

**Literature Review**

**Definition of Epilepsy**

Epilepsy is a chronic disorder characterized by recurrent seizures, which may vary from a brief lapse of attention or muscle jerks, to severe and prolonged convulsions. The seizures are caused by sudden, usually brief, excessive electrical discharges in a group of brain cells (neurons). A seizure is a convulsive episode, which starts of as atypical, excessive hyper-synchronous discharges from an aggregate of neurons in the brain and then recruits surrounding neurons to comprise one or both hemispheres of the brain (Acharya *et al.*, 2008). During the seizure the person may experience the change or loss of consciousness, involuntary movements such as jerking, shaking or twitching.

**Epidemiology of Epilepsy**

Epilepsy is the commonest serious neurological condition affecting 0.5-1% of the population. Today, an estimated 50 million people live with epilepsy (PWE), 80% of whom in developing countries. Those most affected often do not come forward. Stigma, misconceptions and beliefs attached to this condition influence the open presentation of affected individuals in public meetings. The public health significance is particularly high in these settings because of its high prevalence, its seizure acuteness and frequency, and the sociological, psychosocial and financial consequences for the households it affects. Resource poor countries share demographic, sociological and economic features. They are particularly marked by ethnic, linguistic and religious richness, and their populations are frequently threatened by political instability and economic uncertainties. As a consequence health systems are typically weak and lack efficiency in addressing health needs (Quet *et al.*, 2008).
Etiology of Epilepsy

Epilepsy is often the result of an underlying brain disease. The most common etiologic factors of epilepsy that can predispose a person to epilepsy are head traumas, neoplasms, degenerative diseases, infections, metabolic diseases, ischemia and hemorrhages (Vinters et al., 1993). In view of the fact that only a proportion of people who have a brain disease experience seizures as a symptom of that disease, it is suspected that those who do have such symptomatic seizures are more vulnerable due to biochemical/neurotransmitter reasons. The underlying cause may be structural, including a brain injury such as a contusion, infection such as encephalitis, lack of oxygen to one part of the brain as occurs in a stroke, or a tumor. In some cases, there is a brain malformation that developed before birth. In other cases, the cause is a more generalized dysfunction of the brain that is not primarily structural, such as a genetic or metabolic disorder. In a large number of patients, the ultimate cause is not found at all, despite extensive testing.

Certain brain areas, i.e. temporal and frontal lobes are more susceptible to produce epileptic seizure activity than the other regions. However, there are also patients with unresolved etiology of epilepsy (Hauser, 1997). Etiology of epilepsy is also a factor in determining cognitive function and intellectual changes over time. The main distinction is between symptomatic epilepsy which has an identified cause such as stroke or cortical dysplasia and idiopathic epilepsy which has no identified cause other than genetic factors. Lennox et al., (1942) recognized that cognitive function was twice as likely to deteriorate in the presence of a known cause of epilepsy even if the idiopathic group had more frequent seizures. Idiopathic epilepsy is a type of epilepsy whose causes have not been identified. In such cases, the theory most commonly accepted is that this epilepsy is the result of an imbalance of certain chemicals in the brain (especially neurotransmitters) causing them to have a low convulsive threshold. Children and adolescents are more likely to have epilepsy of unknown or genetic origin. The older the patient, the more likely it is that the cause is
an underlying brain disease, such as a brain tumour or cerebrovascular disease, or is the result of head injury.

Trauma and brain infection can cause epilepsy at any age and as mentioned previously may account for a higher incidence of epilepsy in developing countries. For example, a common cause in Latin America is neurocysticercosis cysts on the brain caused by tapeworm infection, while in Africa, malaria and meningitis are common causes, and in India neurocysticercosis and tuberculosis often lead to epilepsy. Febrile illness of any kind can trigger seizures in young children. About 3% of children who have febrile convulsions go on to develop epilepsy in later life.

**Mortality**

Mortality data of PWE in developing countries are scarce. A recent effort in China to address this gap revealed that PWE had 3–4 times higher mortality than the general population (Ding et al., 2006). Most probably is the epilepsy-associated mortality also elsewhere considerably elevated.

**Classification of Epileptic Seizures**

The International Classification of Epileptic Seizures (1981) recognizes two general categories of seizures based on the origin of the abnormal electrical discharge. Two broad categories of seizures are recognized, partial and generalized, with each category having different subtypes.

1) Partial seizures, referred to as focal or local seizures, originate in one location in the brain and then may or may not spread to other brain areas. Partial seizures are further subdivided into simple partial and complex partial. In simple partial seizures consciousness is preserved. In complex partial seizures there is an alteration in consciousness, the person does not recall having the seizure and may be very confused and fatigued in the aftermath. A partial seizure may also progress into a generalized motor seizure.
2) Generalized seizures, referred to as "grand mal" seizures, begin simultaneously in all areas of the brain. Consciousness is altered and the person may or may not show convulsions.

Other commonly used terms include ictal (of seizure itself) and interictal (between seizures). Convulsion implies ictal behaviour with vigorous motor activities. Status epilepticus denotes a very prolonged seizure or series of seizures occurring so frequently that full recovery of brain function does not occur interictally.

**Pilocarpine**

Pilocarpine is a potent cholinergic agonist originally isolated from the leaflets of *Pilocarpus microphyllus* belonging to the Rutaceae family. It is commonly used in the treatment of acute glaucoma in humans (Hardman et al., 1996). Systemic administration of pilocarpine has been used as an animal model for temporal lobe epilepsy and has several features in common with the human complex partial seizures. The most striking similarity was probably that pilocarpine produced marked changes in morphology, membrane properties and synaptic responses of hippocampal rat neurones, comparable to those observed in human epileptic hippocampal neurones (Isokawa & Mello, 1991). Single systemic high dose (300-400 mg/Kg) of pilocarpine injection as a novel animal model of TLE was established (Turski et al., 1983). The systemic administration of this pilocarpine produced electroencephalographic and behavioural seizures, accompanied by widespread brain damage similar to that observed in autopsied brains of human epileptics. These electroencephalographic findings indicate that one of the most sensitive structures to the convulsant effect of pilocarpine is the hippocampus, while other structures remain unaffected or only slightly affected at early time points following injection. Studies confirmed that the hippocampus is the earliest structure to be activated according to electroencephalographic recordings (Turski et al., 1983, 1989). One of the main features of the pilocarpine model that makes it very relevant for comparison to the
human epileptic condition is the reproducible occurrence of spontaneous recurrent seizures (SRS) in rats injected with pilocarpine following a delay or silent period of about 2 weeks (Turski et al., 1983, 1989; Cavalheiro et al., 1991; Mello et al., 1993).

Spontaneity is one of the prominent signs of human epilepsy, therefore strengthening the clinical importance of this model (Turski et al., 1983; Loscher & Schmidt, 1988). Pilocarpine seizures also provide an opportunity to study the involvement of the cholinergic system in the onset, propagation and pathological consequences of limbic seizures (Clifford et al., 1987). Behaviourally, pilocarpine seizures resemble other models of limbic seizures beginning with facial automatisms, head nodding and progressing to forelimb clonus with rearing and falling (Clifford et al., 1987). In terms of neuropathology, the cell damage that results from seizures was identical whether they are initiated with a high-dose pilocarpine injection or a lower dose of pilocarpine administered with lithium (Clifford et al., 1987). Lithium-pilocarpine is an analogous model to pilocarpine injection alone, except that lithium in combination with pilocarpine has been reported to produce a 20-fold shift in the pilocarpine dose response curve for producing seizures (Clifford et al., 1987) thereby permitting the use of a much lower dose of pilocarpine. In terms of cell damage reported at the light microscope level, pilocarpine-induced seizures consistently produce damage in the olfactory nucleus, pyriform cortex, entorhinal cortex, thalamus, amygdala, hippocampus, lateral septum, bed nucleus of stria terminalis, claustrum, substantia nigra and neocortex (Clifford et al., 1987; Turski et al., 1989; Turski et al., 1983). In the hippocampus, the CA3 and CA1 regions are involved and damage has been noted to be greater in ventral as opposed to dorsal hippocampal regions. Interestingly, the highest cholinergic receptor densities are in CA1 and the dentate gyrus, while the region most consistently and severely damaged is CA3 (Clifford et al., 1987). This clearly indicates that the spread of seizure activity beyond the initial focus must entail activation of non-cholinergic pathways. Electron microscopic studies indicate the cellular changes include swelling of dendrites,
swelling or vacuolar condensation of neuronal cell bodies and marked dilatation of astroglial elements with relative sparing of axonal components (Clifford et al., 1987). The neuropathology reported with the pilocarpine model is consistent with prolonged seizures produced by other means (Ben-Ari, 1985; Kapur et al., 1989; Hajnal et al., 1997). These findings support that pilocarpine SE model is useful in studying the molecular mechanisms of neuropathology and screening neuroprotectants following cholinergic agonist exposure (Tetz et al., 2006).

Role of Neurotransmitters in Epilepsy

**Epinephrine and Norepinephrine**

The modifications of the seizure activity by the noradrenergic system were reported early (Chen et al., 1954). Four major observations have supported an anticonvulsant role for norepinephrine (NE): (1) selective lesioning of noradrenergic neurons with 6-hydroxydopamine or DSP-4 increases seizure susceptibility to a variety of convulsant stimuli (Arnold et al., 1973; Jerlicz et al., 1978; Mason & Corcoran, 1979; Snead, 1987; Trottier et al., 1988; Sullivan & Osorio, 1991; Mishra et al., 1994) (2) direct stimulation of the locus coeruleus (LC), the major concentration of noradrenergic cell bodies in the CNS and the subsequent release of NE reduce CNS sensitivity to convulsant stimuli (Turski et al., 1989) (3) genetically epilepsy-prone rats (GEPRs), a widely used animal model of epilepsy, have deficient presynaptic NE content, NE turnover, tyrosine hydroxylase levels, dopamine β-hydroxylase (DBH) levels and NE uptake (Jobe et al., 1984; Dailey & Jobe, 1986; Lauterborn & Ribak, 1989) (4) adrenergic agonists acting at the α₂ adrenoreceptor (α₂-AR) have anticonvulsant action (Baran et al., 1985; Loscher & Czuczwar, 1987; Fletcher & Forster, 1988; Jackson et al., 1991). α₂-AR is known to have a regulatory role in the sympathetic function (Das et al., 2006). The lesioning studies i.e., chemical destruction of noradrenergic terminals, reduce the amount of NE release; this manipulation also reduces the release of other transmitters released with NE. The
neuropeptides galanin and neuropeptide Y (NPY) and the neurotransmitter adenosine i.e., ATP, are released at noradrenergic terminals and have been shown to exert anticonvulsant effects against several convulsant stimuli (Murray et al., 1985; Mazarati et al., 1992, 1998; Dichter, 1994; Erickson et al., 1996; Baraban et al., 1997).

