CHAPTER 1

INTRODUCTION
Derangement in cerebral functioning is a consistent finding in man in conditions of impaired liver function. Wide spectrum of neurological and neuropsychiatric alterations seen in conditions of hepatic inadequacy are termed as Hepatic *Encephalopathy* (HE). Initial stages of HE can be associated with personality and emotional disturbances. As encephalopathy progresses, hyperventilation, confusion, drowsiness, hyperthermia and coma are exhibited. In later stages of encephalopathy, decerebrate rigidity, decortical posture, convulsions and finally death may occur. If proper medical care is not provided in time, such patients may become comatose and terminally ill (Schenker *et al.*, 1974). Mental retardation was also observed in certain mild cases and in patients who survived (Conn and Lieberthal, 1979; Cooper and Plum, 1987). There are also reports of structural changes in both neurons and glial cells of both patients and laboratory animals with HE (Baraldi, 1990).

In **man**, HE occurs in two major forms: an acute (fulminant) type and a chronic type. Fulminant hepatic failure (FHF) is a clinical syndrome resulting from severe inflammatory and/or necrotic liver disease with rapid onset. The neurological disorder progresses from altered mental status to coma, generally within hours or days. Death frequently results from increased intracranial pressure caused by massive brain oedema (Rakela, 1983; Blei *et al.*, 1992). Chief reason for acute hepatic necrosis was reported to be viral infections (Hepatitis; Saunders *et al.*, 1972). Infections related to viral hepatitis type B accounts for 74% of cases under this category. Contribution of **non-A**, non-B type of viral hepatitis accounts for 24% while type A accounts for 2% (Papas-Venegeleu *et al.*, 1984). Drug induced hepatic
necrosis is the next major cause of FHF. Most frequently used drug, acetaminophen (paracetamol) was found to be the major culprit and the next is halothane (Barnard et al., 1981). FHF may also result due to the extensive accumulation of fat in the liver as in Reye's syndrome, in pregnancy, toxicity due to drugs such as tetracycline or valproic acid and in patients with surgically constructed jejunoileal bypass for morbid obesity (Burroughs et al., 1982; DeLong and Glick, 1982).

Management of FHF patients is less rewarding. Supportive intensive care is very essential. Better survival rates observed at present have been attributed to improvements in the intensive care methods rather than any specific treatment (Hoyumpa and Schenker, 1985).

Therapeutic procedures adopted to treat FHF are several and are based on several principles. These include administration of lactulose, lactulose + neomycin sulphate, infusion of synthetic amino acid mixtures, dialysis and providing artificial liver support and finally liver transplantation (Saunders et al., 1972; Schenker et al., 1974; Conn and Lieberthal, 1979; Cooper and Plum, 1987).

Actual mechanism in the encephalopathy in FHF is still enigmatic and uncertain. Reasons for this uncertainty are:

(i) Lack of appropriate animal model which closely resemble the human disease condition.

(ii) Regional and cellular heterogeneity of the brain which renders the studies more difficult, if not impossible. This heterogeneity is seen in the
structure, function and metabolism at cellular and subcellular levels of cerebral organization.

(iii) Multiplicity of factors implicated in the aetiology of cerebral dysfunction in HE has further complicated the issue. These factors include ammonia, false neurotransmitters, mercaptans and short chain fatty acids etc., (Zieve, 1981). Though none of these factors can satisfactorily explain the aetiology of all the symptoms in HE, a brief description is given about these factors.

**AMMONIA**:

Intestine is the major site of ammonia production in the body (Weber and Veach, 1979). Bacterial action on dietary nitrogenous compounds (proteins, amino acids, nucleic acids etc.,) and intestinal metabolism of glutamine are responsible for the production of ammonia in the intestine. Ammonia, so formed, is transported to liver via portal blood and is converted to urea in periportal hepatocytes and to glutamine in perivenous hepatocytes (Haussinger et al, 1984). In conditions of hepatic insufficiency, ammonia is not detoxified in the liver and enters the systemic circulation. Elevation in blood and brain ammonia level is known to be neurotoxic (Schenker et al., 1974; Conn and Lieberthal, 1979; Cooper and Plum, 1987; Rukmini and Murthy, 1993).

Ammonia exists in two forms - unprotonated \( \text{NH}_3 \) form and protonated \( \text{NH}_4^+ \) (ionic) form. Interconversion of these two forms is extremely rapid and depends on pH of the medium. At physiological pH, about 97% of ammonia is in ionic form which is impermeable across the biological membranes. Unprotonated \( \text{NH}_3 \) form is lipid soluble and is highly permeable. However, in the present study, unless otherwise mentioned specifically, the term ammonia represents both the forms.
Once again, several mechanisms have been proposed to explain cerebral dysfunction in hyperammonemic states and none of the proposed mechanisms are equivocal (Cooper and Plum, 1987). Some investigators believe that excess ammonia levels in the brain exerts an adverse influence on cerebral energy metabolism (Bessman and Bessman, 1955), either by depleting α-ketoglutarate (α-KG) from citric acid cycle or by disrupting the transport of reducing equivalents from cytoplasm to mitochondria through malate-aspartate shuttle (Siesjo, 1978). It has also been proposed that ammonia disrupts Blood-Brain Barrier (BBB) and affects the transport of several substances including ions resulting in cerebral oedema (Ware et al., 1971). Another group of investigators believe that ammonia might be exerting its toxic effects by affecting the synthesis, release, reuptake and post-synaptic action of neurotransmitters (Schaffer and Jones, 1982; Rao and Murthy, 1991; Rao et al., 1992). Though, there are some studies in this direction no reports are available in the condition of FHF.

