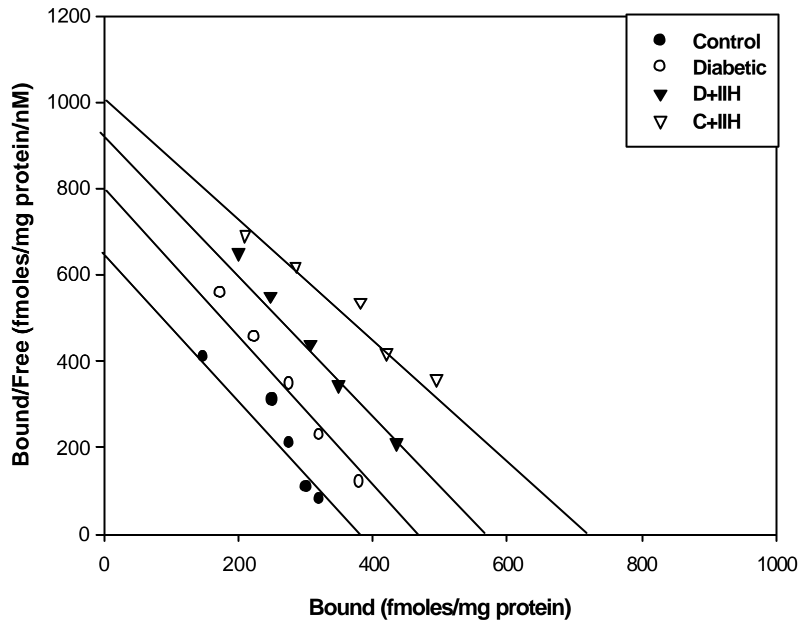


Table-49

Scatchard analysis of NMDA receptors using [^{3}H]MK801 binding against MK801 in the pancreas of control and experimental old rats

Animal status	Bmax (fmoles/mg protein)	Kd (nM)
Control	385.5 ± 13.2	0.6 ± 0.1
Diabetic	493.3 ± 15.6 [a]	0.6 ± 0.1
D+IIH	576.0 ± 6.0 [a b]	0.6 ± 0.1
C+IIH	741.2 ± 9.0 [a b c]	0.7 ± 0.1

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats, [c] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure-52

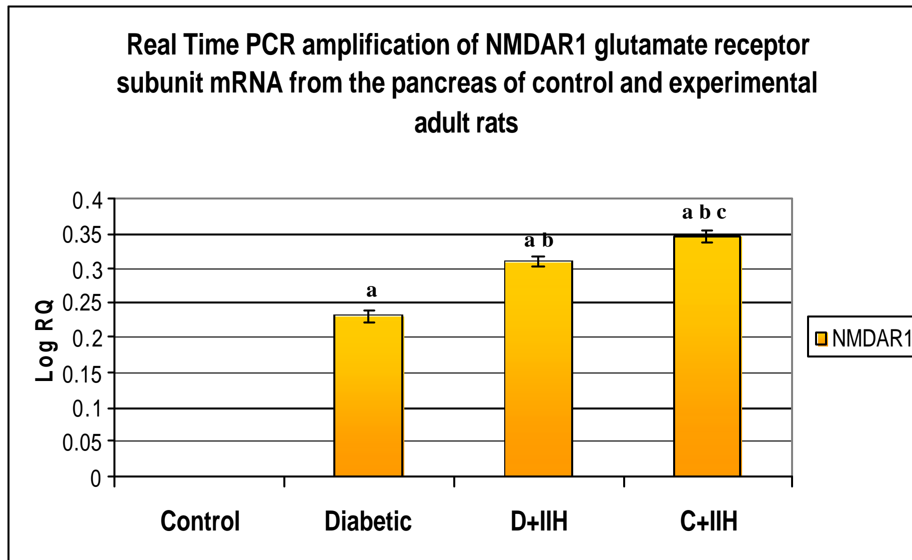


Table-50

Real Time PCR amplification of NMDAR1 glutamate receptor subunit mRNA from the pancreas of control and experimental adult rats

Animal Status	NMDAR1 Log RQ
Control	0
Diabetic	0.23 ± 0.01^{a}
D + IIH	$0.31 \pm 0.01^{a\,b}$
C + IIH	$0.35 \pm 0.01^{a\,b\,c}$

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats, [c] p<0.001 when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-53

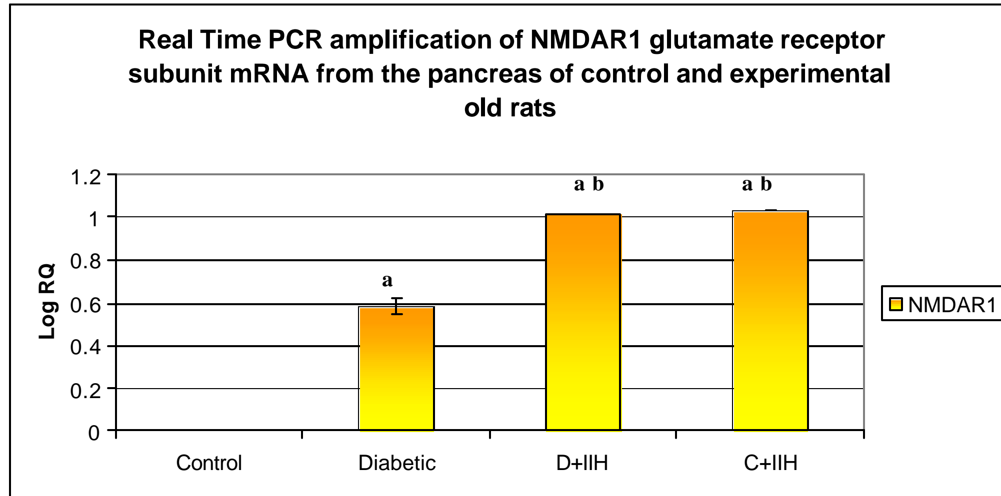


Table-51

Real Time PCR amplification of NMDAR1 glutamate receptor subunit mRNA from the pancreas of control and experimental old rats

Animal Status	NMDAR1 Log RQ
Control	0
Diabetic	0.58 ± 0.04 [a]
D + IIH	1.02 ± 0.00 [a b]
C + IIH	1.03 ± 0.00 [a b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-54

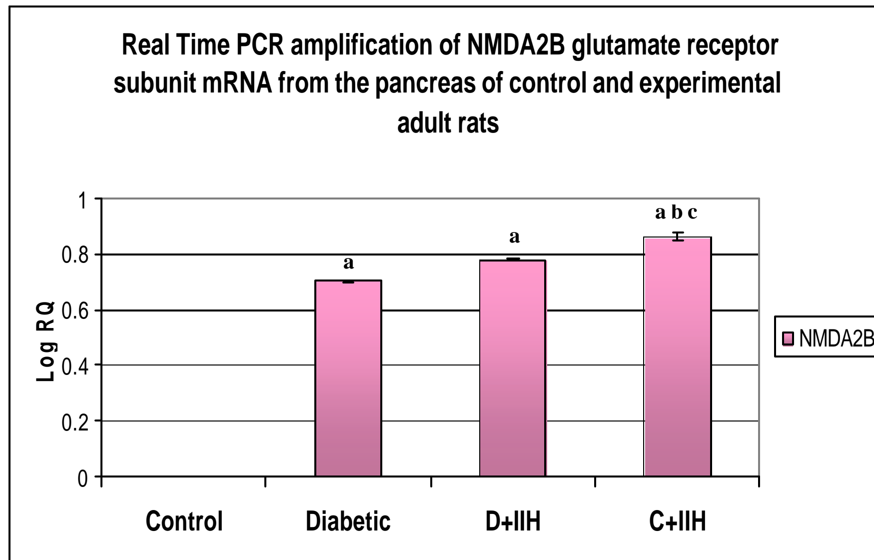


Table-52

Real Time PCR amplification of NMDA2B glutamate receptor subunit mRNA from the pancreas of control and experimental adult rats

Animal Status	NMDA2B Log RQ
Control	0
Diabetic	0.70 ± 0.01^{a}
D + IIH	0.78 ± 0.01^{a}
C + IIH	$0.86 \pm 0.02^{a\,b\,c}$

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [b] p<0.001 when compared with diabetic adult rats, [c] p<0.001 when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure -55

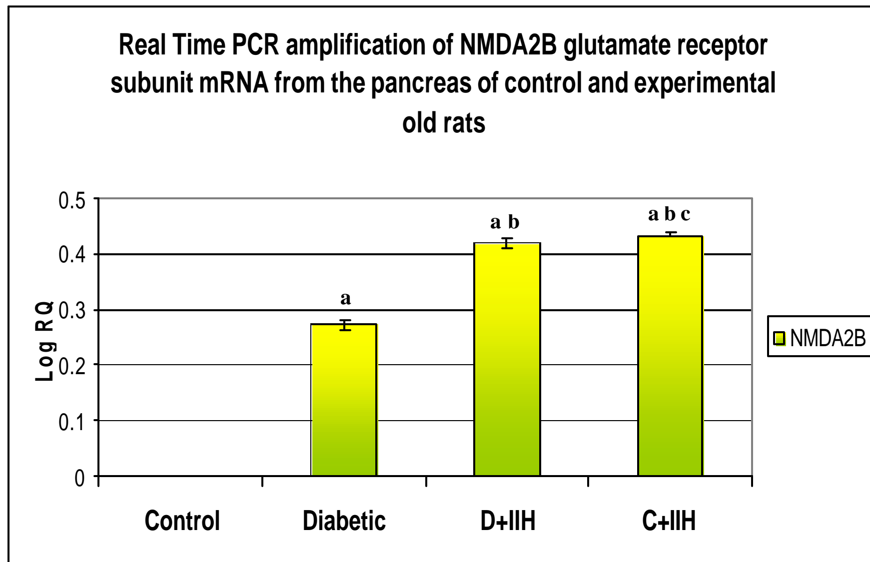


Table-53

Real Time PCR amplification of NMDA2B glutamate receptor subunit mRNA from the pancreas of control and experimental old rats

Animal Status	NMDA2B Log RQ
Control	0
Diabetic	0.27 ± 0.01^{a}
D + IIH	$0.42 \pm 0.01^{a\,b}$
C + IIH	$0.43 \pm 0.01^{a\,b\,c}$

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. ^{a} p<0.001 when compared with control old rats, ^{b} p<0.001 when compared with diabetic old rats, ^{c} p<0.05 when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-56