**Dopamine**

The mammalian prefrontal cortex (PFC) receives a substantial dopaminergic innervation from the midbrain ventral tegmental area (VTA) (Bjorklund & Lindvall, 1984). Dopamine is an endogenous neuromodulator in the cerebral cortex and is believed to be important for normal brain processes (Bjorklund & Lindvall, 1984; Williams & Goldman-Rakic, 1995). There is strong evidence that alterations in dopamine function play a role in pathogenesis of a number of neuropsychiatric diseases including epilepsy (Starr, 1996). *In vivo* studies have shown that dopamine increase and decrease spontaneous firing of neocortical neurons (Bunney & Aghajanian, 1976; Reader et al., 1979; Ferron et al., 1984; Bradshaw et al., 1985; Sesack & Bunney, 1989; Bassant et al., 1990; Yang & Mogenson, 1990; Thierry et al., 1992; Pirot et al., 1992). Dopamine favour long-lasting transitions of PFC neurons to a more excitable up state (Lewis & O’Donnell, 2000). *In vitro* electrophysiological experiments suggest that dopamine has multiple effects on PFC neurons. Both increases (Penit-Soria et al., 1987; Yang & Seamans, 1996; Ceci et al., 1999; Wang & O’Donnell, 2001; Gorelova & Yang, 2000; Henze et al., 2002; Gonzalez-Burgos et al., 2002; Tseng & O’Donnell, 2004) and decreases (Geijo-Barrientos & Pastore, 1995) in postsynaptic excitability of pyramidal neurons have been reported following DA D\(_1\) receptor activation. In addition, changes in excitability mediated by DA D\(_2\) receptors have been reported (Gulledge & Jaffe, 2001; Tseng & O’Donnell, 2004). The effects of dopamine on synaptic responses are also complex and species-specific. AMPA receptor mediated excitatory postsynaptic currents (EPSCs) in layer V
pyramidal cells are depressed by a DA D₁ receptor–mediated effect of dopamine (Law-Tho et al., 1994; Seamans et al., 2001) whereas N-methyl-D-aspartate (NMDA) responses have been reported to be both enhanced (Seamans et al., 2001) and depressed (Law-Tho et al., 1994). EPSCs in layers II/III are enhanced by dopamine in rats (Gonzalez-Islas & Hablitz, 2003) but decreased in primates (Urban et al., 2002). The cerebral cortex contains interconnected local and distant networks of excitatory and inhibitory neurons. Stability of activity in such networks depends on the balance between recurrent excitation and inhibition (Durstewitz et al., 2000; Shu et al., 2003).

A shift of the balance toward excitation leads to the generation of epileptiform activity. The presence of massive recurrent excitatory connections that depend on inhibition for regulation has been implicated in the susceptibility of the neocortex and the hippocampus to develop epileptiform activity and seizures (McCormick & Conteras, 2001). Modulatory influences strongly influence activity in thalamocortical (McCormick et al., 1993; McCormick & Pape, 1990) and neocortical circuits (McCormick et al., 1993). Dopamine is known to modulate epileptiform discharges both in vivo (Alam & Starr, 1993, 1994; George & Kulkarni, 1997) and in vitro (Alam & Starr 1993, 1994; Cepeda et al., 1999; Siniscalchi et al., 1997; Suppes et al., 1985). In vivo studies in different models of epilepsy have suggested that dopamine may have a pro-convulsant effect mediated by DA D₁ receptors and an anti-convulsant effect via DA D₂ receptors (Starr, 1996). Dopamine-mediated recruitment of neurons in local excitatory circuits and synchronization of activity in these neurons underlie these effects of dopamine in neocortex. Local excitatory neocortical networks are complexes of interconnected pyramidal neurons. Several anti-epileptic drugs increase extracellular levels of dopamine DA and/or serotonin (5-HT) in brain areas involved in epileptogenesis (Smolders et al., 1997). Behavioural and electrocorticographic studies in rats have shown that DA controls hippocampal excitability via opposing actions at DA D₁ and DA D₂ receptors (Bo et al., 1995). Seizure enhancement is
presumed to be a specific feature of D₁ receptor stimulation, whereas DA D₂ receptor stimulation is anticonvulsant (Alam & Starr, 1992, 1993). Decreased DA D₂ receptor binding in the brainstem were reported in other neurological diseases like diabetes (Shankar et al., 2007).

GABA

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the CNS. It exerts an inhibitory action in all forebrain structures and plays a role in the physiopathogenesis of certain neurological conditions, including epilepsy. Impairment of GABA functions produces seizures, whereas enhancement results in an anticonvulsant effect. In tissue resected from patients with temporal lobe epilepsy, the number of GABA receptors are reduced in areas of hippocampus showing neuronal cell loss (McDonald et al., 1991; Johnson et al., 1992). Reduced benzodiazepine (BZD) binding to GABA, receptors in mesial temporal lobe of such patients can be detected in vivo by noninvasive positron emission tomography imaging (Savic et al., 1988). These changes are likely secondary to cell loss and not specific for GABA-receptive cells. Recent studies have shown some changes in GABAₐ receptors that occur in the neocortex of patients undergoing epilepsy surgery. These patients had TLE with severe damage and sprouting in limbic structures.

Increased levels of steroid modulation of GABA, receptor ligand binding in neocortex were detected in patients with TLE. Increase in binding of diazepam-insensitive sites for the BZD ligand [³H]Ro15-45 13 associated with the a₄ GABA receptor subunit was also observed (Van Ness et al., 1995). Therefore, changes in the properties, rather than the number of GABA receptors possibly related to plastic changes in subunit combinations result in an altered regulation of inhibitory function. Human focal epilepsy occurs commonly in the mesial temporal lobe often associated with Ammon’s horn sclerosis. This is accompanied by severe gliosis and a sprouting in the molecular layer of the dentate gyr (Babb et al., 1989) as well as a dispersion of
the granule cell layer (Houser et al., 1990). This loss of neurons in the hippocampal formation is evident in CA3 and hilus, especially hilar mossy cells as evidenced by several neuronal markers including glutamic acid decarboxylase (GAD) and GABA receptors. One can mimic these changes in animals by producing lesions or using massive stimulation of hippocampal input (Sloviter et al., 1991), kindling paradigms (Cavazos et al., 1991), or systemic kainite (Cronin et al., 1992) or pilocarpine (Cavalheiro et al., 1991). Like the human condition, these models involve end-folium sclerosis, including hilar interneuron loss and dentate granule cell hyperexcitability. The granule cells normally are inhibited laterally by hilar interneurons, which are excited by mossy cells that innervate them longitudinally. Loss of these mossy cells has been proposed to make the surviving GABAergic basket cells “dormant,” thus disinhibiting long stretches of granule cells (Sloviter et al., 1991). In the pilocarpine model, there is loss of hilar cells, including GABAergic interneurons accompanied by decreased levels of mRNA and immunoreactivity of the GABA_A receptor a5 subunit in CA1/2 (Houser et al., 1995). Loss of a5 and a2 mRNA was also observed by another group of investigators (Rice et al., 1996) who demonstrated decreased GABA, synaptic activity in CA1. Therefore, in several of these animal models, there is evidence of reduced GABA-mediated inhibition.

**Acetylcholine**

The cholinergic system plays a crucial role in modulating cortical and in particular hippocampal functions including processes such as learning and memory (Ashe & Weimberger, 1991; Dunnett & Fibiger, 1993; Huerta & Lisman, 1993; Shen et al., 1994; Winkler et al., 1995). Cholinergic actions are involved in the physiopathogenesis of epileptic discharges as suggested by the ability of some cholinergic agents to induce limbic seizures and histopathological changes resembling those seen in patients with temporal lobe epilepsy (Dickson & Alonso 1997; Liu et al., 1994; Nagao et al., 1996; Turski et al., 1989). Cholinergic stimulation of cortical
neurons, including those located within the hippocampal formation, results in excitatory effects that are mediated mainly through the activation of muscarinic receptors (Krnjević et al., 1993; McCormick et al., 1993).

Cholinergic innervation is present in the subiculum, which is a major synaptic relay station between the hippocampus proper and several limbic structures that are involved in cognitive processes (Amaral & Witter, 1989; Lopes da Silva et al., 1990). Subicular neurons are also involved in the spread of seizure activity within the limbic system (Lothman et al., 1991). To date little is known about the effects of cholinergic agents in the subiculum. The EC is known to be a “gateway” for the bi-directional passage of information in the neocortical hippocampal-neocortical circuit (Van Hoesen, 1982; Witter et al., 1989; Lopes da Silva et al., 1990) via a cascade of cortico-cortical projections, the superficial layers of the EC (II and III) receive an extensive input from polymodal sensory cortices (Jones & Powell, 1970; Van Hoesen & Pandya, 1975; Amaral et al., 1983; Deacon et al., 1983; Room & Groenewegen, 1986; Insauti et al., 1987; Reep et al., 1987) that is then conveyed to the hippocampal formation via the perforant path (Steward & Scoville, 1976). In turn, the hippocampal formation projects back on the deep layers of the Entorhinal Cortex (EC) which provide output paths that reciprocate the input channels (Swanson & Cowan, 1977; Swanson & Kohler, 1986; Insauti et al., 1997). In addition, the deep layers of the EC also project massively on the EC superficial layers (Kohler, 1986) thereby closing an EC–hippocampal loop. Thus, by virtue of its extensive projection systems, the EC network acts powerfully in the generalization of temporal lobe seizures. The EC is also known to receive a profuse cholinergic input from the basal forebrain that terminates primarily in layers II and V (Lewis & Shute, 1967; Mellgren & Srebro, 1973; Milner et al., 1983; Alonso & Kohler, 1984; Lysakowski et al., 1989; Gaykema et al., 1990), precisely those layers that gate the main hippocampal input and output. It is well known that the cholinergic system promotes cortical activation and the expression of normal population oscillatory dynamics. In the EC, in vivo
electrophysiological studies have shown that the cholinergic theta rhythm is generated primarily by cells in layer II (Mitchell & Ranck, 1980; Alonso & Garcia-Aust, 1987a, b; Dickson et al., 1995). In addition, in vitro studies have also shown that muscarinic receptor activation promotes the development of intrinsic oscillations in EC layer II neurons (Klink & Alonso, 1997). On the other hand, some evidence indicates that altered activity of the cholinergic system is relevant to epileptogenesis.