**Plasma Amino Acids And False Neurotransmitters:**

In some patients with HE and in animal models, a decrease in the level of branched-chain amino acids (BCAA; leucine, isoleucine and valine) and an increase in the levels of aromatic amino acids (AAA; phenylalanine, tyrosine and tryptophan) were observed (James et al., 1979). As these amino acids are transported across the BBB by a single carrier, it was postulated that mere would be an increased influx of AAA into brain in the absence of functional liver. As a consequence, cerebral AAA content would exceed the Km of the AAA hydroxylases (rate limiting reactions in the conversion of AAA into biogenic monoamines) and are directly decar-
boxylated to aromatic amines, such as **tyramine**, tryptamine, β-**pheylethylamine**, octopamine etc., which are called False Neurotransmitters (FNT).

These amines displace the real neurotransmitter amines (dopamine, epinephrine, norepinephrine and serotonin) from nerve terminals and alter the neurotransmitter balance in brain and thus cerebral function. A decrease in the dopamine and norepinephrine levels and an accumulation of false neurotransmitters have been found in brain during GALN induced FHF (Ferenci et al., 1984). As FNT might competitively inhibit the binding of dopamine to its receptors, it has been suggested that abnormal dopaminergic neurotransmission might be implicated in the mediation of HE (Fischer and Baldessarini, 1971). Similarly, cerebral levels of serotonin were reported to be increased and the number of serotonin receptors on neurons were observed to be decreased in HE and this might contribute to the neural inhibition in HE (Cummings et al., 1976; Riederer et al., 1982). It was observed that in some human subjects with hepatic inadequacy and in experimental animals that perfusion of BCAA or their keto analogues resulted in an improvement of clinical picture (Beaubernard et al., 1984).

However, in some cases of HE, no changes were observed in the levels of dopamine, noradrenaline and other minor amines, inspite of a neurological impairment (Fischer and Baldessarini, 1971; Cuilleret et al., 1980). Moreover, this hypothesis fails to explain some of the changes observed in the brain in hepatic insufficiency such as Alzheimer **astrocytosis** II.

Role of other **etiological** factors, such as mercaptans (Phear et al., 1956; Chen et al., 1970; Zeive, 1980; Zeive and Brunner, 1985), short
chain fatty acids (Samson et al, 1956; Zeive, 1983; 1985) etc., have not been worked out in detail. Increased blood levels of short chain fatty acids have been reported in the patients with hepatic insufficiency (Zieve, 1981). The reason for this increase is not clear and many factors seem to be involved. Short chain fatty acids uncouple oxidative phosphorylation and were shown to decrease the ATP and creatine phosphate content in the brainstem regions which is the seat for reticular activating system (McClain et al, 1980; Pappas-Venugeleu, 1984; Cooper and Plum, 1987). Injection of short chain fatty acids is known to induce coma (Zieve et al, 1974).

It is believed that other neurotransmitters are involved in the pathophysiology of HE. Altered metabolism of GABA, the major inhibitory neurotransmitter, has been implicated in the pathogenesis of HE. GABA hypothesis was proposed by Schaffer and Jones (1982) to explain HE. It is believed that GABA is produced not only in CNS but also in the intestine due to bacterial action on dietary glutamic acid. During liver dysfunction, GABA produced in the intestine is not detoxified in liver and enters into systemic circulation. Moreover, under these conditions, due to a selective disruption of the BBB, GABA enters the brain, binds to cerebral GABA receptors thus inhibiting the neuronal activity. In support of this hypothesis, these investigators observed an increase in the serum concentrations of GABA like compound in patients with HE (Maddison et al., 1987; Mullen et al., 1988). Schaffer and Jones (1982) reported an increase in the number of GABA receptors in rabbits with HE due to galactosamine (GALN) induced FHF. Baraldi and Zeneroli (1982) reported an increase in the number of GABA binding sites in a rat model during GALN induced FHF. An
increase in the number of cerebellar GABA binding sites in hyper-ammonemnic states with a functional liver was reported by Rao et al., (1991).

It has been suggested that ammonia and other toxins accumulated in the absence of a functional liver might alter the functions of biological membranes (Hoyumpa and Schenker, 1985). Neuronal membranes are known to be the seats of the neurotransmitter receptors, various types of ionic channels (ion gated, voltage gated) and carrier proteins for the transport of amino acids (Amara and Kuhar, 1993). However, there are no conclusive evidences or systematic studies in this area.

This may be vital as the neurotransmitter receptors mediate communication between the neurons, while the ionic channels maintain the proper ionic environment in and around the neuron for its efficient functioning. In fact, opening of ionic channels is mediated by binding of neurotransmitters to the receptors. Alteration in neurotransmitter receptors (affinities and densities) would lead to the disruption of neuronal communication and cerebral functions.