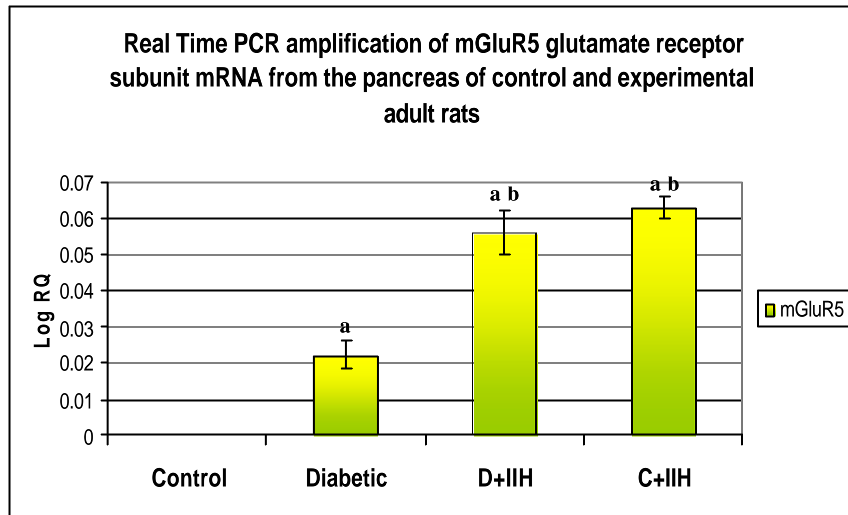


Table-54

Real Time PCR amplification of mGluR5 glutamate receptor subunit mRNA from the pancreas of control and experimental adult rats

Animal Status	mGluR5 Log RQ
Control	0
Diabetic	0.02 ± 0.01 [a]
D + IIH	0.06 ± 0.01 [a b]
C + IIH	0.06 ± 0.01 [a b]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.001$ when compared with diabetic adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-57

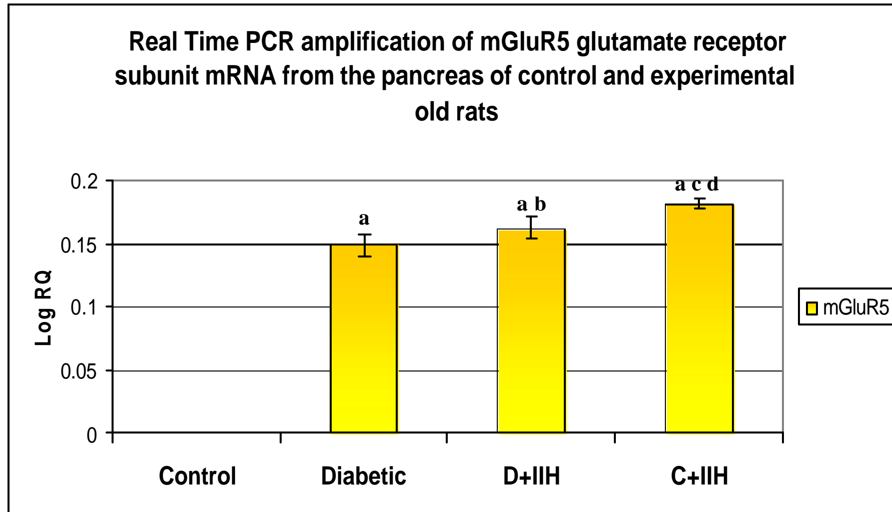


Table-55

Real Time PCR amplification of mGluR5 glutamate receptor subunit mRNA from the pancreas of control and experimental old rats

Animal Status	mGluR5 Log RQ
Control	0
Diabetic	0.149 ± 0.009^{a}
D + IIH	$0.162 \pm 0.009^{a\ b}$
C + IIH	$0.182 \pm 0.004^{a\ c\ d}$

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control old rats, [b] p<0.05, [c] p<0.001 when compared with diabetic old rats, [d] p<0.01 when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-58

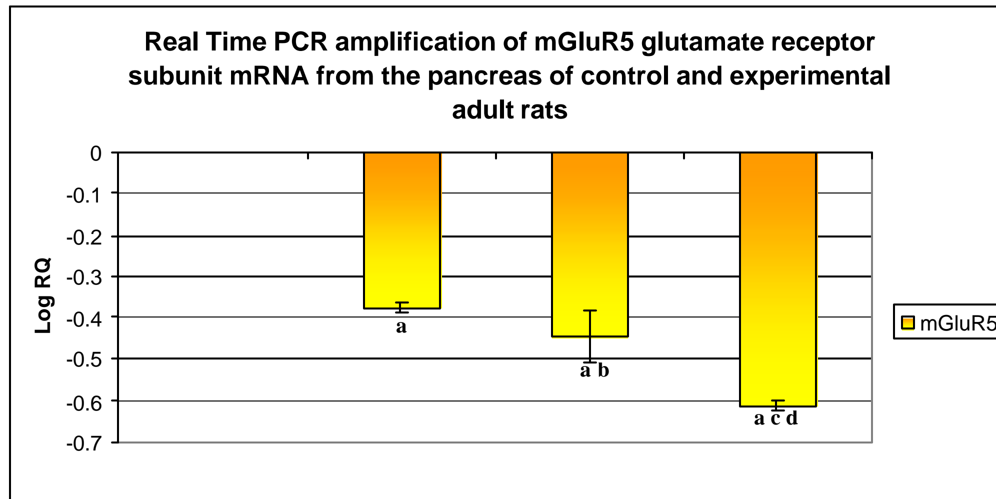


Table-56

Real Time PCR amplification of GLAST glutamate transporter mRNA from the pancreas of control and experimental adult rats

Animal Status	GLAST Log RQ
Control	0
Diabetic	-0.375 ± 0.013 [a]
D + IIH	-0.447 ± 0.064 [a b]
C + IIH	-0.613 ± 0.006 [a c d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.05$, [c] $p<0.001$ when compared with diabetic adult rats, [d] $p<0.001$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-59

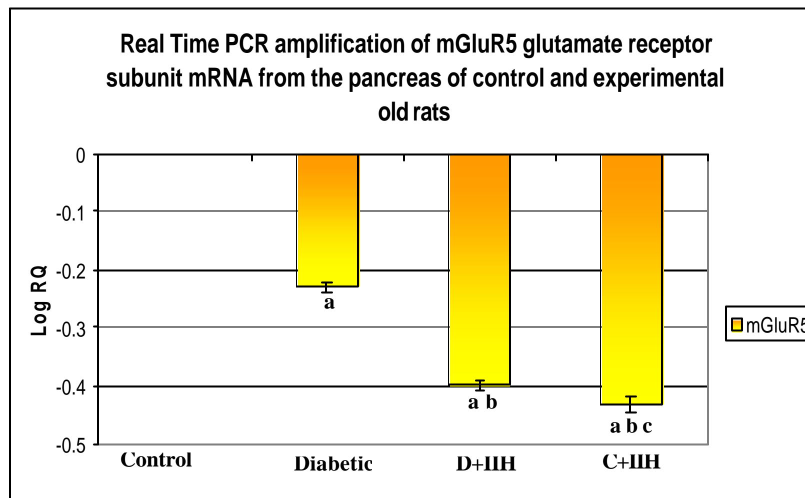


Table-57

Real Time PCR amplification of GLAST glutamate transporter mRNA from the pancreas of control and experimental old rats

Animal Status	GLAST Log RQ
Control	0
Diabetic	-0.229 ± 0.007 [a]
D + IIH	-0.398 ± 0.008 [a b]
C + IIH	-0.432 ± 0.013 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats, [c] $p<0.01$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-60

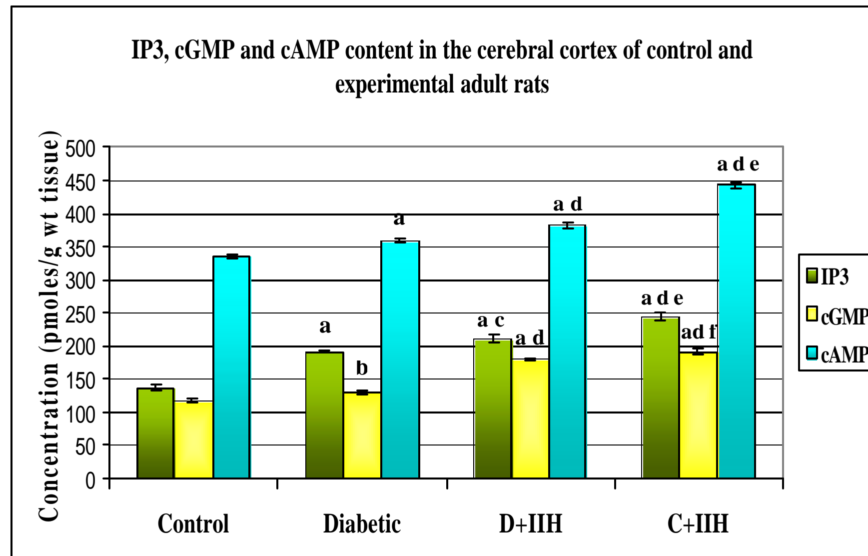


Table-58
IP3, cGMP and cAMP content in the cerebral cortex of control and experimental adult rats

Animal Status	Concentration (pmoles/g wt tissue)		
	IP3	**cGMP**	**cAMP**
Control	136.7 ? 4.7	116.1 ? 2.0	335.7 ? 3.6
Diabetic	191.0 ? 2.1[a]	130.3 ? 3.1[b]	359.1 ± 3.5[a]
D+IIH	210.9 ? 5.0[a c]	179.2 ? 2.7[a d]	382.5 ? 4.6[a d]
C+IIH	244.2 ? 4.6[a d e]	191.6 ? 6.0[a d f]	443.2 ± 4.5[a de]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [b] $p<0.01$, [a] $p<0.001$ when compared with control adult rats, [c] $p<0.05$ [d] $p<0.001$ when compared with diabetic adult rats. [f] $p<0.01$, [e] $p<0.001$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure -61

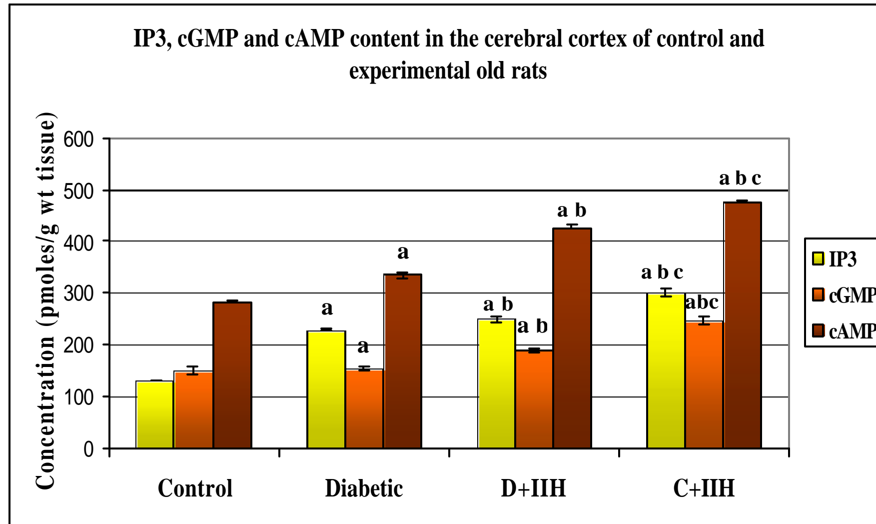


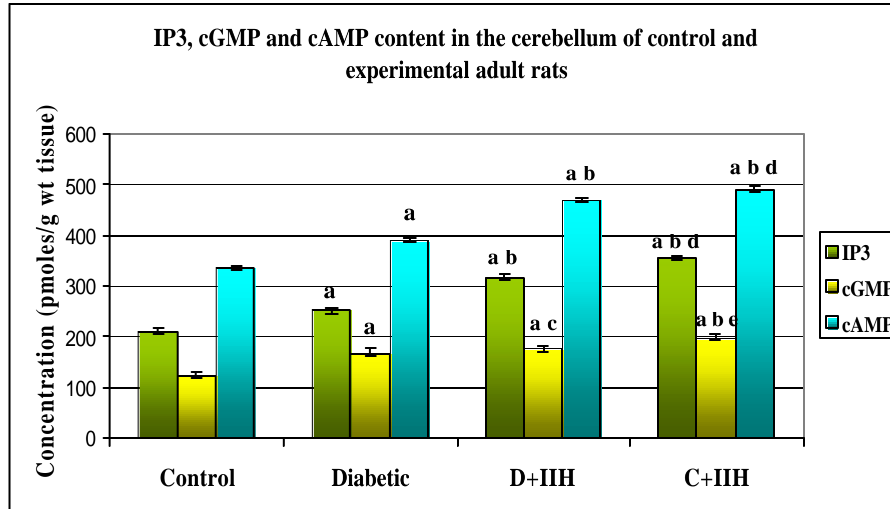
Table -59

IP3, cGMP and cAMP content in the cerebral cortex of control and experimental old rats