**Serotonin synthesis and metabolism**

Serotonin was initially discovered as a vasoconstrictor substance in blood and later in blood vessel walls, platelets and in enterochromafine cells of the gastrointestinal system, the lungs and the heart (Rapport et al., 1948). Outside the CNS, 5-HT acts on autonomic smooth muscle cells, e.g. in blood vessels and the digestive tract (Zifa & Fillion, 1992). More than 50 years ago the chemical structure of 5-HT was identified and it was synthesised (Twarog & Page, 1953). Later, the function of 5-HT as a neurotransmitter in the CNS was proposed (Bogdanski et al., 1956) and 5-HT has been studied intensively since its identification in the pituitary gland (Hyyppa & Wurtman, 1973). In the CNS, serotonin is a two step pathway from the essential amino-acid tryptophan. Serotonin is synthesised in the perikarya of the neuron where tryptophan is hydroxylated to the 5-HT precursor 5-hydroxytryptophan (5-HTP) which is then decarboxylated to 5-HT (Hamon et al., 1982). To avoid immediate enzymatic oxidation to 5-hydroxy-indol acetic acid (5-HIAA) by monoamine oxidase (MAO), 5-HT is contained in neuronal vesicles until it is released into the synaptic cleft. Serotonin then activates either postsynaptic or presynaptic receptors or is reuptaken via the 5-HT transporters molecule into the neuron (Hamon et al., 1982). The principle route of metabolism of 5-HT involves monoamine oxidase forming 5-Hydroxyindole acetic acid by a two step process. In addition to metabolism by MAO, a Na⁺ dependent carrier mediated uptake process exists and is involved in terminating the action of 5-HT. The 5-HT transporters are localized in the outer
membrane of serotonergic axon terminals and in the outer membrane of platelets. This uptake system is the only way that platelets acquire 5-HT since they do not have the enzymes required for synthesis of 5-HT. The degradation processes are very fast due to a large surplus of monoamine oxidase. Therefore, concentrations of 5-HT in cerebral extra cellular space and in peripheral plasma are low, and do not reflect serotonergic activity.

Anatomy of Serotonin System

The serotonin (5-Hydroxytryptamine; 5-HT) systems are widespread throughout the brain, with most of the cell bodies of serotonergic neurons located in the raphe nuclei of the midline brainstem (Palacios et al., 1990). The largest collections of 5-HT neurons are in the dorsal and median raphe nuclei of the caudal midbrain (Jacobs & Azmitia 1992). The neurons of these nuclei project widely over the thalamus, hypothalamus, basal ganglia, basal forebrain, and the entire neocortex. Interestingly, these 5-HT neurons also provide a dense subependymal plexus throughout the lateral and third ventricles. Activation of this innervations result in 5-HT release into the cerebrospinal fluid (CSF), and measurement of 5-HT content in CSF in disease states will largely reflect this pool (Chan-Palay, 1976). This is another interesting aspect of the 5-HT neuron innervation of forebrain. Descarries et al., (1975) has shown that the terminals of 5-HT neurons in forebrain, unlike terminals from other systems, only infrequently form synaptic complexes. Thus, when 5-HT neurons innervating forebrain are activated, 5-HT will be released into the extracellular fluid and its action will depend on the location of nearby 5-HT receptors. The organization of the ascending 5-HT neuron projections, the nature of their interaction with postsynaptic elements and the widespread distribution of 5-HT terminals in cortical and limbic areas indicate that these projections are most likely to be involved in the regulation of behavioural state and the modulation of more specific behaviours. The second 5-HT neuron system is comprised of 5-HT neurons in the
pontine and medullary raphe with projections principally to brainstem, cerebellum and spinal cord. This system appears primarily to be involved in modulation of sensory input and motor control (Meltzer et al., 1998). During brain development, 5-HT provides essential neurotrophic signal. 5-HT is known to play an important role in several physiological functions (Jackson & Paulose, 1999). Evidence from animal and human studies suggests that 5-HT is linked to many functions, such as mood, aggression, feeding, and sleep. Dysregulation of 5-HT function is believed to be involved in depression, impulsivity and suicide (Meltzer, 1998). Additionally, modulation of cholinergic neuronal activity by 5-HT plays a role in higher cognitive processes such as memory and learning (Altman et al., 1990; Richter-Levin & Segal, 1990). Accordingly, alterations in serotonergic function accounts for behavioural disturbances commonly observed during epilepsy. There is conflicting evidence from animal studies, post mortem work and limited clinical trails as to the direction, magnitudes and significance of these findings.

**Serotonin Receptors**

The diverse effects of this neurotransmitter are related to the extensive projections of serotonergic neurons throughout the brain and the large number of distinct serotonin receptor subtypes. At least 14 distinct serotonin receptor subtypes are expressed in the mammalian CNS, each of which is assigned to one of seven families, 5-HT\(_1\) to 5-HT\(_7\). Serotonin receptors have been classified into families designated 5-HT\(_{1-7}\) on the basis of their molecular biological characteristics (Hoyer & Martin, 1997; Peroutka, 1994; Saudou & Hen, 1994). The 5-HT\(_{1B/D}\) receptors are found largely presynaptically, the 5-HT\(_{1A}\) receptor exists in both a presynaptic and postsynaptic form and the remaining receptor subtypes are expressed predominately postsynaptically with their distribution and density regulated with respect to individual brain area and functional state. Another protein important in serotonergic neurotransmission is the 5-HT transporter. This protein is localized on the membrane
of 5-HT nerve terminals and is responsible for reuptake of released 5-HT into the terminals. The distribution of the 5-HT transporter conforms closely to the distribution of 5-HT nerve terminals (Dawson & Wamseley, 1983; Fuxe et al., 1983) and thus serves as a marker for the integrity of serotonergic projections. The 5-HT₁A and 5-HT₂A receptors and the 5-HT reuptake site are the most frequently targeted sites of action for antidepressant medications and have been well-characterized physiologically. Thus, these sites have also been the focus of radiochemistry development. The 5-HT₂ sub-family of serotonin receptors is composed of three subtypes, the 5-HT₂A, 5-HT₂B and 5-HT₂C receptors. All three receptors are G-protein coupled to the activation of the phospholipase C functionally linked to phosphatidyl inositol (PI) hydrolysis and subsequent mobilization of intracellular calcium (Barnes & Sharp, 1999).

**Classification of Serotonin receptors**

The various effects of 5-HT on the central nervous system and peripheral organs are mediated through activation of multiple types of receptors (Hoyer & Martin, 1997). 5-HT receptors can be classified into seven classes from 5-HT₁ to 5-HT₇, based upon their pharmacological profiles, cDNA-deduced primary sequences and signal transduction mechanisms of receptors (Bradley et al., 1986; Zifa & Fillion, 1992). All 5-HT receptors belong to the superfamily of G-protein coupled receptors containing a seven transmembrane domain structure except 5-HT₃ receptor, which forms a ligand-gated ion channel.

**5-HT₁ Receptor**

At least five 5-HT₁ receptor subtypes have been recognised, 5-HT₁A, 5-HT₁B, 5-HT₁D, 5-HT₁E and 5-HT₁F. All are seven transmembrane, G-protein coupled receptors via Gi or Go, encoded by intronless genes, between 365 and 422 amino acids with an overall sequence homology of 40%. 5-HT₁A receptor subtype which is...
located on human chromosome 5cenq11 is widely distributed in the CNS, particularly hippocampus (Hoyer et al., 1994). The 5-HT$_{1B}$ receptor is located on human chromosome 6q13 and is concentrated in the basal ganglia, striatum and frontal cortex. The receptor is negatively coupled to adenylyl cyclase. The 5-HT$_{1D}$ receptor has 63% overall structural homology to 5-HT$_{1B}$ receptor and 77% amino acid sequence homology in the seven transmembrane domains. The receptor is located on human gene 1p36.3-p34.3 and is negatively linked to adenylyl cyclase. 5HT$_{1D}$ receptor mRNA is found in the rat brain, predominantly in the caudate putamen, nucleus accumbens, hippocampus, cortex, dorsal raphe and locus ceruleus (Hoyer et al., 1994). The 5-HT$_{1E}$ receptor was first characterised in man as a [$^3$H] 5-HT binding site in the presence of 5-carboxyamidotryptamine (5-CT) to block binding to the 5-HT$_{1A}$ and 5-HT$_{1D}$ receptors. Human brain binding studies have reported that 5-HT$_{1E}$ receptors are concentrated in the caudate putamen with lower levels in the amygdala, frontal cortex and globus pallidus. This is consistent with the observed distribution of 5-HT$_{1C}$ mRNA (Hoyer et al., 1994). The receptor has been mapped to human chromosome 6q14-q15, is negatively linked to adenylyl cyclase and consists of a 365 amino acid protein with seven transmembrane domains. 5-HT$_{1F}$ receptor subtype is most closely related to the 5-HT$_{1E}$ receptor with 70% sequence homology across the 7 transmembrane domains. mRNA coding for the receptor is concentrated in the dorsal raphe, hippocampus and cortex of the rat and also in the striatum, thalamus and hypothalamus of the mouse (Hoyer et al., 1994). The receptor is negatively linked to adenylyl cyclase.