It is believed that derangement of cerebral function is usually due to alterations in cerebral energy metabolism or in the electrical activity of brain. These two parameters are interrelated and influence each other. Earlier studies have shown that liver dysfunction results in (i) an elevation in brain ammonia levels (Hoyumpa and Schenker, 1985) (ii) ammonia disposal in brain is by way of glutamate and glutamine fonnation and mis requires energy and also drains α-ketoglutarate from citric acid cycle (Weil-
Malherbe, 1950) (iii) these reactions deplete cerebral energy stores either directly or indirectly thus disrupting cerebral function (Bessman and Bessman, 1955).

However, studies on cerebral energy metabolism gave contradictory results (Hawkins et al., 1973 Schenker et al, 1974; Bates et al., 1989). Ratnakumari et al., (1986) showed that failure of cerebral energy metabolism may not be the chief reason for cerebral dysfunction at least in acute hyperammonemic conditions.

Above information, in summary, indicates that (i) hepatic insufficiency is of common occurrence in man and has variable clinical consequences (ii) the precise aetiology of the cerebral dysfunction under these conditions is not known (iii) various toxins accumulate under these conditions, of which ammonia is still supposed to be the chief culprit and (iv) survival in this condition depends on the proper management of patients and intensive care techniques rather than on a rational therapeutic mechanism.

In the present study, efforts were made to develop an animal model for FHF using galactosamine (GALN) as a hepatotoxin. Since the initial report of Keppler and Decker (1969) on the hepatotoxicity of GALN, considerable amount of work has been done to using this compound.

**Mechanism of Galactosamine Toxicity:**

Galactosamine, an aminosugar, is a naturally occurring component of heteroglycans in cells. However, very little amount of free GALN is detectable either in the liver or in other tissues. In liver, administered GALN is first phosphorylated to GALN-1-phosphate by galactokinase. This is con-
verted to UDP-GALN by \textit{UDPglucose:galactose-1-phosphate} uridylyltransferase. UDP-GALN is further converted to \textit{UDP-n-acetylGALN} or epimerized to UDP-glucosamine.

UDP-hexosamines, so formed during GALN administration, do not act as uridylate donors and prevent the replenishment of UTP levels in liver. When UTP content is decreased to a critical level (20% the normal values), degenerative reactions are initiated in the hepatocytes. Moreover, UDP-GALN and UDP-glucosamine are also incorporated into glycogen resulting in the formation of aminoglycogen (Mandl \textit{et al.}, 1979). It has been shown that aminoglycogen precipitates ribosomal subunits and acts as an effective inhibitor of protein synthesis (Mandl \textit{et al.}, 1979). However, despite the knowledge of the biochemical events, leading to GALN toxicity, it is not exactly known as to how the changes in the uridine nucleotide levels and of the inhibition of protein synthesis brings about hepatocellular degeneration.

In the later part of this work, another animal model for hyperammonemia was used. In this model, ammonium acetate was administered to animals with a functionally normal and active liver. This model was used to elucidate the role of ammonia in the changes observed in neurotransmitter functions. In some experiments, animals (with functionally active liver) starved for 36 hr were also used. This is to delineate the effects of starvation from those of FHF (as animals with FHF stop feeding).

\textbf{Neurotransmitter Functions of Glutamate and GABA}:

Using these animal models, studies were carried out on the neurotransmitter functions of glutamate and GABA. These two amino acids have
both metabolic and functional roles in brain (Mayer and Westbrook, 1987). They occupy a unique position in brain biochemistry as they constitute more than 50% of the total amino nitrogen present in brain (McIlwain and Bachelard, 1971). Moreover, their metabolism is closely associated with that of glucose and they (especially glutamate) are supposed to serve as alternate fuels in brain (Erecinska and Silver, 1990). Besides these, glutamate and GABA also play a pivotal role in neurotransmission - glutamate as an excitatory neurotransmitter (Fonnum, 1984) and GABA as an inhibitory neurotransmitter (Roberts, 1986). As neurotransmitters, they have been implicated in long term potentiation and long term depression, memory and learning (Kennedy, 1989; Ito, 1989). Another interesting aspect of glutamate is its neurotoxicity. Prolonged exposure to glutamate (or its analogues) leads to neuronal degeneration and death and this has been implicated in the aetiology of several neurodegenerative disorders (Choi, 1988; Wroblewski and Danysz, 1989, Choi and Rothman, 1990; Blackstone et al., 1992). A brief survey of literature pertaining to major metabolic and functional aspects of these two amino acids is given below.

**Metabolic Aspects of Glutamate and GABA:**

Studies carried out on glutamate metabolism revealed that it is closely related to the metabolism of glucose. Carbons from glucose rapidly equilibrate with the carbons of glutamate and GABA (Balazs et al., 1972). Further studies revealed that in brain, glutamate is present in two pools - a large pool and a small pool. Large pool of glutamate contains most of the (80-92%) tissue glutamate and has a slow turnover rate while small pool of glutamate contains less glutamate (8-20%) and has a rapid turnover rate.
These two pools do not mix with each other. Glutamate in the large pool is metabolized by oxidative deamination while in the small pool it is involved in the synthesis of glutamine and in transamination. Rate of synthesis of glutamine was found to be higher in the small pool than in the large pool. Precursor for the larger pool of glutamate has been shown to be glucose while in small pool glucose, β-hydroxybutyrate, acetate, CO₂, butyrate, propionate, citrate, leucine etc., would serve as precursors (Balazs et al., 1972; Clarke et al., 1974; Mohler et al., 1974; Van den Berg et al., 1975).