Animal Status	Concentration (pmoles/g wt tissue)		
	IP3	cGMP	cAMP
Control	129.5 ? 1.0	150.0 ? 7.5	282.9 ? 3.6
Diabetic	228.2 ? 2.6[a]	154.4 ? 4.2[a]	335.7 ± 5.1[a]
D+IIH	249.7 ? 4.7[a b]	189.3 ? 3.2[a b]	428.1 ? 4.7[a b]
C+IIH	301.3 ? 8.0[a b c]	245.8 ? 6.7[a b c]	477.4 ± 3.9[a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control old rats, [b] p<0.001 when compared with diabetic old rats. [c] p<0.001 when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure -62



Tables-60

IP3, cGMP and cAMP content in the cerebellum of control and experimental adult rats

Animal Status	Concentration (pmoles/g wt tissue)		
	IP3	cGMP	cAMP
Control	210.7 ? 5.8	124.3 ? 5.5	335.8 ? 4.0
Diabetic	252.4 ? 4.6[a]	167.7 ? 8.0[a]	389.4 ± 3.4[a]
D+IIH	318.2 ? 3.6[a b]	175.3 ? 4.0[a c]	470.1 ? 4.5[a b]
C+IIH	356.8 ? 3.4[a b d]	197.8 ? 5.1[a b e]	491.3 ± 4.4[a b d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] p<0.001 when compared with control adult rats, [c] p<0.05, [b] p<0.001 when compared with diabetic adult rats, [e] p<0.01, [d] p<0.001 when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-63

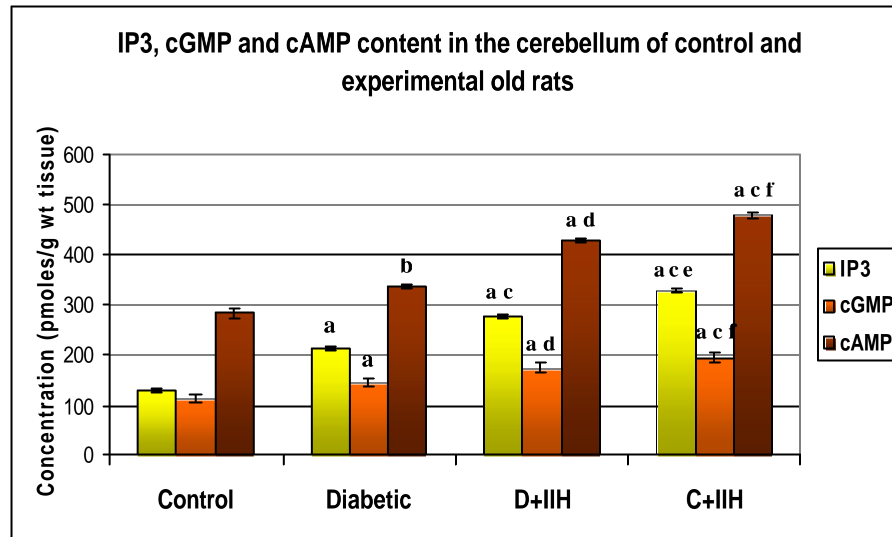


Table-61

IP3, cGMP and cAMP content in the cerebellum of control and experimental old rats

Animal Status	Concentration (pmoles/g wt tissue)		
	IP3	cGMP	cAMP
Control	131.0 ? 3.1	112.7 ? 6.3	260.6 ? 9.6
Diabetic	212.4 ? 6.4[a]	145.4 ? 7.4[a]	298.6 ± 6.3[b]
D+IIH	277.7 ? 3.6[a c]	173.0 ? 9.2[a d]	318.9 ? 5.0[a d]
C+IIH	327.7 ? 5.1[a c e]	194.7 ? 8.8[a c f]	332.3 ± 5.5[a c f]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [b] $p<0.01$, [a] $p<0.001$ when compared with control old rats, [d] $p<0.01$, [c] $p<0.001$ when compared with diabetic old rats, [f] $p<0.05$, [e] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia

Figure -64

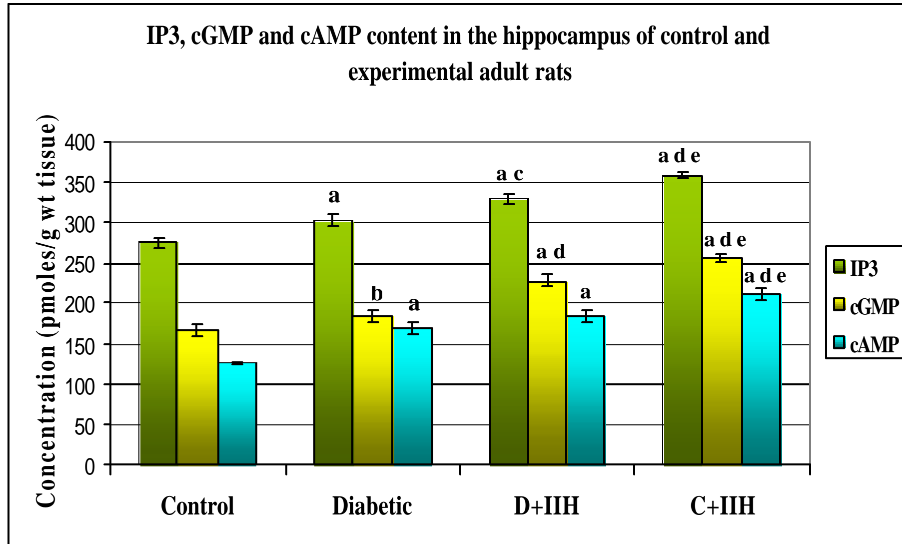


Table -62

IP3, cGMP and cAMP content in the hippocampus of control and experimental adult rats

Animal Status	Concentration (pmoles/g wt tissue)		
	IP3	**cGMP**	**cAMP**
Control	274.6 ? 5.5	167.2 ? 6.3	125.6 ? 1.5
Diabetic	303.0 ? 6.8 [a]	183.8 ? 7.2 [b]	168.8 ± 6.8 [a]
D+IIH	328.4 ? 7.0 [a c]	227.3 ? 7.6 [a d]	183.5 ? 8.3 [a]
C+IIH	358.0 ? 4.5 [a d e]	254.7 ? 4.2 [a d e]	210.3 ± 7.6 [a d e]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [b] $p<0.05$, [a] $p<0.001$ when compared with control adult rats, [c] $p<0.01$, [d] $p<0.001$ when compared with diabetic adult rats. [e] $p<0.001$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-65

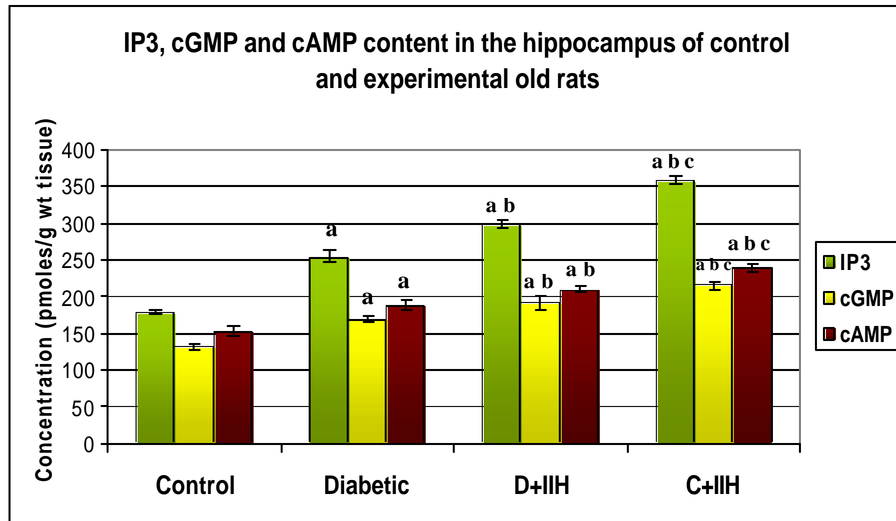


Table-63
IP3, cGMP and cAMP content in the hippocampus of control and experimental old rats

Animal Status	Concentration (pmoles/g wt tissue)		
	IP3	cGMP	cAMP
Control	178.2 ? 3.8	130.4 ? 4.4	152.4 ? 7.2
Diabetic	253.7 ? 8.2[a]	168.7 ? 4.2[a]	186.8 ± 6.5[a]
D+IIH	297.4 ? 5.6[a b]	192.3 ? 9.5[a b]	209.2 ? 3.2[a b]
C+IIH	357.6 ? 4.2[a b c]	214.2 ? 6.0[a b c]	238.8 ± 5.1[a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats. [c] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-66

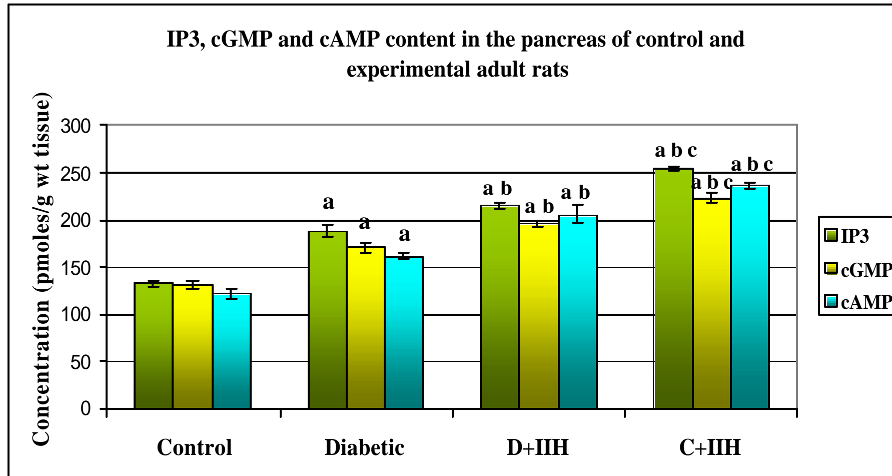


Table-64

IP3, cGMP and cAMP content in the pancreas of control and experimental adult rats