5-HT$_2$ Receptor

The 5-HT$_2$ receptor family consists of three subtypes namely 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$. 5-HT$_{2C}$ was previously termed as 5-HT$_{1C}$ before its structural similarity to the 5-HT$_2$ family members was recognized. All three are single protein molecules of 458 - 471 amino acids with an overall homology of approximately 50% rising to
between 70-80% in the seven transmembrane domains. All three are thought to be linked to the phosphoinositol hydrolysis signal transduction system via the $\alpha$ subunit of Gq protein. In human pulmonary artery endothelial cells, 5-HT$_{3C}$ receptor stimulation causes intracellular calcium release via a mechanism independent of phosphatidylinositol hydrolysis (Hagan et al., 1995). 5-HT$_{2A}$ receptor previously termed as 5HT$_2$ receptor is located on human chromosome 13q14-q21 and is widely distributed in peripheral tissues. It mediates contractile responses of vascular, urinary, gastrointestinal and uterine smooth muscle preparations, platelet aggregation and increased capillary permeability in both rodent and human tissue (Hoyer et al., 1994). The 5-HT$_{2B}$ receptor located on chromosome 2q36-2q37.1 mediates contraction of the rat stomach fundus and endothelium dependent relaxation of the rat and cat jugular veins and possibly of the pig pulmonary artery, via nitric oxide release (Choi & Maroteaux, 1996). 5-HT$_{2B}$ receptor mRNA has been detected throughout the mouse, rat and guinea pig colon and small intestine. 5-HT$_{2C}$ specific antibodies have recently been used to show the presence of the receptor protein in the choroid plexus (highest density) and at a lower level in the cerebral cortex, hippocampus, striatum, and substantia nigra of rat and a similar distribution in man. The receptor has been mapped to human chromosome Xq24. No splice variants have been reported but the receptor is capable of post translational modification whereby adenosine residues can be represented as guanosine in the second loop to yield 4 variants.

5-HT$_3$, Receptor

The 5-HT$_3$ receptor binding site is widely distributed both centrally and peripherally and has been detected in a number of neuronally derived cells. The highest densities are found in the area postrema, nucleus tractus solitarius, substantia gelatinosa and nuclei of the lower brainstem. It is also found in higher brain areas such as the cortex, hippocampus, amygdala and medial habenula but at lower densities. Unlike other 5-HT receptors, 5-HT$_3$ receptor subunits form a pentameric
cation channel that is selectively permeable to Na⁺, K⁺ and Ca²⁺ ions causing depolarisation. The 5-HT₃ receptor is a member of a superfamily of ligand-gated ion channels, which includes the muscle and neuronal nicotinic acetylcholine receptor (AChR), the glycine receptor, and the γ aminobutyric acid type A receptor (Karlin & Akabas, 1995; Ortells & Lunt, 1995). Like the other members of this gene superfamily, the 5HT₃ receptor exhibits a large degree of sequence similarity and thus presumably structural homology with the AchR (Maricq et al., 1991).

**5-HT₄ Receptor**

Receptor binding studies have established that the 5-HT₄ receptor is highly concentrated in areas of the rat brain associated with dopamine function such as the striatum, basal ganglia and nucleus accumbens. These receptors are also located on GABAergic or cholinergic interneurons and/or on GABAergic projections to the substantia nigra (Patel et al., 1995). The receptor is functionally coupled to the G protein.

**5-HT₅ Receptor**

Two 5-HT receptors identified from rat cDNA and cloned were found to have 88% overall sequence homology, yet were not closely related to any other 5-HT receptor family (Erlander et al., 1993). These receptors have thus been classified as 5-HT₅A and 5-HT₅B and their mRNAs have been located in man (Grailhe et al., 1994). In cells expressing the cloned rat 5-HT₅A site, the receptor was negatively linked to adenylyl cyclase and acts as terminal autoreceptors in the mouse frontal cortex (Wisden et al., 1993).

**5-HT₆ Receptor**

Like the 5-HT₃ receptor, the 5-HT₆ receptor has been cloned from rat cDNA based on its homology to previously cloned G protein coupled receptors. The rat
receptor consists of 438 amino acids with seven transmembrane domains and is positively coupled to adenylyl cyclase via the Gs G protein. The human gene has been cloned and has 89% sequence homology with its rat equivalent and is coupled to adenylyl cyclase (Kohen et al., 1996). Rat and human 5-HT₆ mRNA is located in the striatum, amygdala, nucleus accumbens, hippocampus, cortex and olfactory tubercle, but has not been found in peripheral organs studied (Kohen et al., 1996).

5-HT₇ Receptor

5-HT₇ receptor has been cloned from rat, mouse, guinea pig and human cDNA and is located on human chromosome 10q23.3-q24.4. Despite a high degree of interspecies homology (95%) the receptor has low homology (<40%) with other 5-HT receptor subtypes. The human receptor has a sequence of 445 amino acids and appears to form a receptor with seven transmembrane domains.

5-HT₂C Receptor Structure

The 5-HT₂C receptor was identified as a tritiated-5-HT binding site in the choroid plexus, tissue involved in production of CSF of various species that could also be labeled by tritiated-mesulergine and tritiated-lysergic acid diethylamide (LSD). Originally this site was seen as a new member of the 5-HT₁ receptor family and termed 5-HT₁C, because of its high affinity for [³H]5-HT (Pazos et al., 1999). However, once the receptor was cloned and more information about its characteristics became available, a shift to the 5-HT₂ receptor family and reclassification as 5-HT₂C receptors became accepted (Humphrey et al., 1993). The partial cloning of the mouse 5-HT₂C receptor (Lubbert et al., 1987) was shortly followed by the sequencing of the full length clone in the rat (Julius et al., 1989), mouse (Yu et al., 1991) and human (Saltzman et al., 1991). Additionally, a splice variant of the 5-HT₂C receptor has been observed in brain tissues of the rat, mouse and human (Canton et al., 1996). The functional significance of this variant is however, unclear as the protein product is
truncated and lacks a 5-HT binding site. More recently, it has been reported that 5-HT$_{2C}$ mRNA undergoes post-transcriptional editing to yield multiple 5-HT$_{2C}$ receptor isoforms with different distributions in brain. In functional terms, this is potentially of great significance as the amino acid sequences predicted from the mRNA transcripts indicate that the isoforms if expressed endogenously in significant amounts in brain tissue have different regulatory and pharmacological properties (Burns et al., 1997).

The gene for the 5-HT$_{2C}$ receptor is located on the human X chromosome at position q 24 (Xq24). The 5-HT$_{2C}$ receptor gene has three introns rather than two as in the case of the 5-HT$_{2A}$ and 5-HT$_{2B}$ produce a protein product with eight rather than seven transmembrane regions, which, if proven, would be unusual for a G-protein coupled receptor (Yu et al., 1991). There is high sequence homology, >80% in the transmembrane regions, between the mouse, rat and human 5-HT$_{2C}$ receptors. The mouse and rat 5-HT$_{2C}$ receptors possess six potential N-glycosylation sites, four of which are conserved in the human sequence. The rat 5-HT$_{2C}$ receptor has eight serine/threonine residues representing possible phosphorylation sites, all of which are conserved in the human sequence (Barnes & Sharp, 1999).

**Functional Effects Mediated via the 5-HT$_{2C}$ Receptor Signal Transduction**

Agonist binding to the 5-HT$_{2C}$ receptor activates phospholipase C via activation of a G protein (Gq11). Phospholipase C catalyzes the hydrolysis of phosphatidylinositol-4, 5-bisphosphate to inositol 1,4,5-triphosphate and diacylglycerol. Inositol 1,4,5-triphosphate, acting as a second messenger, diffuses through the cell cytoplasm and stimulates the release of calcium sequestered in the endoplasmic reticulum which in turn activates numerous cellular processes through the intermediacy of calmodulin and its homologs. The diacylglycerol remains associated with the plasma membrane where it activates protein kinase C to phosphorylate and thereby modulate the activities of a number of cellular proteins. It has been suggested that 5-HT$_{2C}$ receptors in choroid plexus may regulate CSF
formation as a result of their ability mediate cyclic guanosine monophosphate (cGMP) formation (Schulzen et al., 1988; Boess & Martin, 1994; Conn et al., 1987; Kaufman et al., 1995).

Genetic and molecular events regulate the creation of variants of 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors whose diversity has important functional significance. Overall sequence identity between the 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors is quite high and it is not surprising that the mechanisms of regulation for these two receptors are similar. There is, however, one striking difference between these two receptors, which involves RNA editing a mechanism for generating molecular diversity by altering the genetic code at the level of RNA. The 5-HT\textsubscript{2C} receptor is the only known G protein-coupled receptor whose mRNA undergoes post-transcriptional editing to yield different receptor isoforms (Sander-Bush et al., 2003). The different 5-HT\textsubscript{2C} receptor isoforms generated from RNA editing have demonstrated altered dynamics of agonist-induced calcium release. These distinctions in agonist-induced calcium release imply that edited 5-HT\textsubscript{2C} receptors may produce distinct physiological responses within the CNS (Price & Sanders-Bush, 2000). It has been shown that editing sites are located on the second intracellular loop, which contains a consensus sequence for G-protein interaction (Niswender et al., 1999). It is therefore clear that changes in amino acid sequence affect the coupling ability between the receptor and its protein. In this regard, it was recently reported that depletion of serotonin increases expression of 5-HT\textsubscript{2C} mRNA isoforms encoding receptors with higher sensitivity to serotonin. These results indicate that mRNA editing serves as a mechanism whereby 5-HT\textsubscript{2C} receptor activity is stabilized in the face of changing synaptic serotonergic input (Gurevich et al., 2002).