Further studies revealed that larger pool of glutamate is localized in neurons and the small pool in glia (Garfinkel, 1966, 1972; Benjamin and Quastel, 1972; Clarke et al., 1974; Van den Berg et al., 1974). Later studies revealed the existence of subcompartments in these two pools of glutamate. For e.g., the large compartment was shown to have a subcompartment from which glutamate is released on depolarization (releasable pool of glutamate; Benjamin and Quastel, 1972). This is most probably localized in the synaptic vesicles of glutamatergic neurons. Similarly, a subcompartment of the large compartment was believed to be involved in the synthesis and release of GABA (most probably) in the GABAergic neurons (Fyske and Fonnum, 1988). It is suggested that glutamate metabolism might be different not only in neurons and glia but even among different types of neurons.

Glutamate or GABA is released (from a subcompartment of the large compartment), especially in response to depolarizing stimuli. Glutamate/GABA that is released (referred as extracellular glutamate/GABA) is transported to the astrocytes. In these cells, major portion of glutamate is converted to glutamine and the rest is transaminated to α-KG and oxidized in
the citric acid cycle (Benjamin and Quastel, 1974). Glutamine, so formed, in the glia, would be transported to nerve endings where it serves as a precursor for the releasable pool of glutamate and GABA (Ward and Bradford, 1979). This process of recycling of glutamate (thus of α-KG/glucose) carbons between glia and neurons would prevent the perpetual loss of glutamate carbons from neurons (Benjamin and Quastel, 1974). This concept received support from histochemical and biochemical studies on the localization of the enzymes - glutamine synthetase and glutaminase in astrocytes and nerve terminals, respectively (Martinez-Hernandez et al., 1977; Norenberg and Martinez-Hernandez, 1979; Weiler et al., 1979).

GABA, like glutamate, has two major functions in brain tissue - it serves as a neurotransmitter and also as an intermediate in energy metabolism. In synaptic terminals, it functions as a neurotransmitter whereas in the non-synaptic regions (perikarya and dendrites) GABA functions as a metabolic intermediate (Martin and Rimvall, 1993). Studies carried out on GABA metabolism revealed that it is synthesized in brain at least in two compartments, commonly called as the transmitter and metabolic compartments (Garfinkel, 1966; Baxter, 1970; Patel et al., 1970; Balazs et al., 1972; Van den Berg, 1972; Iadarole and Gale, 1981).

In brain, GABA is synthesized principally from glutamate in a reaction mediated by glutamate decarboxylase, which uses pyridoxal-5-phosphate as a cofactor. In the subsequent step, GABA transaminates with a-KG to form succinic semialdehyde. This is oxidized to succinate by a mitochondrial NAD$^+$ dependent succinic semialdehyde dehydrogenase, thus providing a
pathway for the entry of GABA into the TCA cycle. This alternate route is called the GABA shunt. In brain, about 10-20% of the α-KG is diverted from TCA cycle through GABA shunt (Baxter, 1970; 1976). The transmitter compartment contains ~30% of tissue GABA and the metabolic compartment contains the rest (~70%) of GABA (Patel et al., 1970; Balazs et al., 1972). Although presence of metabolic pool of GABA is well established, its physiological significance is unclear.

**Glutamate and GABA as Neurotransmitters:**

**Release:**

In isolated neural preparations, release of glutamate is evoked by electric pulses (deBellaroche and Bradford, 1972; Potashner, 1978), by depolarizing chemical stimuli such as high external K⁺ levels (30-56 mM; Naddler et al., 1978), by ergot alkaloids (like veratridine; Toggenburger et al., 1982), by A23187 (calcium ionophore) along with Ca²⁺ (Levi et al., 1976), tityus toxin, p-bungarotoxin (Smith et al., 1980), verrucologen (Norris et al., 1980). It is believed that glutamate is released from the nerve terminals by two processes (Haycock et al., 1978; Sandoval et al., 1978). The first process is the Ca²⁺ dependent exocytosis of amino acids from the synaptic vesicles (Douglas, 1974; Strom-Matheison et al., 1983; Nicholls et al., 1987). Second one is the Ca²⁺ independent release. This is believed to be mediated by the high affinity uptake system acting in reverse direction - transporting cytosolic pool of glutamate to cell exterior (Erecinska, 1987; Nicholls and Attwell, 1990). Ca²⁺ dependent exocytosis of glutamate is ATP dependent (Nicholls et al., 1987) whereas the Ca²⁺ independent release occurs when energy levels (ATP/ADP) are decreased and ion gradi-
ents across plasma membranes are reduced (Sanchez-Prieto and Gonzalez, 1988). During normal neuronal activity, $\text{Ca}^{2+}$ dependent system is likely to dominate while $\text{Ca}^{2+}$ independent release occurs during pathological conditions associated with low intracellular ATP levels.