Animal Status	Concentration (pmoles/g wt tissue)		
	IP3	cGMP	cAMP
Control	132.7 ? 3.1	130.9 ? 4.7	121.7 ? 4.6
Diabetic	188.3 ? 6.2[a]	171.2 ? 5.7[a]	161.7 ± 3.5[a]
D+IIH	214. 9 ? 3.6[a b]	196.0 ? 2.1[a b]	205.1 ? 9.1[a b]
C+IIH	254.4 ? 3.0[a b c]	222.9 ? 5.0[a b c]	236.4 ± 3.8[a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.001$ when compared with diabetic adult rats, [c] $p<0.001$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure -67

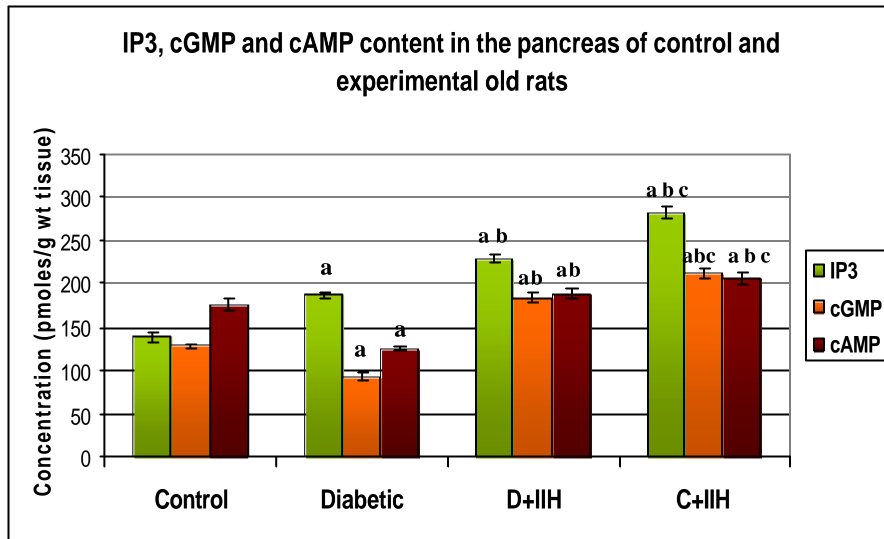


Table-65

IP3, cGMP and cAMP content in the pancreas of control and experimental old rats

Animal Status	Concentration (pmoles/g wt tissue)		
	IP3	cGMP	cAMP
Control	138.4 ? 5.5	128.2 ? 3.0	176.6 ? 6.1
Diabetic	187.4 ? 3.7 [a]	92.5 ? 5.0 [a]	124.4 ± 2.1 [a]
D+IIH	229.5 ? 3.8 [a b]	184.6 ? 6.7 [a b]	188.2 ? 6.4 [a b]
C+IIH	282.4 ? 6.5 [a b c]	212.1 ? 5.9 [a b c]	206.2 ± 7.0 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control old rats, [b] $p<0.001$ when compared with diabetic old rats. [c] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-68

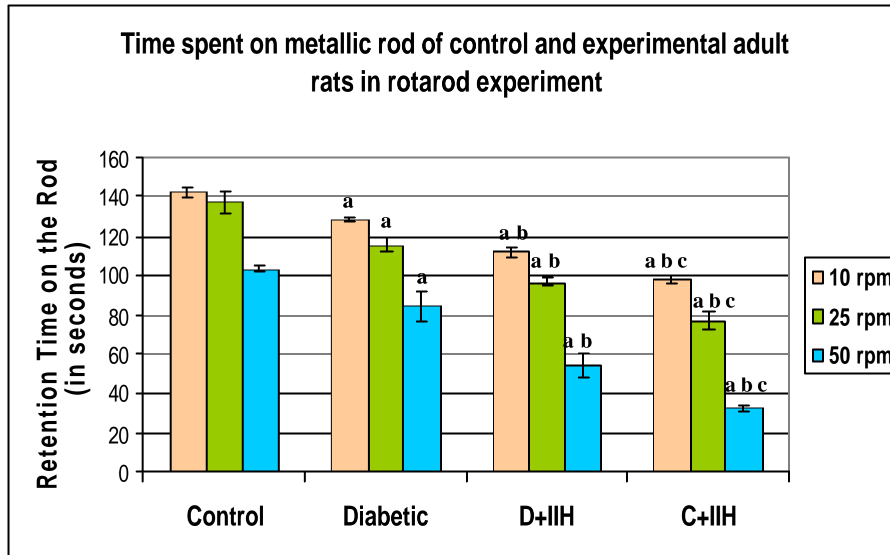


Table-66
Time spent on metallic rod of control and experimental adult rats in Rotarod experiment

Animal Status	Retention Time on the Rod (in seconds)		
	10 rpm	**25 rpm**	**50 rpm**
Control	142.0 ± 2.8	137.3 ± 5.1	103.3 ± 1.2
Diabetic	128.3 ± 1.1 [a]	115.3 ± 3.2 [a]	84.3 ± 7.4 [a]
D+IIH	112.0 ± 2.4 [a b]	96.7 ± 2.4 [a b]	54.0 ± 6.1 [a b]
C+IIH	98.0 ± 2.0 [a b c]	76.7 ± 4.6 [a b c]	32.3 ± 1.6 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control adult rats, [b] $p<0.001$ when compared with diabetic adult rats, [c] $p<0.001$ when compared with D+IIH adult rats. IIH - Insulin Induced Hypoglycaemia.

Figure-69

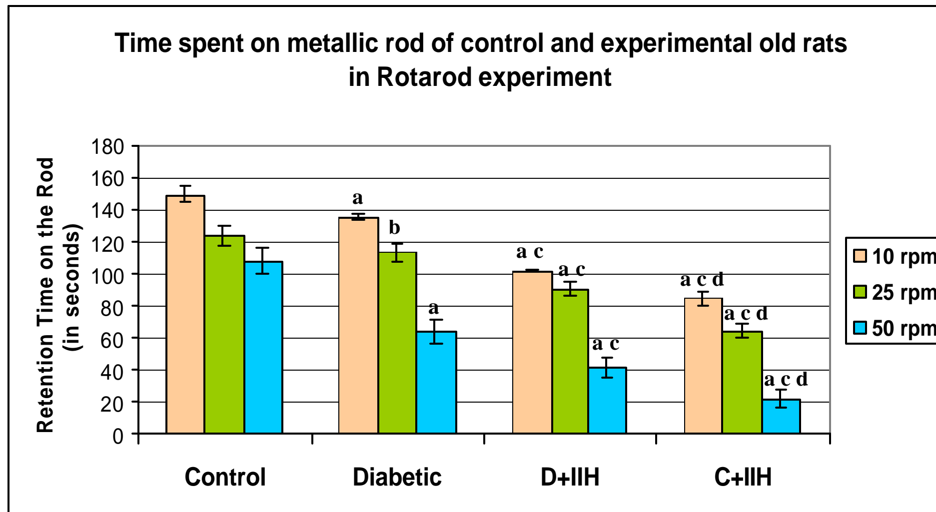


Table-67

Time spent on metallic rod of control and experimental old rats in Rotarod experiment

Animal Status	Retention Time on the Rod (in seconds)		
	10 rpm	**25 rpm**	**50 rpm**
Control	150.0 ± 4.56	124.5 ± 6.3	108.5 ± 8.3
Diabetic	135.7 ± 2.5[a]	113.4 ± 5.4[b]	64.6 ± 7.5[a]
D+IIH	102.0 ± 1.8[a c]	90.7 ± 4.4[a c]	42.4 ± 6.2[a c]
C+IIH	85.0 ± 5.0[a c d]	64.5 ± 4.9[a c d]	22.2 ± 5.6[a c d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [b] $p<0.01$, [a] $p<0.001$ when compared with control old rats, [c] $p<0.001$ when compared with diabetic old rats, [d] $p<0.001$ when compared with D+IIH old rats. IIH - Insulin Induced Hypoglycaemia.

Figure-71
NMDA2B Receptor Expression in the Cerebral Cortex of Control and Experimental Rats

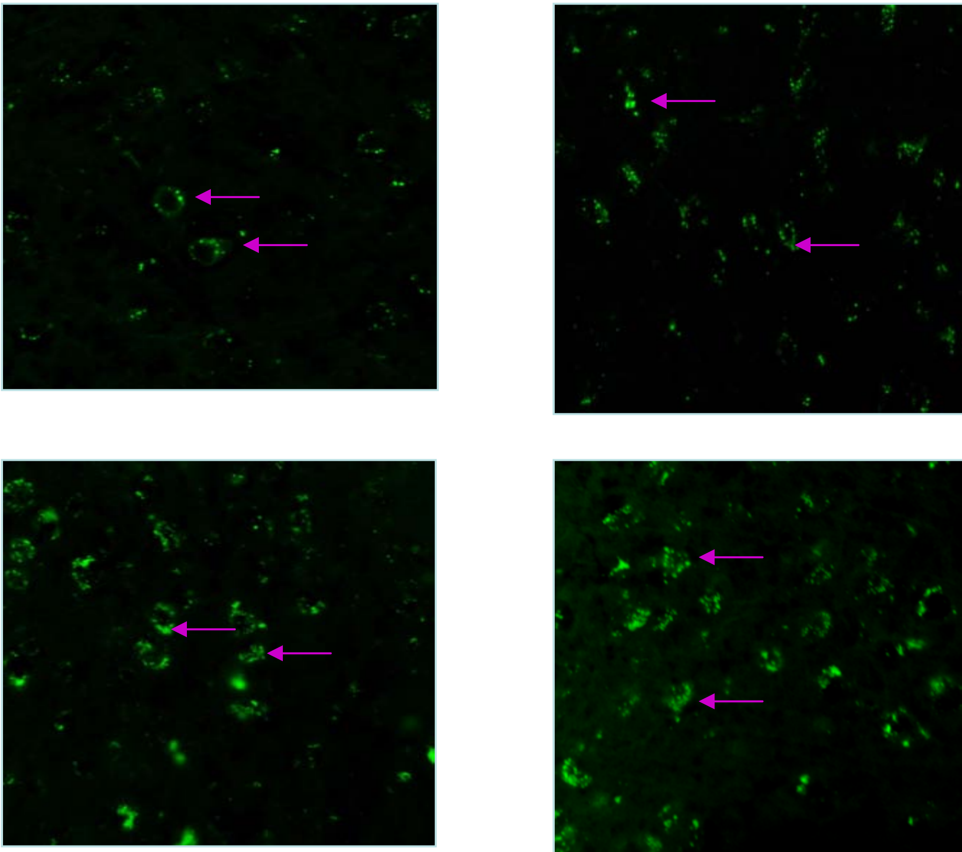


Figure-72
mGluR5 Receptor Expression in the Cerebral Cortex of Control and Experimental Rats