Serotonin and Serotonin Receptors in Epilepsy

The general recognition that serotonin plays a role in epileptic mechanisms is based on several lines of evidence from studies in both animal models of epilepsy and humans. In the genetically epilepsy-prone rat (GEPR) model of generalized epilepsy,
a decrease is found in brain concentration of serotonin (Dailey et al., 1989) as well as decreased $V_{max}$ for $[^{1}H]$ serotonin uptake by synaptosomes and tryptophan hydroxylase activity (Statnick et al., 1996). Pharmacologic treatments that facilitate serotonergic neurotransmission inhibit seizures in many animal models of epilepsy, including the GEPR rat, maximal electroshock model, pentylenetetrazol administration, kindling, and bicuculline microinjections in the anterior piriform cortex (area tempestas) (Statnick et al., 1996). Conversely, reduction of brain serotonin concentrations leads to an increase in seizure susceptibility in animal models of epilepsy (Wenger et al., 1973, Lazarova et al., 1983) as well as in humans. In human brain tissue surgically removed for seizure control, the level of 5-HIAA, which is a breakdown product of serotonin, was found to be higher in actively spiking temporal cortex as compared with normal tissue (Pintor et al., 1990). Finally, increased serotonin immunoreactivity has been reported in human epileptic brain tissue resected for the control of epilepsy (Trottier et al., 1996). Serotonergic neurotransmission exerts a considerable influence on hippocampal function. It is influenced powerfully by serotonergic projections from midbrain raphe nuclei (Moore & Halaris, 1975; Lidov et al., 1980), which modulate hippocampal electrical activity, hippocampal-dependent behaviours, and long-term potentiation (LTP), a form of hippocampal plasticity that has been implicated in memory formation (Winson, 1980; Bliss et al., 1983). Studies of the serotonergic modulation of hippocampal function have been complicated by the marked heterogeneity of 5-HT receptor subtypes, with at least 14 distinct subtypes expressed in the central nervous system. Determining the contributions of individual 5-HT receptor subtypes to the serotonergic regulation of hippocampal function is hindered by a paucity of subtype-selective drugs (8-12). Studies indicate 5-HT consistently hyperpolarized theophylline-treated hippocampal CA3 neurons and abolished theophylline-induced epileptiform activity. Efforts to determine the mechanisms through which serotonin (5-HT) systems regulate CNS excitability have been complicated by the marked diversity of 5-HT receptor subtypes
and the paucity of available selective agonists and antagonists. At least 14 distinct 5-HT receptor subtypes have been identified in the CNS and there are few selective pharmacological agents available to determine the functional roles of individual receptor subtypes.

Central 5-HT$_{1A}$ receptors function both as somatodendric presynaptic autoreceptors in the raphe nuclei as postsynaptic receptors in terminal field areas such as the hippocampus and many have different functional and regulatory characteristics, depending on the structures innervated (Barnes et al., 1999). In the raphe nuclei activation of 5-HT$_{1A}$ autoreceptors produces inhibition of serotonergic neurons and decreases 5-HT release and neurotransmission. In contrast, postsynaptic 5-HT$_{1A}$ receptor activation in the hippocampus increases 5-HT neurotransmission (Clarke et al., 1996). The 5-HT$_{1A}$ somatodendric autoreceptors and postsynaptic receptors differ in their adaptive response to prolonged stimulation during long term treatment with selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, which has antiseizure effects in several models (Hernandez et al., 2002). The fluoxetine effect is not dependent on GABA receptors are mediated by multiple receptor subtypes and shows regional variation (Pasini et al., 1996). Rats treated over the long term with fluoxetine showed desensitization of 5-HT$_{1A}$ somatodendric autoreceptors in the dorsal raphe nucleus but not of postsynaptic 5-HT$_{1A}$ receptors in the hippocampus (Le Poul et al., 2000). 5-HT$_{1A}$ receptor activation elicits a membrane hyper polarization response related to increase potassium conductance (Beck et al., 1991) and has an anticonvulsant effect in various experimental in vivo as well as in vitro seizure models. Including hippocampal kindled seizures in cats, intrahippocampal kainic acid induced seizures in freely moving rats and picrotoxin-bicuculline and kainic acid induced seizures in rat hippocampal slice preparations (Wada et al., 1992). The anticonvulsant effects of 5-HT$_{1A}$ receptor activation differ from region to region and from model to model. 5-HT is reported to inhibit low Mg$^{2+}$-induced epileptiform activity, by reduction of NMDA receptor-mediated excitatory postsynaptic potentials
in the subiculum and entorhinal cortex but not on areas CA3 and CA1 of hippocampus (Behr et al., 1996). The genetically epilepsy prone rat model (GEPR) illustrates 5-HT effects on seizure susceptibility. GEPRs have decreased 5-HT1A receptor density in the hippocampus compared to non-epileptic control rats (Statnick et al., 1996). In addition the SSRI sertraline produces a dose dependent reduction in the intensity of audiogenic seizures in GEPRs, correlating with increased extracellular thalamic 5-HT concentrations (Yan et al., 1995). However the model is complex and other neurotransmitters play a role, as 5-HT receptor activation increases release of catecholamines (Yan et al., 1998). 5-HT1B receptor was reported to inhibit rat ventral tegmental GABA release and 5-HT1B/1D activation increases nucleus accumbens dopamine release (Yan et al., 2001). Other receptor subtypes have received less attention. One study suggested an excitatory role of 5-HT3 receptors in a rat kindling model (Wada et al., 1997). Several knock out mouse models suggest a relation between 5-HT, hippocampal dysfunction and epilepsy. 5-HT1A knockout mice display lower seizure thresholds and higher lethality in response to kainic acid administration. Furthermore, 5-HT1A knockout mouse demonstrate impaired hippocampal dependent learning and enhanced anxiety related behaviours. Interactions between serotonergic and other neurotransmitters contribute to the behaviourial phenotype (Sarnyai et al., 2000). 5-HT2C receptor knockout mice showed a combination of obesity and sound induced seizures. Other receptor types are not altered in this model suggesting that the clinical effects are receptor subtype specific (Heiser et al., 1998). In contrast activation of 5-HT2C receptors potentiates cocaine induced seizures (O’Dell et al., 2000). The up-regulation of 5-HT2C receptors was reported in the brain stem which induces sympathetic stimulation was reported (Pyroja et al., 2007).

5-HT2C Receptors and Epilepsy

The distribution of 5-HT2C receptors and the action of non-selective drugs have prompted speculations that these receptors participate in the processing and
integration of sensory information, regulation of central monoaminergic system and modulation of neuroendocrine regulation, feeding behaviour anxiety and cerebrospinal fluid production. Mice devoid of 5-HT$_{2C}$ receptors showed epileptic phenotype (Tecott et al., 1995) associated with sporadic spontaneous seizures that occasionally result in death. Thus 5-HT$_{2C}$ receptors are involved in the neuronal network excitability which alters the threshold of seizure activity. Mice lacking these receptors exhibit increased focal excitability and facilitated propagation of seizure activity within the forebrain seizure system. These mice also exhibit lower thresholds for the expression of generalized seizures of either the tonic or clonic type. Importantly, the 5-HT receptor antagonist, mesulergine (2 or 4 mg/Kg), administered prior to electroshock testing, recapitulated the mutant phenotype in wild-type mice suggests that the seizure susceptibility profiles observed in 5-HT$_{2C}$ deficient mice are not secondary to developmental abnormalities caused by deletion of the gene (Applegate & Tecott, 1998). Together, these data strongly implicate a role for serotonin and the 5-HT$_{2C}$ receptors in the modulation of neuronal network excitability and seizure propagation throughout the CNS. Reduction in seizure activity has been observed for the 5-HT$_{2C}$ receptor agonists meta-chlorophenylpiperazine (mCPP) and 3-Trifluoromethylphenylpiperazine monohydrochloride (TFMPP) when microinjected bilaterally into the rat substantia nigra. This indicates that the 5-HT$_{2C}$ receptors in the substantia nigra contribute to seizure regulation (Gobert et al., 2000; Hutson et al., 2000). Serotonin 5-HT$_{2C}$ receptors, which play an important role in the control of mood, reward, motor function, and appetite, are implicated in the etiology and management of depression, anxiety, schizophrenia and other psychiatric disorders (Giorgetti & Tecott, 2004; Millan, 2005; Di Giovanni et al., 2006; Dutton & Barnes, 2006). Functional status of 5- HT$_{2C}$ receptors is elevated in depressed patients, in rat models of depression and upon experimental depletion of 5-HT (Heslop & Curzon, 1999; Millan, 2006).
Glutamate

Glutamate is a fast excitatory neurotransmitter in the CNS and has been shown, with GABA, to interact primarily with receptors in the synaptic cleft (Dingledine & McBain, 1999). L-Glutamate is a non-essential dicarboxylic amino acid synthesized mainly from 2-oxoglutarate by transamination reactions. Glutamate has strong excitatory effects on neurons. High affinity uptake systems move them into nerve endings. The occurrence of their receptors is known from sites where radiolabeled glutamate selectively binds on neural cell surfaces and through the discovery of their antagonists. Both procedures identify and characterize their receptors on neuronal and glial cells. These amino acids open sodium and potassium ion channels and cause a rapid excitatory response in most neurons at a very low concentration (Dingledine & McBain, 1999). Electrical stimulation of brain slices and cultured neurons releases glutamate in a Ca\textsuperscript{2+} dependent manner.

Glutamate is the main excitatory amino acid neurotransmitter in central and peripheral nervous systems. Its concentration in brain is higher than in other body tissues. In the brain, the concentration of glutamate is 3 to 4-fold greater than that of aspartate, taurine, or glutamine. The most abundant amino acid in synaptosomes is glutamate, followed by glutamine, aspartate, γ-aminobutyric acid and taurine. Glutamate cannot cross the blood-brain-barrier. The main source of glutamate carbon is glucose with synthesis of glutamate from glucose and other metabolites of the citric acid cycle. It appears however, that aspartate aminotransferase and glutaminase account for a majority of glutamate production in brain tissue (McGeer et al., 1987). Enzymes responsible for the synthesis of glutamate are in neurons as well as glial cells. 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. Many experiments have supported the view that the extent of glutamate release during epileptic seizures is so great that uptake is not able to re-establish the normal glutamate concentration and the excess of glutamate spreads by diffusion, activating neurons via extra-synaptic receptors. Increased glutamatergic transmission is regarded as one of the possible causes of seizure origination (Bradford, 1995). Most of the damage induced by seizure activity is generated by glutamatergic neurotransmission-driven excitotoxicity (Whetsell, 1996).