GABA is released from nerve terminals by a $\text{K}^+$ stimulated, $\text{Ca}^{2+}$ dependent mechanism (deBellaroche and Bradford, 1972; Osborne et al., 1973; Redburn and Cotman, 1974) which is blocked by Tetanus toxin (Osborne et al., 1973). Sustained release of GABA from cortical slices or synaptosomes is elicited by raising the extracellular $\text{K}^+$ concentration in the absence of $\text{Ca}^{2+}$ (Redburn et al., 1976; Vargas et al., 1977; Haycock et al., 1978). GABA released under these conditions is derived from recently accumulated GABA pools, whereas the $\text{Ca}^{2+}$ dependent $\text{K}^+$ stimulus facilitate the release from both pools (Haycock et al., 1978).

**Receptor Binding:**

Glutamate and GABA, once released from the nerve terminals into the synaptic cleft, bind to their receptors and exert their action. These receptors are membrane proteins present either on the post-synaptic or on the pre-synaptic neuron. Recently, receptors have also been reported to be present on astrocytes (Pearce, 1993). When neurotransmitter binds to the receptors on the post-synaptic neuron, then information transfer is accomplished. However, binding of neurotransmitter to a receptor on the pre-synaptic receptor (autoreceptor), it regulates the release of neurotransmitter itself. Binding of neurotransmitter to the receptor may open up ion channels associated with the receptor (ionotropic action) or may result in the synthe-
sis of a second messenger (metabotropic action). As the binding of glutamate results in the depolarization of the neuronal membranes and the genesis of an action potential, this amino acid is called as excitatory neurotransmitter (Fonnum, 1984). In contrast to this, GABA binding results in hyperpolarization of neuronal membranes and suppresses the neuronal activity. Hence, it is considered as an inhibitory neurotransmitter (Roberts, 1986; McGeer and McGeer, 1989). A brief description of glutamate and GABA receptors is given below.

**Glutamate Receptor**

Based on pharmacological specificity, several subtypes of glutamate receptors have been described. This classification is based on their preference for agonists and antagonists of glutamate. These include N-methyl-D-aspartate (NMDA), kainate (KA) and quisqualate (QA) receptors (Watkins and Evans, 1981; Honore et al., 1989). More recently the QA receptor has been renamed as AMPA receptor, as quisqualate is non-selective when compared to the selectivity of AMPA (a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) as an antagonist for this receptor subtype (Watkins et al., 1990). In addition, evidence for two more glutamate receptor subtypes has been obtained. One of the two new receptor subtypes (termed the L-AP4 receptor) has been defined on the basis of its sensitivity to low concentrations of glutamate analogue, 1-2-amino-4-phosphonobutanoate (L-AP4) (Monaghan et al., 1989). The second (referred as Glu_C receptor) is linked to an intracellular second messenger systems via a GTP binding protein (G protein) in the post-synaptic neuron (Schoepp et al., 1990). Molecular biological studies, conducted in the past few years, have
confirmed the existence of all five glutamate receptor subtypes in brain and lead to the identification of multiple isoforms of each receptor subtype with distinct functional properties (Foster and Fagg, 1993).

NMDA receptor is the best characterized acidic amino acid receptor subtypes. This receptor is associated with an ionic channel (hence an ionotropic receptor). This channel is opened when glutamate and other related agonists bind to the receptor. When opened, the channel is permeable to both $\text{Na}^+$ and $\text{Ca}^{2+}$ (in addition to $\text{K}^+$; MacDermott et al, 1986).

NMDA receptor complex consists of four different domains: (a) an NMDA recognition domain with a unique high affinity for glutamate, (b) a glycine recognition domain, (c) a polyamine recognition domain, and (d) an ion channel domain permeable to $\text{Ca}^{2+}$ and $\text{Na}^+$. Ion channel domain has four independent sites which are sensitive to inhibition by $\text{Mg}^{2+}$, $\text{Zn}^{2+}$, $\text{H}^+$ and allosteric blockers such as MK-801 and TCP (Nowak et al, 1984).

$\text{Ca}^{2+}$ permeability of the NMDA receptor has been implicated in the physiological and pathological roles of NMDA receptor. Activation of NMDA receptor is believed to play a role in the synaptic plasticity associated with learning and memory (Iversen, 1994) and in information processing (Daw et al, 1993). Entry of $\text{Ca}^{2+}$ through NMDA receptor channel has also been shown to play a crucial role in long term potentiation in many neural pathways in the brain (Collingridge and Bliss, 1995), epileptiform activity (Dingledine and McBain, 1994) and excitotoxic neuronal degeneration (Choi, 1988; Meldrum, 1990) and cell death (Ghosh and Greenberg,
Fig. 1: NMDA receptor ion channel complex.
A recent investigation indicated that activation of a protein tyrosine kinase followed by phosphorylation of a putative microtubule-associated protein (MAP2) kinase might be one of the early consequences of Ca^{2+} entry via the NMDA receptor channel (Bading and Greenberg, 1991). Increased formation of arachidonic acid and synthesis of nitric oxide have also been reported to occur due to NMDA receptor stimulation (Dumuis et al., 1988).