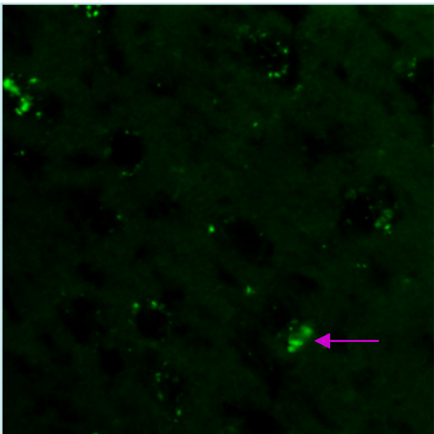
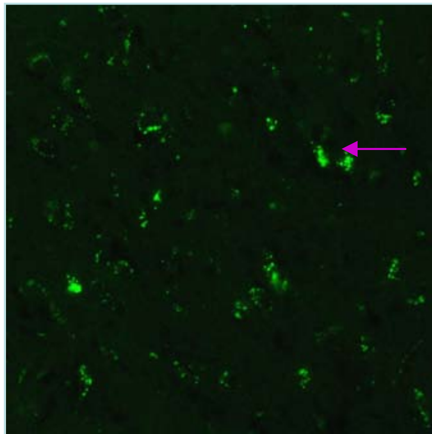
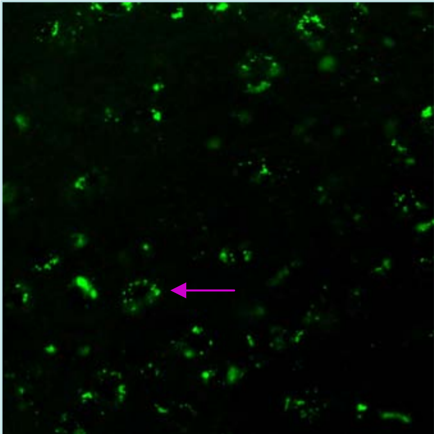
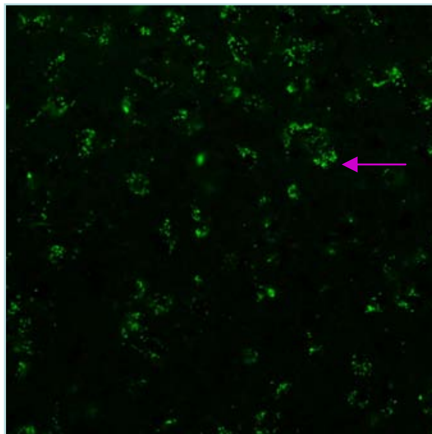





Figure-73
IP3 Receptor Expression in the Cerebral Cortex of Control and Experimental Rats

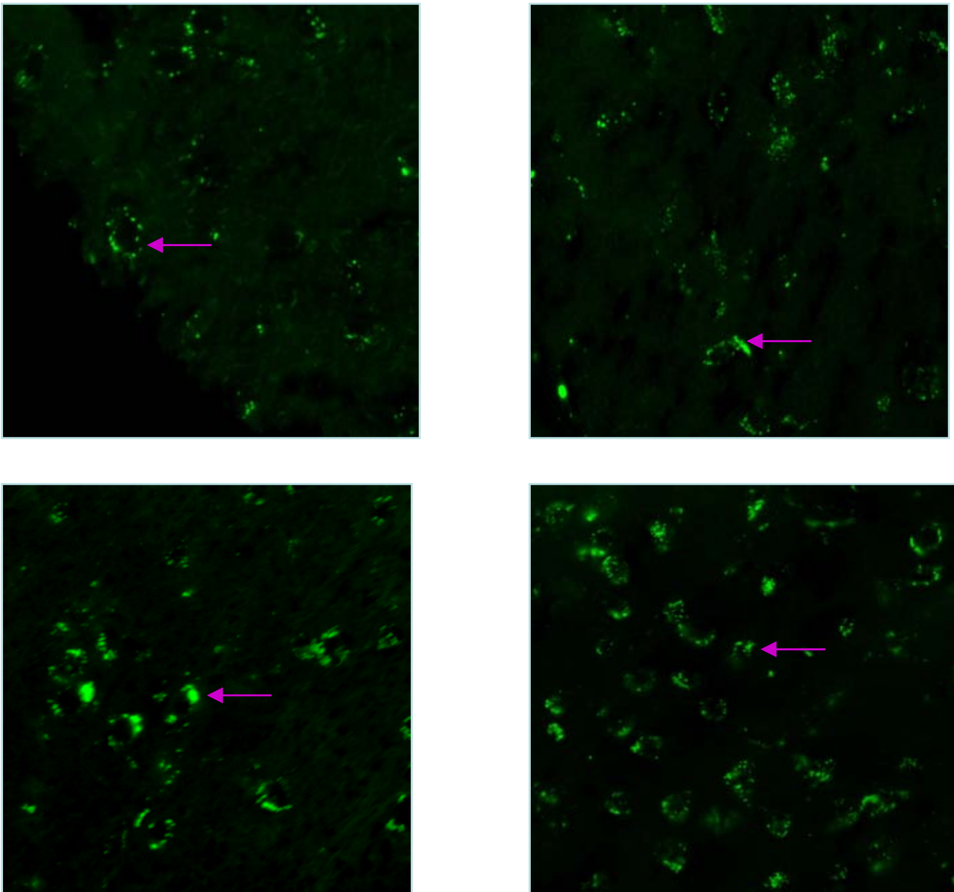


Table-68

Glutamate Receptor Subtype and IP3 Receptor Expression in the Cerebral Cortex of Control and Experimental Rats

Condition	Pixel Intensity			
	NMDAR1	NMDA2B	mGluR5	IP3
Control	174367 ± 100	198675 ± 57	163276 ± 139	189537 ± 116
Diabetic	202790 ± 110 [a]	218376 ± 43 [a]	197832 ± 150 [a]	202413 ± 127 [a]
D+IIH	228761 ± 109 [a b]	267243 ± 60 [a b]	224362 ± 163 [a b]	237645 ± 132 [a b]
C+IIH	263298 ± 116 [a b c]	299684 ± 54 [a b c]	288769 ± 162 [a b c]	279732 ± 102 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control rats, [b] $p<0.001$ when compared with diabetic rats, [c] $p<0.001$ when compared with D+IIH rats. IIH - Insulin Induced Hypoglycaemia.

Figure-74
NMDAR1 Receptor Expression in the Cerebellum of Control and Experimental Rats

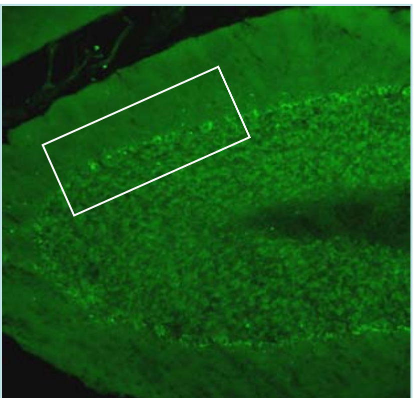
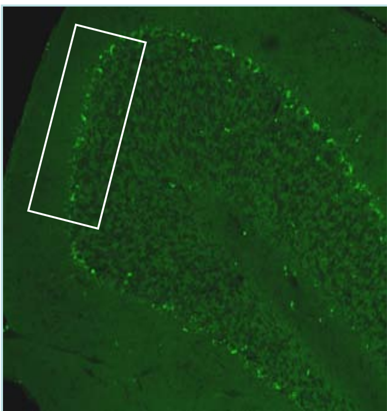
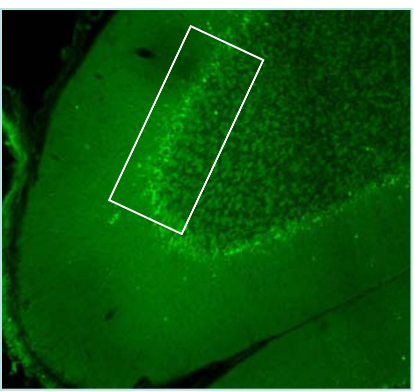
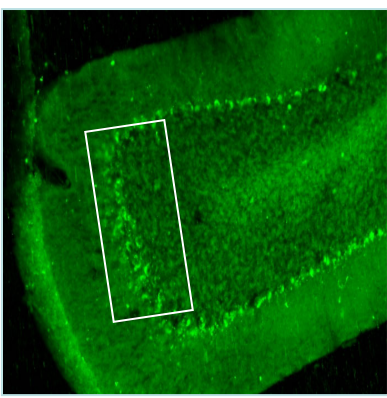





Figure-75
NMDAR2B Receptor Expression in the Cerebellum of Control and Experimental Rats

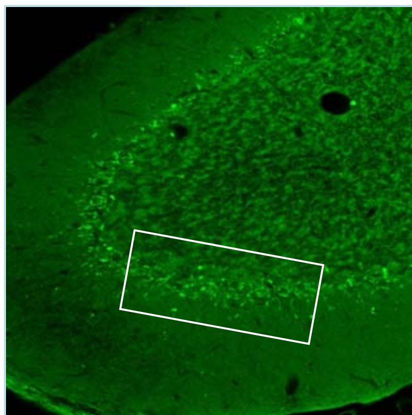
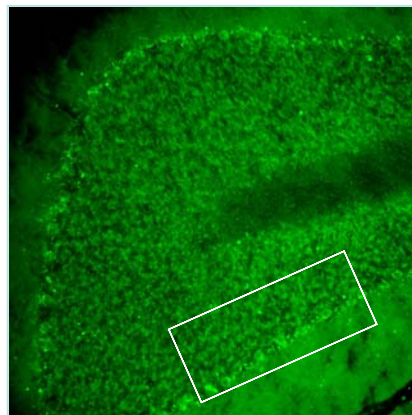
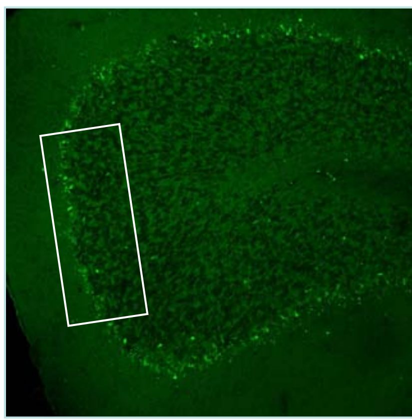
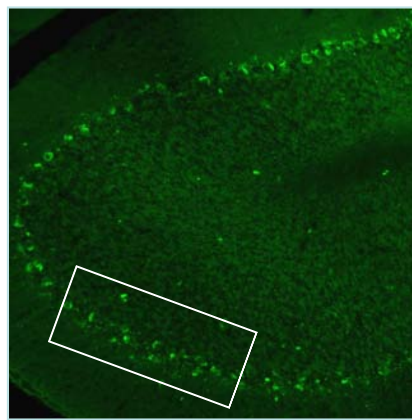
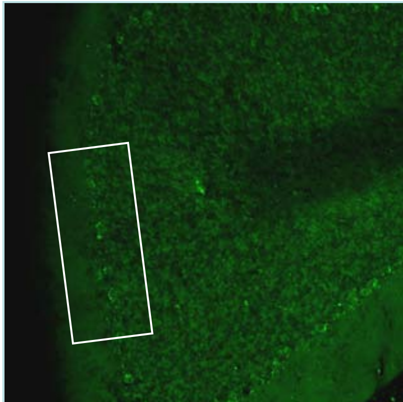
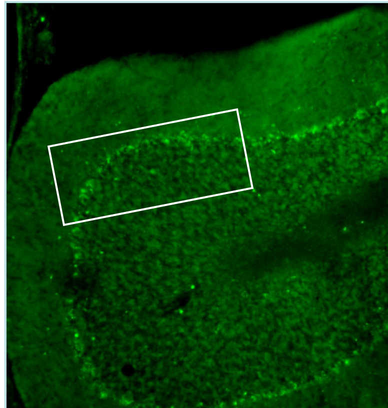
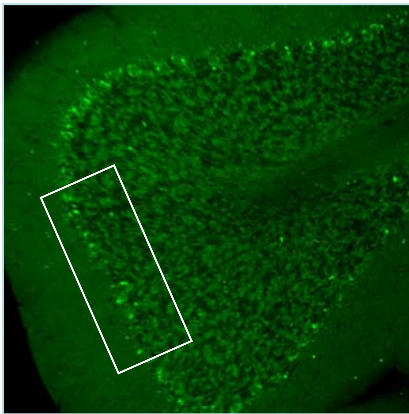