It activates two main types of postsynaptic receptors: (i) ionotropic glutamate receptors (iGluRs) that are ligand-gated channels, and (ii) metabotropic glutamate receptors (mGluRs) that are receptors coupled to GTP binding proteins. The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA–KA (Choi, 1988). Ionotropic glutamate receptors are divided into three major subtypes, on the basis of their affinity for glutamate selective structural analogues, notably for N-methyl-D-aspartate (NMDA). A distinction is made between those glutamate receptors activated by NMDA receptors and those not activated by NMDA, non-NMDA receptors. Cloning studies have demonstrated that these non-NMDA receptors can be further distinguished in AMPA and kainate receptors.
Glutamate and Glutamate Receptors in Epilepsy

Glutamate can cause convulsions when administered focally or systemically to experimental animals. Glutamate exerts its excitatory action via ligand-gated ion channels, NMDA and non-NMDA receptors, to increase sodium and calcium conductance. Reciprocal regulatory interactions exist between the activation of glutamatergic receptors and other transmitter systems, ion transport, gene activation and receptor modification. The flexibility and complexity of these interactions place glutamate-mediated transmission in a pivotal position for modulating the excitatory threshold of pathways involved in seizure generation. All classes of NMDA receptor antagonists, competitive NMDA antagonists, channel site antagonists, glycine site antagonists, polyamine site antagonists, as well as competitive and noncompetitive AMPA/kainate antagonists, display wide-spectrum anticonvulsant properties in acute and chronic animal epilepsy models, with varying degrees of behavioural side effects, ranging from minimal for some of the glycine site or competitive NMDA antagonists, to extensive for some of the high affinity open-channel NMDA antagonists. Transgenic mice with an editing-deficient AMPA receptor subunit, GluR2, display early onset of epilepsy. The GluR2 subunit confers an almost complete block of calcium conductance in homomeric or heteromeric AMPA receptors. Both the GluR2 receptor level and the RNA editing process are reduced significantly and the corresponding AMPA-evoked calcium current in pyramidal neurons increased significantly in accordance with the enhanced seizure susceptibility in these mice (Brusa et al., 1995). Neuronal (EAAC-1) and glial (GLT-1 and GLAST) glutamate transporters facilitate glutamate and aspartate reuptake after synaptic release. A down regulation of glutamate transporters would be compatible with enhanced excitatory activity. Transgenic mice with GLT-1 knockout display spontaneous epileptic activity (Tanaka et al., 1997) and mice treated chronically with antisense probes to EAAC-1 shows reduced transporter levels and increased epileptic activity (Rothstein et al., 1996). The reported changes in glutamate receptors and transporters subsequent to
sustained or chronic epilepsy are less consistent and frequently transient in nature; some of these changes reflect patterns of cell loss. A functional enhancement of NMDA receptors is observed in amygdala-kindled rats and in resected tissue from humans with temporal lobe epilepsy (Mody et al., 1998). The molecular alterations in the NMDA receptor responsible for this functional up regulation are not clearly defined but probably involve altered phosphorylation. Changes in the editing of the GluR2 AMPA subunit been reported in resected hippocampi from some patients with refractory epilepsy (Grigorenko et al., 1997). The mRNA levels of multiple AMPA subunits are also altered in kindled rats and in rats after sustained seizure activity evoked by kainate or pilocarpine.

**Ionotropic Receptors - 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 (Fagg & Baud, 1988). 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 NRI 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 (Murphy & Miller, 1988; Holopainen et al., 1989, 1990; MacDermott et al., 1986). 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; Lester et al., 1988) associated with synaptogenesis and synaptic plasticity.

**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; Verma & Moghaddam, 1996; 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.

**NMDA receptors and Epilepsy**

Several studies have shown an increase in the density of hippocampal and cortical NMDA receptors in some animal models of epilepsy (Yeh et al., 1989; Corlew et al., 2008). This up regulation reflects one molecular mechanism that maintains neuronal hyperexcitability in the course of the epileptic disease and is implicated in NMDAR-targeted therapies treating seizure disorders (Kalia et al., 2008). Epileptogenesis causes an NMDA/Ca\(^{2+}\)-dependent decrease in Ca\(^{2+}\)/calmodulin-dependent protein kinase II activity in a hippocampal neuronal culture model of spontaneous recurrent epileptiform discharges. When epileptiform activity is acutely induced in vitro, transient partial blockade of NMDA receptor-mediated calcium influx leads to selective long-term depotentiation of the synapses involved in the epileptic activity as well as a reduction in the probability of further epileptiform activity (Hellier et al., 2009). In postsynaptic densities isolated from epileptic human and rat neocortex, however, components of the NMDA-receptor complex were found to be down regulated (Wyneken et al., 2003).

**Metabotropic Glutamate Receptors**

Metabotropic glutamate (mGlu) receptors have a widespread distribution throughout the CNS and are understood to be involved in a number of physiological mechanisms including memory, learning and motor control. The mGluRs comprise a novel family of G-protein coupled receptors, which are characterized by a large ligand-binding N-terminal domain and seven transmembrane domains responsible for
G-protein coupling. The second intracellular loop of mGluRs determines G-protein coupling specificity, while other intracellular loops contribute to coupling efficiency. This differs somewhat from other G-protein coupled receptor families. Metabotropic glutamate receptors are classified into three groups based on their amino acid sequence homology, signal transduction mechanisms and pharmacology (Pin & Duvoisin, 1995).

Group I mGlu receptors (mGlu1/5) are positively coupled to phospholipase C (PLC) and induce the hydrolysis of phosphoinositide and the release of intracellular Ca\(^{2+}\) stores. Group II mGlu receptors (mGlu2/3) are negatively coupled to adenylate cyclase (AC) and have been shown to inhibit the production of cAMP and Ca\(^{2+}\) influx. Group III mGlu receptors (mGlu4, 6–8) are thought to be similarly coupled to AC and inhibit the production of cAMP. At least eight metabotropic glutamate receptors (mGluR1-8) occur in brain tissue. They are members of the group C family of G-protein-coupled receptors, GPCR (Bonsi et al., 2005). Based on sequence homology, agonist pharmacology and coupling to intracellular transduction mechanisms, metabotropic receptors are classified into three groups (Endoh, 2004). In the recent past, evidence accumulated in favour of a central role of group I mGlu receptors (Bonsia, 2008).

Group I consists of mGluR1 and mGluR5, including their splice variants. These receptors are coupled to an inositol phosphate/Ca\(^{2+}\) intracellular signaling pathway. These receptors are found on postsynaptic membranes (Endoh, 2004). In general, group-I mGluRs tend to increase neuronal excitability by inhibiting potassium conductances and activating non-selective cation currents. Conversely, mGluRs in groups II and III reduce neuronal excitability by activating potassium currents. They are involved in the modulation of the permeability of Na\(^+\) channels 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 (Chu & Hablitz, 2000). They can also inhibit glutamate release and can modulate voltage dependent Ca\(^{2+}\) channels (Endoh, 2004).

Metabotropic glutamate mGlu5 receptors have been implicated in the regulation of seizures and have been suggested as a target against which discovery of novel anticonvulsants may be possible. However, the experimental literature is not consistent in reporting anticonvulsant efficacy of mGlu5 receptor antagonists. But studies by Witkin et al., (2008) do not support the idea that mGlu5 receptors play as important a role in seizure control as previously speculated.

**Glutamate Transporter**

Glutamate transport is the major mechanism controlling extracellular glutamate levels, preventing excitotoxicity and averting neural damage associated with epilepsy. (McBean & Roberts, 1985; Rothstein et al., 1992, 1994, 1995; Robinson et al., 1993; Tanaka et al., 1997). Glutamate transporters are localized to the membranes of synaptic terminals and astroglial processes that ensheath synaptic complexes (Kanner & Schuldiner, 1987; Danbolt et al., 1992; Kanai et al., 1993; Rothstein et al., 1994, 1996; Conti et al., 1998). GLAST for glutamate–aspartate transporter, (EAAT-1) for excitatory amino acid transporter-1 (Storck et al., 1992; Arriza et al., 1994) and GLT-1 for glutamate transporter-1, EAAT-2 (Pines et al., 1992; Arriza et al., 1994) are astroglial glutamate transporters, and EAAC1 for excitatory amino acid carrier-1, EAAT-3 (Kanai & Hediger, 1992; Arriza et al., 1994; Shashidharan et al., 1994; Kanai et al., 1995; Bjoras et al., 1996; Nakayama et al., 1996; Velaz-Faircloth et al., 1996; Eskandari et al., 2000), EAAT-4 (Fairman et al., 1995) and EAAT-5 (Arriza et al., 1997) are neuronal proteins.
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 diacylglycerol (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\(^{2+}\)]i, 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-I\(\alpha\) and -I\(\beta\). In the brain, PKG-I is highly expressed in cerebellar Purkinje cells and to a lesser extent, in striatal medium spiny neurons (De Camilli et al., 1984). PKG-II is a membrane-associated protein that is expressed throughout the brain (de Vente et al., 2001). The effects produced by the cGMP signaling pathway modulate drug-induced neural plasticity leading to behavioural alterations (Jouvert et al., 2004).

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

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

**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., 1998), cyclic nucleotide gated channels involved in transduction of olfactory and visual signals (Goulding et al., 1992; Kaupp et al., 1989) and the cAMP-activated guanine exchange factors Epac 1,2 which specifically activate the monomeric G protein Rap (Rooij et al., 1998; Kawasaki, et al., 1998).