Several antagonists, each acting by a different mechanism, have been formulated and identified for NMDA receptors. Competitive antagonists compete for binding at the glutamate recognition sites (e.g. AP-5, CPP); MK-801 and related non-competitive antagonists (e.g. ketamine, phencyclidine) act by occupying a site within the ion channel (distinct from the Mg^{2+} sites). They act most effectively when the receptor is activated (i.e., when the associated channel is open). Binding of non-competitive antagonists such as MK-801 is a good biochemical index of the activity of the open NMDA channel in brain synaptic membranes. It was shown that phosphorylation of one or more amino acid residues on the NMDA receptor might be necessary for sustained activation of the receptor (Dingledine and McBain, 1994).

NMDA receptor was shown to have five subunits. NMDAR1, a subunit of NMDA receptor, was the first characterized subunit from rat brain has a molecular mass of 103 kDa cDNAs for these subunits have been cloned and characterized for both mouse and rat brain (Nakanishi et al., 1990; 1992; Moriyoshi et al., 1991). The open reading frame of the cDNA sequence for NMDAR1 predicts a total of 938 amino acids with a
series of 5 hydrophobic sequences which are thought to represent 4 trans-
membrane domains. A second gene family of NMDA receptor subunits
with four members sharing only 18-20% amino acid sequence homology to
NMDAR1 was isolated from rat. Four subunits of this family share 38 -
53% amino acid sequence identity and were termed NR2A - NR2D
(Monyer et al., 1992; Ishii et al., 1993).

Kainate receptors are a subtype of glutamate receptors associated with
ionic channels which are permeable to Na$^+$ and K$^+$ (Mayer and
Westbrook, 1987; Collingridge and Lester, 1989). Overstimulation of neurons through
kainate receptors induces neurodegenerative response in discrete populations of
neurons (Monaghan et al., 1989). Kainate can also activate the
AMPA receptors (Honore et al., 1988). Screening cDNA libraries for
clones resulted in the identification of five proteins, GluR5, 6 and 7, and
KA1 and 2, which showed properties expected of kainate receptors (Bettler

AMPA receptor associated ionic channels are permeable to Na$^+$ and
K$^+$ but not to Ca$^{2+}$. This receptor is activated by quisqualate and this can
be partially be blocked by the lectin, concanavalin A (Fagg and Foster,
1993). In the absence of other excitatory activity, AMPA receptors may
mediate fast depolarizing responses at most excitatory synapses in the
central nervous system. Four subunits for AMPA receptors have been
cloned. They were identified as GluR1, 2, 3 and 4. cDNA sequencing indi-
cates that GluR1 - GluR4 are 862-889 amino acids in length and are about
approximately 100 kDa (Keinanen et al., 1990; Boulter et al., 1990;
Hollmann et al., 1991).
Recent work using whole cell recording techniques indicated that L-AP4 receptor may act as a G protein coupled receptor which directly inhibits high threshold $\text{Ca}^{2+}$ currents and thereby suppresses transmitter release (Trombley and Westbrook, 1992).

$\text{Glu}_c$ receptors are the only acidic amino acid receptors that have been shown to be directly linked to intracellular second messenger systems. This receptor activates phospholipase $C$ to cause the hydrolysis of phosphoinositides to inositol 1,4,5-triphosphate ($\text{IP}_3$ ), which in turn releases $\text{Ca}^{2+}$ from intracellular stores, increases arachidonic acid and nitric oxide production. $\text{Glu}_c$ receptors have been proposed to participate in excitotoxic neurodegeneration (Choi, 1988; Fagg and Foster, 1993). The first member of the G protein linked receptors for glutamate ($\text{mGluR1}\alpha$) was isolated by expression cloning from rat CB (Masu et al., 1991). This protein contains 1199 amino acids (about 133 kDa) and seven putative membrane spanning domains. Recently, four additional $\text{mGluR}$ clones have been identified. These proteins ($\text{mGluR2-5}$) are 872-1171 amino acids in length and share sequence homology with $\text{mGluR1}$ (Abe et al., 1992; Aramori and Nakanishi, 1992; Tanabe et al., 1992; 1993 ).

Investigations using a variety of animal species indicate that excitatory amino acid receptor antagonists may be of primary value for the treatment of epilepsy and ischemic neurodegeneration (e.g., stroke). Therapeutic role of glutamate antagonists in Parkinson's disease, Schizophrenia and chronic neurodegenerative disorders is also being pursued (Lodge and Collinridge, 1990; Meldrum, 1991).
GABA Receptors:

GABA is the major inhibitory neurotransmitter in mammalian central nervous system. Altered GABAergic function is involved in neurological and psychiatric disorders such as Huntington's chorea, epilepsy, tardive dyskinesia, alcoholism, schizophrenia, sleep disorders and Parkinson's disease. Pharmacological manipulation of GABAergic transmission is being used for the treatment of anxiety (Enna and Mohler, 1987). GABA receptors are currently classified into three groups. GABA_A receptors are associated with a Cl⁻ ion channel (ionotropic receptors) and are antagonized by bicuculline. They are also modulated by a variety of ligands such as benzodiazepines and barbiturates. GABA and benzodiazepine binding sites were found to copurify and benzodiazepine binding site cross react with the antibodies of GABA binding site, indicating that this is an integral part of the GABA receptor-Cl⁻ channel complex (Siegel et al., 1983). GABA receptors are activated by muscimol and THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-3-ol; Enna, 1988).