Figure-76
mGluR5 Receptor Expression in the Cerebellum of Control and Experimental Rats







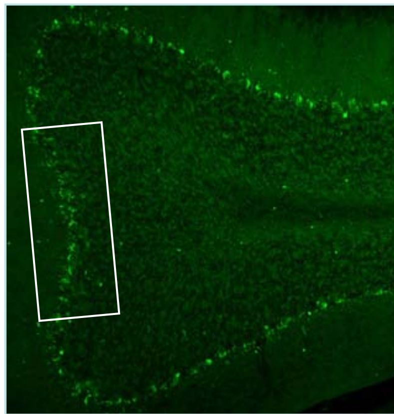


Figure-77
IP3 Receptor Expression in the Cerebellum of Control and Experimental Rats

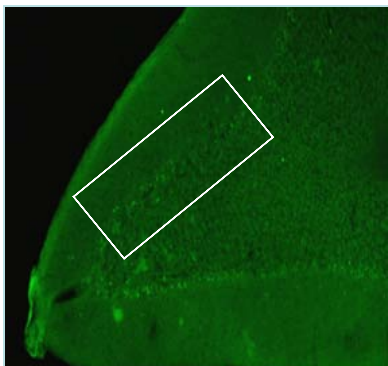
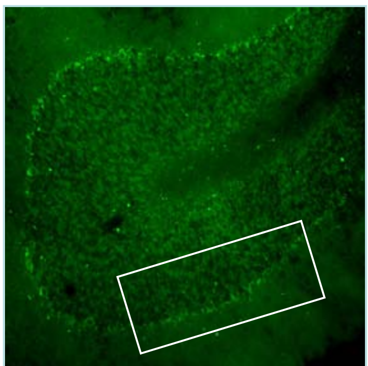
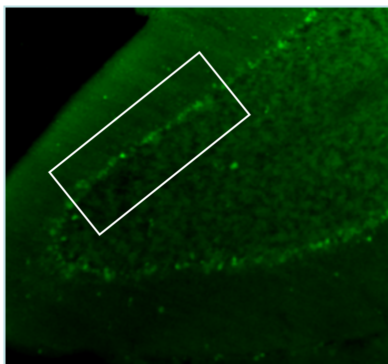
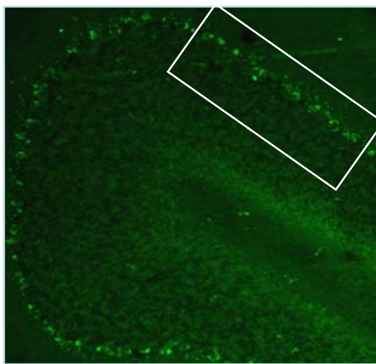





Table-69

Glutamate Receptor Subtype and IP3 Receptor Expression in the Cerebellum of Control and Experimental Rats

Condition	Pixel Intensity			
	NMDAR1	**NMDA2B**	**mGluR5**	**IP3**
Control	236379 ± 90	244453 ± 185	182536 ± 137	190621 ± 59
Diabetic	282156 ± 110 [a]	267836 ± 173 [a]	213569 ± 153 [a]	213982 ± 42 [a]
D+IIH	318754 ± 85 [a b]	288983 ± 156 [a b]	289845 ± 159 [a b]	267843 ± 56 [a b]
C+IIH	348973 ± 116 [a b c]	319987 ± 194 [a b c]	314752 ± 124 [a b c]	286470 ± 53 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control rats, [b] $p<0.001$ when compared with diabetic rats, [c] $p<0.001$ when compared with D+IIH rats. IIH - Insulin Induced Hypoglycaemia.

Figure-78
NMDAR1 Receptor Expression in the Pancreatic Islets of Control and Experimental Rats

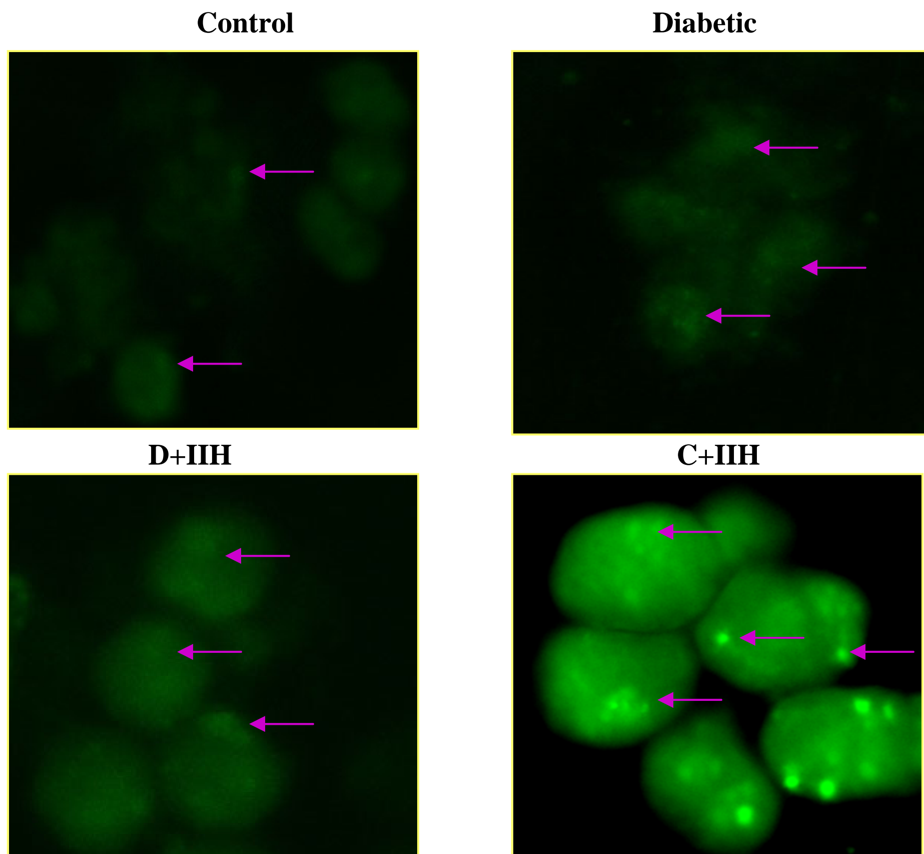


Figure-79
NMDA2B Receptor Expression in the Pancreatic Islets of Control and Experimental Rats

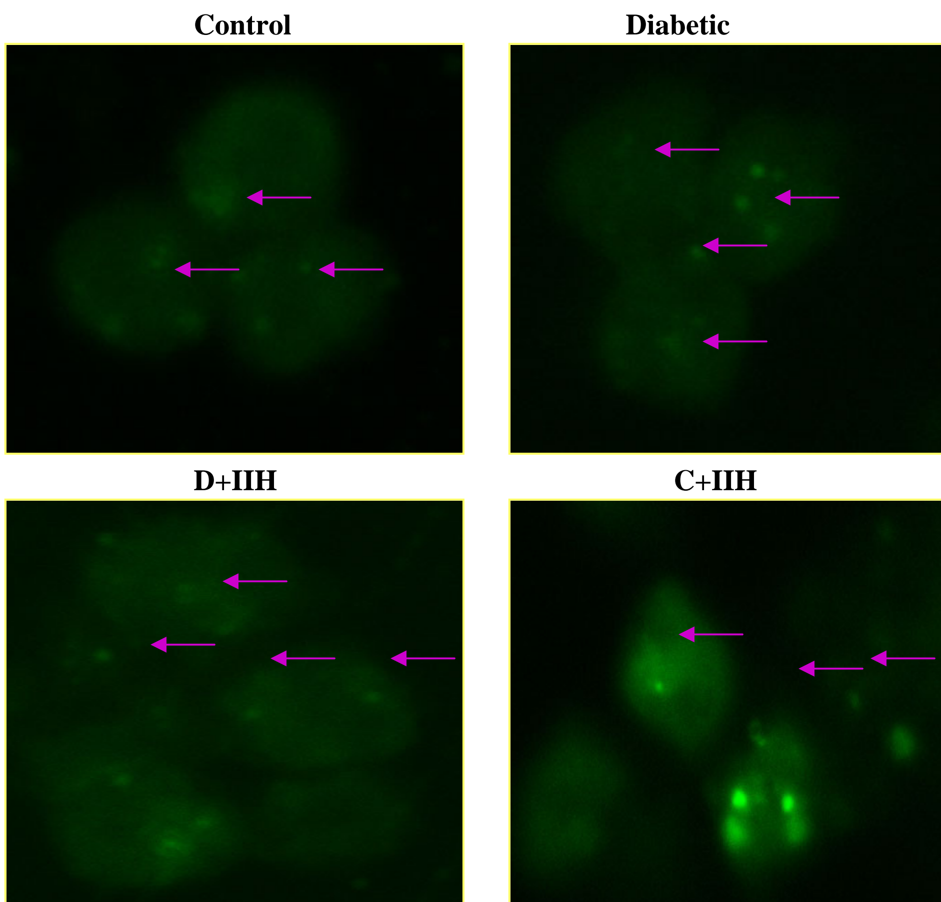


Figure-80
mGluR5 Receptor Expression in the Pancreatic Islets of Control and Experimental Rats