Pathophysiology of Temporal Lobe Epilepsy

EEG studies shows that the hippocampus is one of the earliest structures to be activated during seizures. In addition, the cure of epilepsy by surgical resection of the hippocampus in properly selected individuals led to the idea that hyperexcitability intrinsic to the hippocampus contribute to the development of epilepsy (Bausch & McNamara, 1999). Thus it is not surprising that from the perspective of mechanisms, the best studied form of seizure is the seizure activity in the hippocampus. Recent report states that different neuronal populations react differently to SE induction. For some brain areas most, if not all, of the vulnerable cells are lost after an initial insult leaving only relatively resistant cells and little space for further damage or cell loss (Covolan et al., 2006).
**Cell Loss**

The most frequent lesion in patients with TLE is mesial temporal sclerosis or hippocampal sclerosis, consisting of gliosis and neuronal loss in the CA1, CA3 and the hilus of the dentate gyrus (Houser *et al.*, 1990). This typical pattern of neuronal loss characteristic of hippocampal sclerosis (Kapur *et al.*, 1999; Lewis *et al.*, 1999) can be produced experimentally by repeated or prolonged seizures and results presumably from excitotoxic damage subsequent to excessive activation of glutamate receptors (Olney *et al.*, 1986; Sloviter *et al.*, 1994). There are striking similarities between the pathology produced in experimental animals by prolonged seizures (Sloviter *et al.*, 1991) or head trauma (Coulter *et al.*, 1996) and the pathological changes seen in the hippocampi of many patients with TLE (Meldrum & Bruton, 1992). Seven days and two months post-status epilepticus rats showed significant neuron loss in the pre-endopiriform nucleus, layer III of the intermediate piriform cortex, and layers II and III of the caudal piriform cortex (Chen *et al.*, 2007). There is an extensive loss of dentate hilar neurons (Bausch & Chavkin, 1997) and hippocampal pyramidal cells (De Giorgio *et al.*, 1997). Data also demonstrated cases where some granule cells of experimental animals are also highly vulnerable (Sloviter *et al.*, 1996). Seizure-induced astrocytic damage has also been documented (Schmidt-Kastner & Ingvar, 1996). Interestingly, in contrast to the many studies showing cell loss, a recent study described an increased generation of hippocampal granule cells as a consequence of seizures (Parent *et al.*, 1997). It is hypothesized that the astrocytes in sclerotic tissue have activated molecular pathways that could lead to enhanced release of glutamate by these cells. Such glutamate release may excite surrounding neurons and elicit seizure activity (Janigro, 2008). Induction of limbic epilepsy resulted in an increased proliferation of granule cells using bromodeoxyuridine labelling. Therefore, although death of certain cell populations was suggested as a main event during or as a result of epileptogenesis, there is also evidence of neurogenesis. Mechanistically, neuronal loss can occur with either active or passive participation of cellular constituents. This has
been referred to as apoptosis or necrosis (Kerr et al., 1972). Apoptosis is a form of gene-mediated death characterised by specific morphological features: early nuclear chromatin condensation, cytoplasmic compaction with cell shrinkage, endonuclease-mediated DNA fragmentation into oligonucleosomes, apoptotic body formation and well-preserved organelles. In contrast, necrosis resulting from sudden injury with the cell unable to maintain homeostasis is characterised by early cytoplasmic vacuolization before any nuclear changes occur and is associated with an inflammatory response (Tomei & Cope, 1991). It appears that epileptic neuronal death is primarily but not exclusively apoptotic (Charriaut-Marlangue & Ben-Ari, 1995). Long-term repetitive stimulation of the perforant path induced apoptosis in the granule cells but necrosis in the hilar and pyramidal cells (Sloviter et al., 1996). The surviving granule cells showed dendritic deformations and shrinkage (Isokawa & Mello, 1991).

**Axon sprouting**

In addition to the neuronal loss, the second morphological change induced in the hippocampus by seizures is sprouting of dentate granule cell axons which are commonly referred to as mossy fibres. This occurs in both animal models of epilepsy (Bausch & Chavkin, 1997) as well as in human epilepsy (Babb et al., 1991). Denervation of the inner molecular layer secondary to hilar cell loss is believed to constitute the initial stimulus for sprouting (Tauck & Nadler, 1985). The sprouted mossy fibre axons appear to make synaptic contacts with granule cells and GABAergic basket cells. It has been proposed that seizure induced expression of neurotropic genes which is suggested to underlie the sprouting of axons of the granule cell layer (Sutula et al., 1996). It has been established that nerve growth factor (NGF) protein levels in dentate granule cells are increased by seizure activity (Gall & Isackson, 1989).
**Gliosis**

Reactive gliosis occurs in response to injury, including pilocarpine-induced seizures, in the mature CNS. A salient manifestation of reactive gliosis is an increase in glial fibrillary acidic protein (GFAP), a protein subunit of glial intermediate filaments found exclusively in astrocytes in the CNS (Amaducci et al., 1981). Glial proliferation characteristically accompanies neuronal loss seen in Ammon's horn sclerosis and after various insults including status epilepticus and contributes to epileptogenesis.

**Dendritic Changes**

Dendritic degeneration is another common pathological finding in TLE and its animal models (Isokawa et al., 1998). Neurons from the hippocampus and neocortex from patients with chronic focal epilepsy showed dramatic dendritic abnormalities. Dendritic spine loss has been repeatedly reported and has been suggested to be more severe with an increased duration of a seizure disorder (Multani et al., 1994). Dendrites of pyramidal cells have also been reported to have varicose swellings at irregular intervals along their length (Muller et al., 1993). It was established that following initial acute seizures, surviving neurons undergo substantial changes in the morphology and density of dendrites and spines in the chronic phase, during which the gradual development of spontaneous seizure is established (Isokawa et al., 1998). In the pilocarpine animal model of epilepsy, the membrane time constant of neurons, which can assess a cell's total surface area and geographic extent of dendritic branches was reported to be significantly reduced in rats that experienced many spontaneous seizures in the chronic phase (Isokawa et al., 1996). This suggests that the higher the frequency of spontaneous seizures, the more severe the local dendritic shrinkage.
Mossy fibre sprouting and impaired inhibition.

Mossy fiber sprouting is a form of synaptic reorganization in the dentate gyrus that occurs in human temporal lobe epilepsy and animal models of epilepsy. The axons of dentate gyrus granule cells, called mossy fibers, develop collaterals that grow into an abnormal location, the inner third of the dentate gyrus molecular layer. Electron microscopy has shown that sprouted fibers form synapses on both spines and dendritic shafts in the inner molecular layer, which are likely to represent the dendrites of granule cells and inhibitory neurons. One of the controversies about this phenomenon is whether mossy fiber sprouting contributes to seizures by forming novel recurrent excitatory circuits among granule cells. Sprouting mossy fibers synapse almost exclusively with excitatory neurons in the granule cell layer and molecular layer of the dentate gyrus. Lesioning the synaptic input from the entorhinal cortex to granule cells also triggers mossy fiber sprouting and synaptogenesis in adult rats (Laurberg & Zimmer, 1981; Frotscher & Zimmer, 1983). A variety of experimental treatments that produce epilepsy also induce axon sprouting in other brain regions (Salin et al., 1995; Perez et al., 1996; McKinney et al., 1997; Esclapez et al., 1999). Repeated intense seizures caused an attenuation of GABA mediated inhibition of the granule cells and in the pyramidal cells of the hippocampus (Coulter et al., 1996). This change cannot be explained by a selective loss of GABAergic inhibitory interneuron, since the GABA immunoreactive neurons were shown to be more resistant to seizure-induced injury than other hippocampal neurons (Sloviter et al., 1987). Preservation of GABAergic cells in surgical specimens from patients with epilepsy was confirmed (Babb et al., 1989). The neurons among the most sensitive to the seizure-induced neuronal death are the mossy cells in the dentate hilus (Lowenstrin et al., 1992; Sloviter et al., 1989). These cells receive synaptic input from granule cells via collaterals of mossy fibres and from the entorhinal cortex via the perforant path. To account for the paradoxical loss of GABA-mediated inhibition with preservation of GABAergic neurons, the dormant basket cell hypothesis (Sloviter et
suggests that the seizure-induced loss of hilar excitatory neurons removes tonic excitatory projection to GABAergic basket cells, the inhibitory interneuron in the dentate hilus. Being deafferented these cells then lie dormant with the end result being disinhibition (Sloviter et al., 1987). Loss of mossy cells which govern lateral inhibition in the dentate area cause functional delamination of the granule cell layer and result in synchronous multilamellar discharges in response to excitatory input (Sloviter et al., 1994). Therefore, there are 3 premises to this theory: 1) the general preservation of the inhibitory network. 2) The loss of excitatory afferents to GABAergic interneuron, 3) decreased inhibition on principal cells (Bernard et al., 1998).

**Epilepsy and personality disorder**

An epileptic phenomenon that illustrates this point is the partial epileptic seizure, which can secondarily generalize. The partial epileptic seizure is a neuronal network phenomenon rather than just a simple focus of hyperactive cells, as once thought. When partial seizure begins, the activated network from which the aberrant properties emerge is relatively localized, resulting in a relatively minor, usually non-convulsive pattern of behaviours (e.g. automatisms). However, if the seizure secondarily generalizes, the neuronal network of the seizure undergoes expansion and additional networks (e.g. locomotion networks) become recruited. Dependency is one of the most common psychological characteristics of patients with epilepsy. It is a disabling disorder that induces a sense of decreased control and self-efficacy, social difficulties, a perception of being stigmatized and low self-esteem (Teddman et al., 1994; Collins, 1994). Individuals with epilepsy have higher incidence of psychiatric disorders than do those without epilepsy. Depression is the most frequently reported psychiatric condition in epileptic patients.

Depression represents one of the most common co-morbidities in patients with epilepsy. However, the mechanisms of depression in epilepsy patients are poorly
understood. Establishment of animal models of this co-morbidity is critical for both understanding the mechanisms of the condition, and for preclinical development of effective therapies.

**Neuroprotection and Drugs in Epilepsy**

Neuroprotection following status epilepticus should encompass not only the prevention of neuronal death, but also preservation of neuronal and network function. This is critical because these aims are not necessarily equivalent; prevention of neuronal loss, for example, does not inevitably prevent epileptogenesis. Anticonvulsant drugs prevent or terminate seizures. In so doing, these agents act on the emergent properties of the epileptogenic network to alter or diminish their function (Walker, 2007). This involves modification of specific neuronal components that result in sufficient elevation of seizure threshold that prevents the usual initiating mechanisms from activating the network. Such an action can totally prevent the seizure or result in blockade of specific behavioural components of the seizure thereby reducing seizure severity (Graumlich et al., 1999; Faingold, 1999). Long-lasting changes in neuronal networks are observed following repetitive experiences, including behavioural conditioning and repeated seizures. Experience repetition can induce neurogenesis in susceptible brain sites, resulting in structural and functional network changes. Structural changes are partially mediated by neurotrophic factors and excitation increases and burst firing in network neurons can result.