GABA_A receptor was reported to be made up of 4 subunits -α, 3, γ, and 5. Isoforms of receptor arise by the assembly of subunits in different combinations, for e.g. isoforms α1-α6, β1-β4, γ1-γ3, and 5 have been identified (Burt and Kamatchi, 1991; Wisden and Seeburg, 1992). Each of the polypeptides is encoded by a separate gene which arose by duplication of a common ancestor (Lasham et al., 1991).

GABA_B receptors, for which baclofen is a specific agonist, are coupled to G proteins and acts through the stimulation of phospholipase activity (Feltz et al., 1987) and gate K⁺ or Ca²⁺ or both the channels (Bowery et
a./, 1988). $\text{GABA}_c$ receptors also gate $\text{Cl}^-$ channels and are blocked by picrotoxin but are insensitive to bicuculline and baclofen; they are activated by agonists TACA and CACA (*trans*-* and *cis*- 4-aminocrotonic acid, respectively). A fourth type (baclofen resistant, G protein coupled) of $\text{GABA}$ receptor might also exist (Djamgoz, 1995).

**Termination of Neurotransmitter Activity by Uptake:**

Neurotransmitter activity of both glutamate and GABA is terminated by uptake mechanisms. These uptake mechanisms are very efficient and maintain very low extracellular levels ($<1 \mu\text{M}$) of amino acids under physiological conditions (Nicholls and Attwell, 1990). Investigations on the transport of these amino acids revealed the existence of two types of uptake mechanisms, viz., one with a high affinity and low capacity and other with a low affinity and high capacity.

The high affinity uptake system has an absolute requirement for sodium (Bennet et al., 1973). This system is saturated at very low concentrations of the amino acid and is supposed to be involved in the removal of neurotransmitter glutamate from the synaptic cleft. Low affinity uptake system is not $\text{Na}^+$ dependent and is saturated at relatively high levels of extracellular amino acids ($K_m$ 1-2 mM). Presence of these uptake mechanisms in the brain is well documented in the literature (Logan and Snyder, 1972; Campbell and Shank, 1978; deBary et al., 1982; Levi et al., 1982; Gordon and Balazs, 1983; Hansson, 1986).

Mechanism of glutamate transport by high affinity uptake system is yet to be elucidated. It is not clear whether this process is same in nerve end-
ings and glia. Energy required for this process is provided by a simultaneous downhill movement of sodium ions. **Stoichiometry** of this process is two or more sodium ions and one \( \text{H}^+ \) for each molecule of glutamate transported (Stallcup *et al.*, 1979; Drejer *et al.*, 1982; Kanner and Marva, 1982; Kimelberg *et al.*, 1989). The proton is supposed to neutralize the charge of the amino acid (Erecinska *et al.*, 1983). Transport of glutamate through this carrier seems to be bidirectional and the direction of transport varies with the physiological state. When the magnitude of the driving force (which is a combination of the membrane electrical potential and the \( \text{Na}^+ \) concentration gradient) falls below equilibrium value, outward flux will be greater than inward flux and the carrier will mediate net glutamate release from the cell.

Presence of low affinity transport system for glutamate has been demonstrated in **synaptosomes** (Campbell and Shank, 1978; Weiler *et al.*, 1979). This system transports glutamate with a low affinity but with a high capacity. It may be responsible for the transport of glutamate into GABA-ergic terminals which lack sodium dependent high affinity uptake system (Erecinska and Silver, 1990). Not much information is available on the properties and distribution of these carrier proteins (transporters). The glutamate transporter has been solubilized and partially purified (Gordon and Kanner, 1988; Amara and Kuhar, 1993). In general, studies have shown that these carriers are glycoproteins with apparent molecular weights ranging from 60-85 kDa.

GABA is also transported by two transport systems, the high affinity system and a low affinity systems. Mechanism for the uptake of GABA were shown to be present in the cerebellar neurons, **glial** cells (Hokfelt and
Ljungdahl, 1970), synaptosomes (Weinberger and Cohen, 1983) and oligodendrocytes (Reynolds et al., 1987). GABA is taken up principally into inhibitory neurons viz., Purkinjee, Golgi, stellate and basket cells (East et al., 1980). Uptake of GABA by granule cell population is approximately 6% of that of glutamate. Accumulation of GABA by glial cells was about 20 times of that of granule cells (Campbell and Shank, 1978). High affinity uptake of GABA was more intense in the GABAergic cortical neurons than in cerebellar granule cells and in astrocytes (Yu and Hertz, 1982). Transport of GABA into synaptic vesicles is by a Mg-ATP dependent mechanism which are different from that of the plasma membrane (Fyske and Fonnum, 1988). GABA transporter has been solubilized and reconstituted in proteoliposomes (Radian and Kanner, 1986; Kanner et al., 1989). Studies have shown that these transporters have an apparent molecular weight ranging from 60-85 kDa (Amara and Kuhar, 1993).