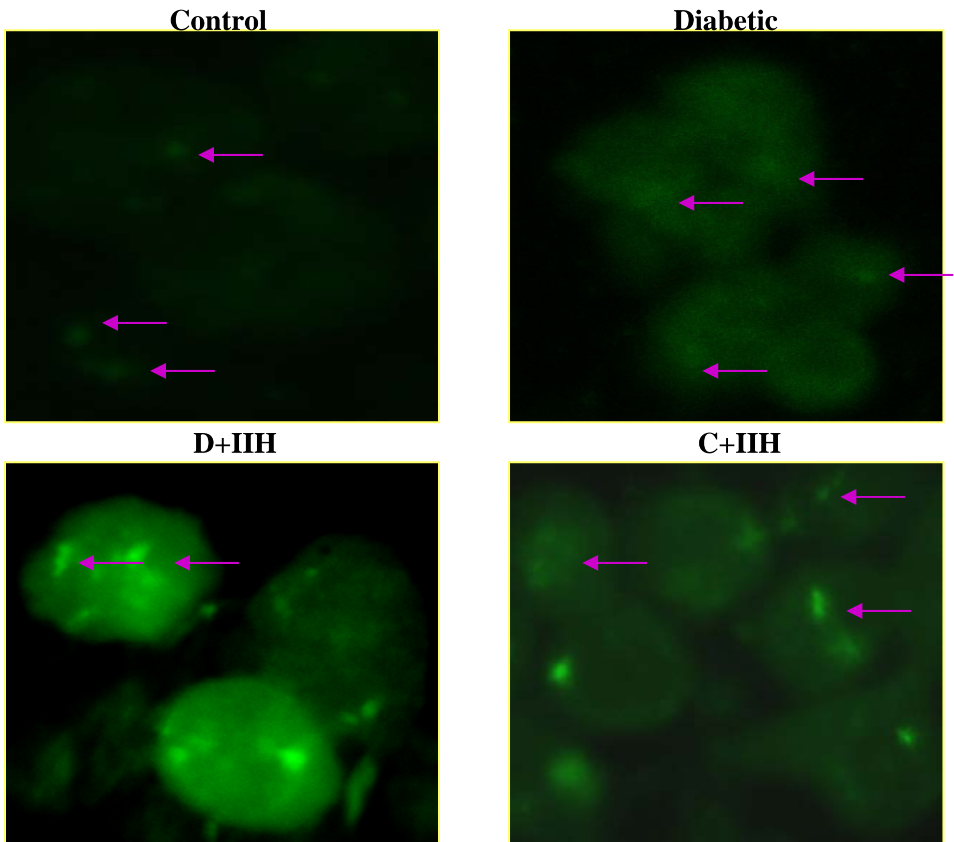


Figure-81
IP3 Receptor Expression in the Pancreatic Islets of Control and Experimental Rats

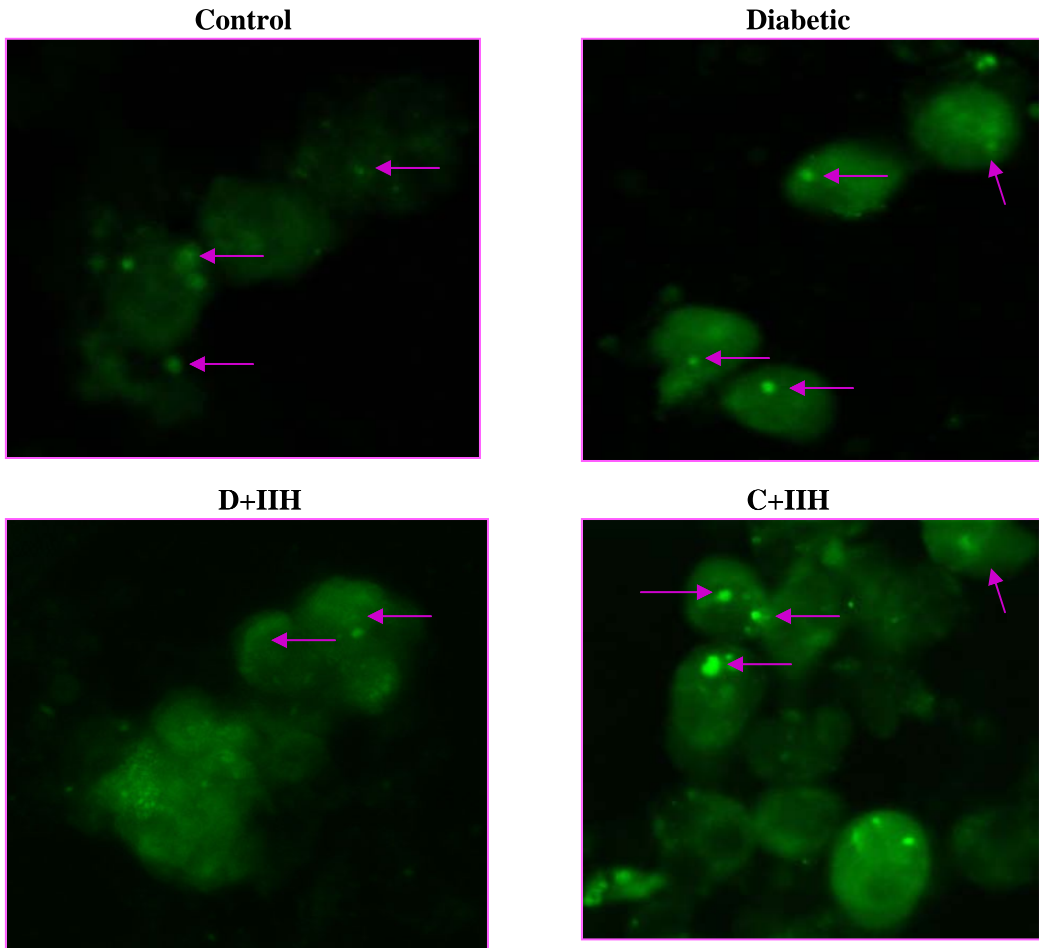


Table-70

Glutamate Receptor Subtype and IP3 Receptor Expression in the Pancreatic Islets of Control and Experimental Rats

Condition	Pixel Intensity			
	NMDAR1	**NMDA2B**	**mGluR5**	**IP3**
Control	219876 ± 103	223476 ± 128	192453 ± 157	208743 ± 98
Diabetic	263489 ± 116 [a]	247869 ± 129 [a]	219874 ± 153 [a]	235764 ± 121 [a]
D+IIH	315372 ± 122 [a b]	298546 ± 146 [a b]	276359 ± 168 [a b]	287646 ± 118 [a b]
C+IIH	353213 ± 110 [a b c]	318423 ± 132 [a b c]	300459 ± 141 [a b c]	312754 ± 135 [a b c]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consists of 6-8 rats. [a] $p<0.001$ when compared with control rats, [b] $p<0.001$ when compared with diabetic rats, [c] $p<0.001$ when compared with D+IIH rats. IIH - Insulin Induced Hypoglycaemia.

Figure-82
Calcium Release from Pancreatic Islets in the presence of 1mM Glucose, Dopamine (DA) (10^{-5}M), DA + Sulpiride (SUL) (10^{-5}M).

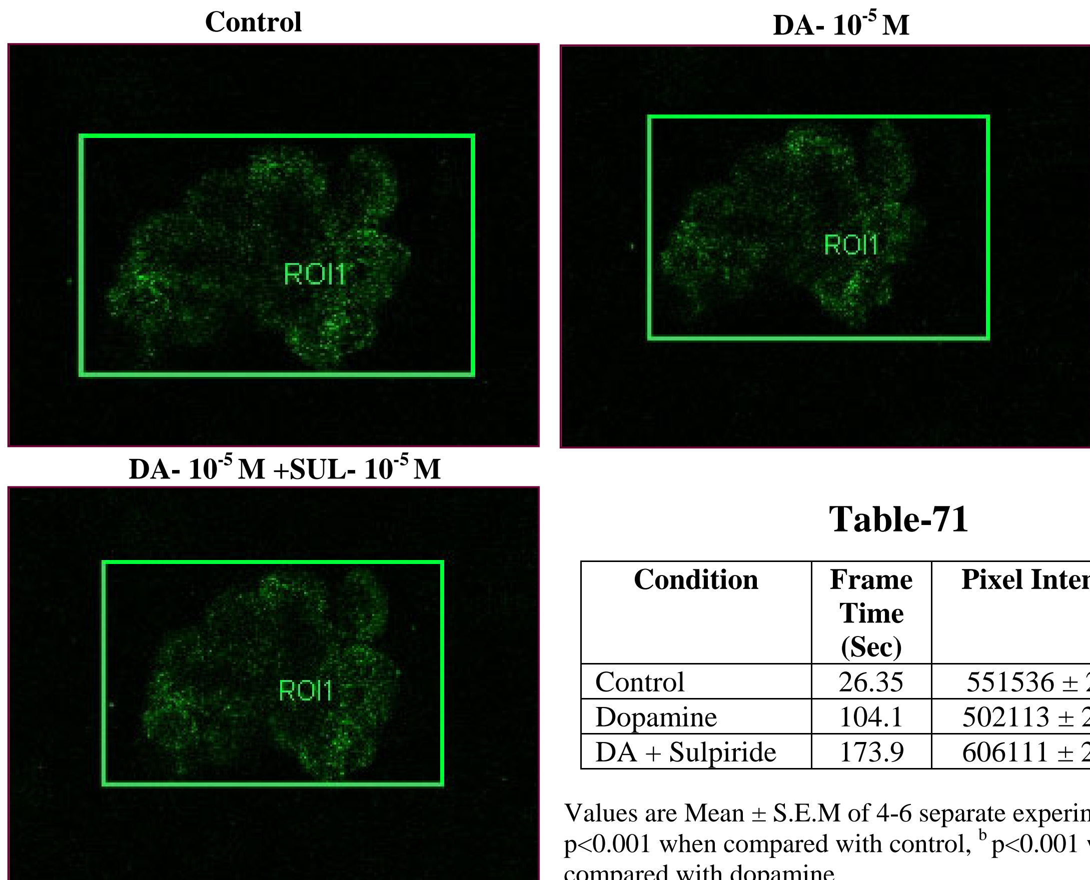


Table-71

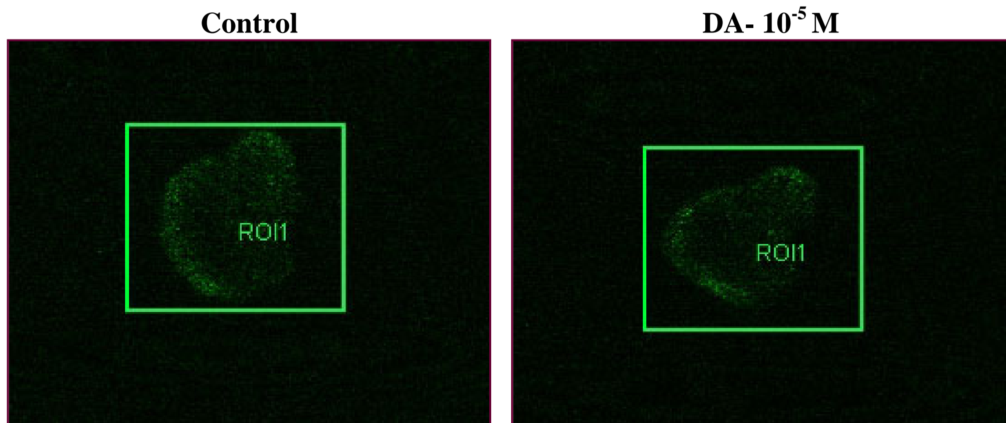
Condition	Frame Time (Sec)	Pixel Intensity
Control	26.35	551536 ± 22.9
Dopamine	104.1	502113 ± 20.9[a]
DA + Sulpiride	173.9	606111 ± 22.2[b]

Values are Mean ± S.E.M of 4-6 separate experiments. [a] $p<0.001$ when compared with control, [b] $p<0.001$ when compared with dopamine.