A brief survey of literature indicated alterations in the neurotransmitter (glutamate and GABA) functions during hepatic encephalopathy. Decreased release of GABA was shown in portacaval shunted rats (Mans et al., 1979; 1990). Diaz-Munoz and Tapia (1988) reported decreased release of GABA in striatum and cerebellum and a significant increase in the hippocampus during CCl4 induced hepatic coma. Rao and Murthy (1991) reported increased in the evoked release of glutamate from cerebellar synaptosomes of hyperammonemic rats. Bosnian et al., (1992) reported a 4-5 fold increase in glutamate release in conditions of complete liver devascularization

Baraldi and Zenroli, (1982) reported that membrane preparations from rats with mild GALN induced hepatic encephalopathy had an increase in
the number of low and high affinity GABA binding sites, whereas only high affinity binding sites were present in those with severe encephalopathy. Increased number of GABA receptors in the brain during GALN induced FHF was also reported by Schafer and Jones (1982). Ferenci et al., (1984) reported that the development of hepatic encephalopathy in rabbits correlates with a decrease in the density of receptors for the excitatory amino acids. Binding of GABA to its receptors was reported to be unchanged in brains of portacaval shunted rats (Zanchin et al., 1984) while loss of low affinity sites for the GABA binding in brains of GALN induced FHF was reported by Zeneroli et al., (1984). Butterworth et al., (1986, 1987) reported increased binding of glutamate in cerebral cortex of portocaval shunted rats. However, Maddison et al., (1987) reported that GABA binding and function in brain, were unaltered in rats with thioacetamide induced hepatic encephalopathy. Olasmaa et al., (1990) reported a 10 fold increase in the cerebral concentrations of endogenous ligands for benzodiazepine receptors in rats suffering from FHF, but not in rats with portacaval shunts. Selective loss of NMDA sensitive $[^3\text{H}]$glutamate binding sites without alterations in the kainic acid (KA) and quisqualate sensitive binding sites in rat brain following portocaval anastomosis was also reported (Peterson et al., 1990). Rao et al., (1991) reported a decrease in the glutamate binding and an increase in the GABA binding in the cerebellum of rats treated with ammonium acetate.

Rao and Murthy, (1991) reported an increase in synaptosomal high affinity uptake systems for glutamate during acute hyperammonemia. uptake was reported to be increased in most of the brain
regions after CCl₄ treatment and this was independent of the presence of coma (Diaz-Munoz and Tapia, 1988).

Review of literature has shown that during acute liver damage the neurotransmitter functions of glutamate and GABA are altered and the information on this aspect during FHF is incomplete. Complete study of temporal and region specific changes in neurotransmitter functions of glutamate and GABA is not yet done during conditions of FHF. Also studies are not yet carried out on the kinetics of these changes (binding and uptake). It is not yet known whether these alterations are occurring due to changes in receptors (upregulation/down regulation or due to conformational changes) and transporters or whether these changes are associated with membrane architecture etc. Similarly, it is not yet worked out whether the changes observed in neurotransmitter functions is due to the action of a single factor (modulator) or a synergistic action of more than one factor. Therefore it was felt essential to understand the changes in the excitatory and inhibitory amino acid neurotransmission (mediated by glutamate and GABA) in conditions of FHF.

Three aspects of neurotransmitter functions were studied viz., (i) release of glutamate from nerve terminals (ii) binding of glutamate and GABA to their respective receptors (representing post-synaptic action of these amino acid neurotransmitters) (iii) uptake of glutamate into nerve terminals (representing the inactivation of glutamate released by nerve terminals). As mentioned earlier, alterations in these neurotransmitter functions of glutamate have been implicated in several neurological disorders, such as Alzheimer’s disease, Huntington’s chorea, epilepsy, Parkinsonism,
AIDS encephalopathy and dementia complex (Gasic and Hollman, 1992; Kitamaru et al., 1993; Dingledine and McBain, 1994; DeLorey and Olsen, 1994). As neurotransmitter functions are membrane associated phenomena, alterations in the membrane functions due to the changes, if any, in the membrane composition or the organization were also studied.

**Scope of the Present Work**

Hepatic failure occurs due to viral infections (Hepatitis) and hepatotoxins. A wide spectrum of neurological and neuropsychiatric disturbances are observed during this condition. Though therapeutic measures are available, survival depends on better intensive care facilities, monitoring and proper management of the patients in the hospitals. This is because of lack of complete knowledge of pathophysiological mechanisms involved in the disease process. Development of animal model for FHF would aid in carrying out temporal and regional studies on the alterations in the cerebral functions and the mechanisms responsible for these changes, which is not possible in human samples. Moreover, pilot studies of drug treatment can also be successfully carried out in animal model.

Present work is aimed to study the alterations in the neurotransmitter functions of glutamate and GABA during FHF and in hyperammonemic states associated with or without a functional liver. This work will help in the design and development of drugs for the altered neurotransmitter functions which might help in preventing the cerebral dysfunction. This will open up new avenues for the development of rational therapeutic mechanisms for FHF.