Figure-83

Calcium Release from Pancreatic Islets in the presence of 4mM Glucose, Dopamine (DA) 10^{-5}M, DA + Sulpiride (SUL) 10^{-5}M

Control **DA- 10^{-5} M**



DA- 10^{-5} M +SUL- 10^{-5} M

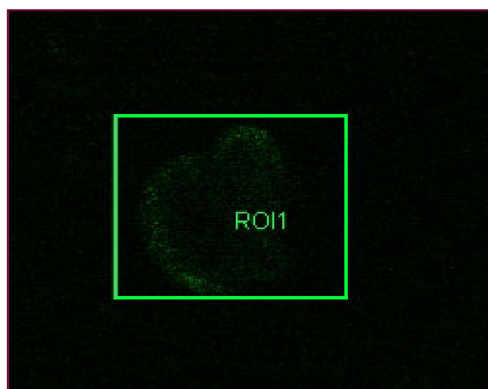


Table-72

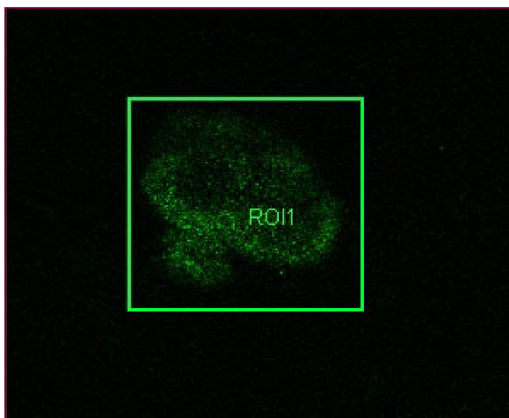
Condition	Frame Time (Sec)	Pixel Intensity
Control	26.35	231472 ± 16.3
Dopamine	104.1	285190 ± 14.6[a]
DA + Sulpiride	173.9	208027 ± 10.8[b]

Values are Mean ± S.E.M of 4-6 separate experiments. [a] $p<0.001$ when compared with control, [b] $p<0.001$ when compared with dopamine

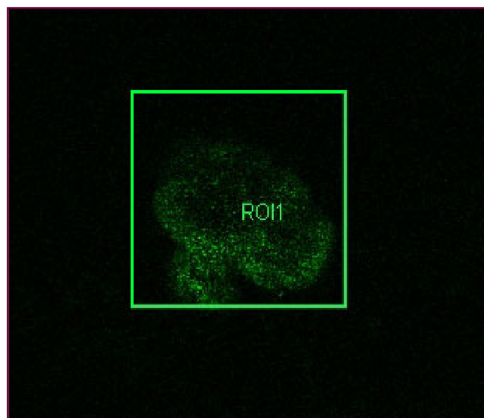
Figure-84

Calcium Release from Pancreatic Islets in the presence of 20mM Glucose, Dopamine (DA) 10^{-5}M, DA + Sulpiride (SUL) 10^{-5}M

Control



DA- 10^{-5} M



DA- 10^{-5} M +SUL- 10^{-5} M

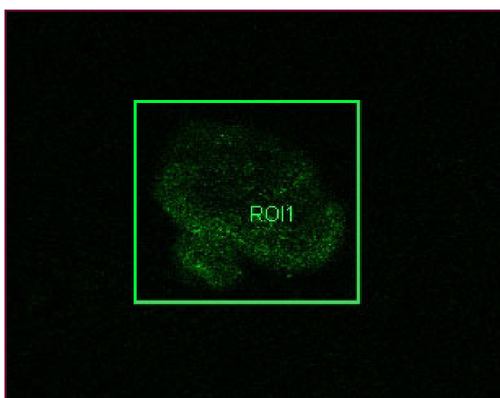


Table-73

Condition	Frame Time (Sec)	Pixel Intensity
Control	26.35	197450 ± 25.4
Dopamine	104.1	222595 ± 22.1^{a}
DA + Sulpiride	173.9	207844 ± 24.6^{b}

Values are Mean ± S.E.M of 4-6 separate experiments. [a] $p<0.001$ when compared with control, [b] $p<0.001$ when compared with dopamine

Figure-85

Calcium Release from Pancreatic Islets in the presence of 1mM Glucose, Glutamate (Glut) 10^{-5}M, Glut + MK 801 (10^{-5}M)

Control

ROI1

Glut- 10^{-5} M

ROI1

Glut- 10^{-5} M +MK801- 10^{-5} M

ROI1

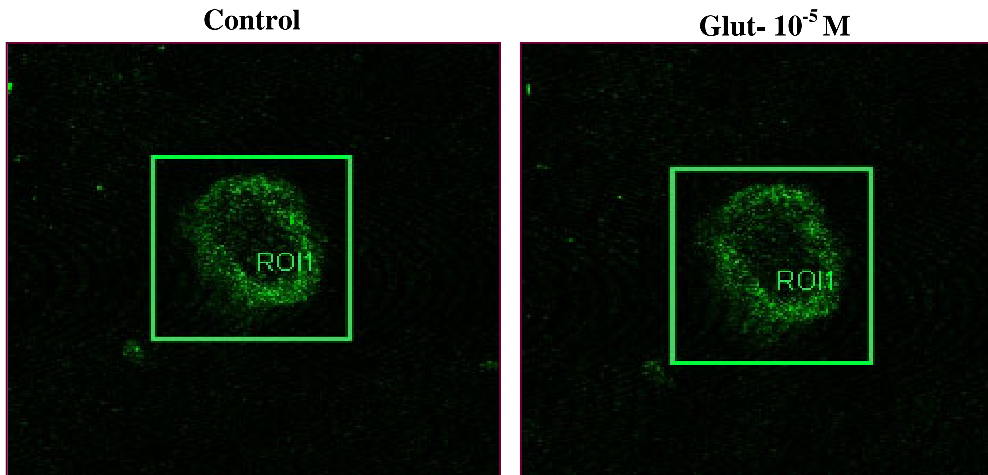
Table-74

Condition	Frame Time (Sec)	Pixel Intensity
Control	26.35	957666 ± 59.1
Glutamate	104.1	1116368 ± 59.8[a]
Glut + MK801	173.9	934772 ± 44.3[b]

Values are Mean ± S.E.M of 4-6 separate experiments. [a] $p<0.001$ when compared with control, [b] $p<0.001$ when compared with glutamate

Figure-86
Calcium Release from Pancreatic Islets in the presence of 4mM Glucose, Glutamate (Glut) 10^{-5}M, Glut + MK 801 10^{-5}M

Control **Glut- 10^{-5} M**



Glut- 10^{-5} M +MK801- 10^{-5} M

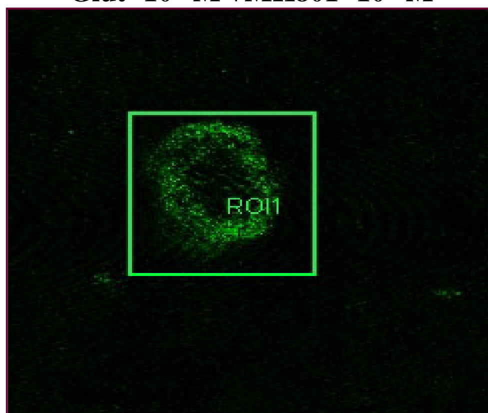


Table-75

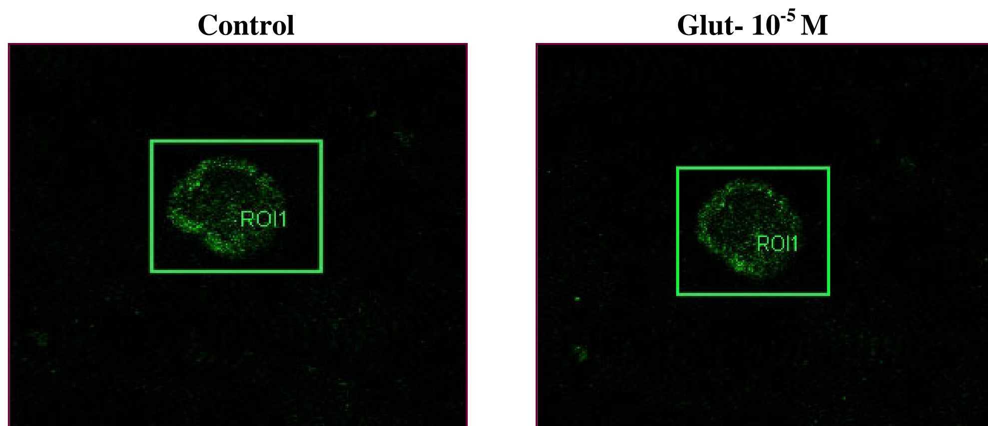
Condition	Frame Time (Sec)	Pixel Intensity
Control	26.35	328035 ± 34.4
Glutamate	104.1	397100 ± 33.1[a]
Glut + MK801	173.9	285142 ± 33.8[b]

Values are Mean ± S.E.M of 4-6 separate experiments. [a] $p<0.001$ when compared with control, [b] $p<0.001$ when compared with glutamate

Figure-87

Calcium Release from Pancreatic Islets in the presence of 20mM Glucose, Glutamate (Glut) 10^{-5}M, Glut + MK 801 (10^{-5}M)

Control **Glut- 10^{-5} M**



Glut- 10^{-5} M +MK801- 10^{-5} M

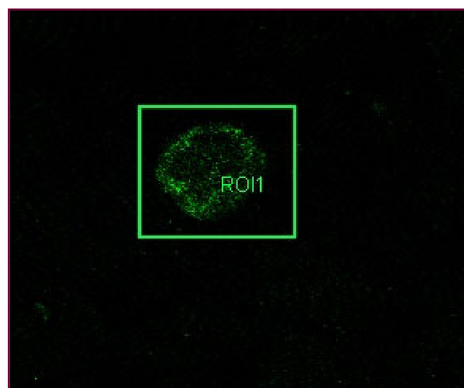
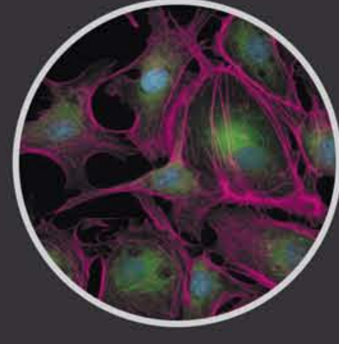


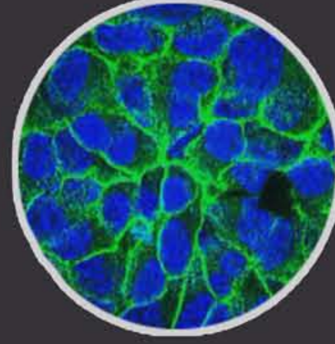
Table-76

Condition	Frame Time (Sec)	Pixel Intensity
Control	26.35	517450 ± 28.7
Glutamate	104.1	583899 ± 25.9[a]
Glut + MK801	173.9	437945 ± 26.2[b]

Values are Mean ± S.E.M of 4-6 separate experiments. [a] $p<0.001$ when compared with control, [b] $p<0.001$ when compared with glutamate

Glutamate Receptor Gene Expression: IP3, cAMP, cGMP Functional Regulation in Hypoglycaemic and Diabetic Rats.





Ph.D. Thesis

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