By classifying epilepsy syndromes - seizure type, age of onset, EEG evidence, associated impairments, - clinicians can begin to rationalise their approach and define the therapeutic options for each patient. When to start and/or stop drug treatment in epilepsy is a major issue, which requires detailed knowledge of the prognosis of the disorder. For 20-30% of sufferers, epilepsy is a chronic and disabling condition, refractory to drug treatment, which has immense social impact. Although currently available drugs are able to prevent seizures, there remains a clear unmet medical need
for new antiepileptic drugs. David Chadwick’s, Walton Centre for Neurology, Liverpool defines criteria for the ideal antiepileptic drug (AED):

- A clearly identified and novel mechanism of action;
- Simple pharmacokinetic profile - no interactions with existing drugs;
- Efficacy across the broad spectrum of seizure types;
- Low toxicity and wide therapeutic window;
- Low cost.

To put things in perspective, 30% of epilepsies are currently uncontrolled. There is little rational basis for use of AEDs and side effects and drug interactions are major problems. Current developments in mechanism based drug design hopefully will overcome these issues and allow for more confidence in therapy. Experiments in intact animals provide a complementary approach for the application of mechanistic information obtained in *in vitro* investigations. *In vivo* techniques allow the exploration of the regional selectivity of these mechanisms, since it has become quite clear that the anticonvulsant effects of certain drugs involve actions on a combination of specific currents and cellular properties selectively expressed in specific brain networks (Kao & Coulter, 1997). Whether such actions observed with acute anticonvulsant drug treatment continue during the duration of treatment is unknown, since it is becoming clear that chronic treatment with a number of drugs that modify neuronal properties often results in compensatory mechanisms that lead to tolerance or to additional chronic effects that contribute to the action of the drug (Chen et al., 1999).
Bacopa monnieri (Linn.) Pennel

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Lamiales
Family: Scrophulariaceae
Genus: Bacopa
Species: Bacopa monnieri

The drugs of plant origin are gaining importance and are being investigated for remedies of a number of disorders. Since the introduction of adaptogen concept (Lazarev, 1947), several plants have been investigated, which were used earlier as tonics due to their adaptogenic and rejuvenating properties in traditional medicine (Rege et al., 1999). Effective treatments such as anxiolytic drug therapy or cognitive-behavioural therapy exist but many patients remain untreated, experience adverse effects of benzodiazepines or do not benefit from full symptom control (Ernst, 2006). Commonly known as ‘Brahmi’, the plant has been used by Ayurvedic medical practitioners in India for almost 3000 years and is classified as a medhya rasayana, a drug used to improve memory and intellect (medhya). Bacopa monnieri is highly esteemed as a Rasayana drug in Ayurvedic medicine.

Bacopa monnieri (Linn) (family: Scrophulariaceae) is a perennial creeping annual plant found throughout the Indian subcontinent in wet, damp and marshy areas (Sheikh et al., 2007). The main stem is green or slightly purplish, obtuse-angular and 10-30 cm long with rooting at nodes. Leaves are opposite, short-petioled, obovate-oblong and somewhat succulent 1-2.5 cm long and 0.4-1 cm broad, glabrous on both sides and dotted with minute black specks. Flowers are solitary axially, white or purple-tinged. Fruits are ovoid capsules, about 5 mm long and glabrous. The plant
flowers and bears fruits throughout the year, though mostly during February to April. Both fresh and dried whole plant is used for drug preparation.

The plant elaborates several tri-terpenoids of dammarane group which occur mostly as glycosides (saponins) and are present to the extent of 2-3% on dry herb basis and are considered medicinally valuable. Around 10 of these have been obtained pure (Bacosaponins A-F, Bacoposides III-IV). Betulinic acid a tri-terpene with known anticancer activity has also been obtained from the plant (Brown et al., 1960). Four glycosides based on phenylethanol as basic unit have been isolated (Chakravarthy et al., 2002). Of other secondary metabolites attention is drawn to flavonoids like luteolin and its glycosides, sugars (D-mannnitol), usual sterols - β-sitosterol, stigmasterol and its esters and paraffins – heptacosane and hentriacontane.

The pharmacological properties of Bacopa monnieri were studied extensively and the activities were attributed mainly due to the presence of characteristic saponins called as Bacosides (Deepak & Amit, 2004). Bacosides are complex mixture of structurally closely related compounds, glycosides of either jujubogenin or pseudojujubogenin. Bacosides have been found to offer protective role in the synaptic functions of the nerves in hippocampus (Kishore et al., 2005). There are few methods reported in the literature for quantification of Bacosides in plant extracts and formulations. Spectrophotometric methods (Pal & Sarin, 1992; Prakash et al., 2008) developed based on the hydrolysis of Bacosides to an aglycone that has an absorption maximum at 278 nm. A high performance thin-layer chromatographic method was developed for the estimation of Bacoside A in Bacopa monnieri plant and its formulations (Shrikumar et al., 2004). A few high performance liquid chromatographic (HPLC) methods were also developed for the quantification of Bacosides in Bacopa monnieri extracts and formulations (Pal et al., 1998).

The whole plant is a potent nerve tonic and is well known for its neuropharmacological effects. It is used in the treatment of epilepsy, insanity, hysteria and other neural disorders. It is claimed to improve memory and mental function. Its
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neuropharmacological activity (Singh & Dhawan, 1997) and anti-oxidant activities (Singh et al., 2006) has been also reported. After clinical trials in human volunteers, a chemically standardized extract of *Bacopa monnieri* has been now made available for clinical use by the Central Drug Research Institute in India (Dhawan & Singh, 1996). *Bacopa monnieri* has been reported to possess anxiolytic, antidepressant and memory enhancing activity (Bhattacharya & Ghosal, 1998; Bhattacharya et al., 1999; Sairam et al., 2002; Das et al., 2002). Studies by Rai et al., (2003) identified the potential of *Bacopa monnieri* as an effective adaptogen that normalizes the stress induced elevation in plasma glucose, creatine kinase and adrenal gland weight similar to the effects of *Panax quinquefolium* (PQ), commonly known as American ginseng, a standard adaptogenic plant. Tri-terpenoid saponins and bacosides present in *Bacopa monnieri* are considered to be responsible for enhancing cognitive function (Russo & Borrelli, 2005), however the detailed mechanisms for its adaptogenic activity are yet to be explored. The standardized extract of *Bacopa monnieri* was reported earlier to have significant anxiolytic activity () and improve memory retention in Alzheimer’s disease (Bhattacharya & Ghosal, 1998; Bhattacharya et al., 1999) but the biochemical basis for the observed behavioural changes was not established. During stressful conditions, changes in monoamines -NA, DA and 5-HT, are well associated with transient behavioural aberrations in memory, learning and other mood disorders. Since *Bacopa monnieri* normalizes stress mediated transient deregulation of plasma corticosterone and monoamine changes in brain is one of the reasons for its adaptogenic activity (Rai et al., 2003) and mild anxiolytic effects, respectively (Shanker & Singh, 2000). Deregulated function of monoamines is one of the principle reasons for memory dysfunction during stressful conditions (Rachel et al., 2003)

Anti-cancer activity of the alcoholic extract (Elangovan et al., 1995) and analgesic activity of bacosine, a tri-terpenoid isolated from the plant (Vohora et al., 1997) have also been reported. Alkaloids, steroids, tri-terpenoids, hydrocarbons, flavanoids, amino acids and saponins were reported from the plant. Studies also indicated the
protective effect of *Bacopa monnieri* extract on morphine induced brain mitochondrial enzyme activity. The dammarane tri-terpene glycosides bacoside A and bacoside B isolated from the plant have been shown to be responsible for the neuropharmacological activities of the plant. It has been found to be well tolerated and without any untoward reaction or side effects in regulatory pharmacological and toxicological studies. The LD50 of aqueous and alcoholic crude extracts of *Bacopa monnieri* in rats were 1000 mg and 15 g/Kg by intraperitoneal route, respectively (Martis et al., 1992). The aqueous crude extract given orally at a dose of 5 g/Kg did not show any toxicity. The LD50 of the alcoholic crude extract was 17 g/Kg given orally.

Animal behaviour is the bridge between the molecular and physiological aspects of biology and the ecological. Behaviour is the link between organisms and environment as well as between the nervous system and the ecosystem. Behaviour is one of the most important properties of animal life. Behaviour plays a critical role in biological adaptations. Behaviour is that part of an organism by which it interacts with its environment. Neuroethology, the integration of animal behaviour and the neurosciences, provides important frameworks for hypothesizing neural mechanisms. Careful behavioural data allow neurobiologists to narrow the scope of their studies and to focus on relevant input stimuli and attend to relevant responses. In many cases the use of species specific natural stimuli has led to new insights about neural structure and function that contrast with results obtained using non-relevant stimuli.

Anxiety like behaviours in rats were measured using two pharmacologically well-validated exploration-based tests, elevated plus-maze (Komada *et al*., 2008) and novel open field (Cryan & Holmes, 2005; Rodgers, 1997). The elevated plus maze test is one of the most popular tests of all currently available animal models of anxiety (Crawley, 2007). This test for anxiety-like behaviour has been used for screening and phenotyping transgenic and knockout mice (Horii *et al*., 2008; Tsujimura *et al*., 2008) and for drug discovery. The elevated plus maze test has a strong predictive validity for
screening anxiolytic drugs (Mechiel & De Boer, 2003); anxiolytic drugs specifically increase, and anxiogenic drugs specifically decrease, the number of entries into the open arms and the time spent there. Depression-related behaviour was tested using a pharmacologically-validated test, the forced swim test (Rodgers, 1997; Olivier et al. 2008), sucrose consumption test and social interaction test (Sano et al., 2008). Rotarod test has been previously been employed to assess the motor skills in rodents (Samad et al., 2008).

The present work was carried out to investigate the alterations of the 5-HT$_{2c}$ and NMDA receptors in the brain regions of pilocarpine induced epileptic rats. The work focuses on the evaluation of the antiepileptic activity of extracts of *Bacopa monnieri* in vivo acting through 5-HT$_{2c}$ and NMDA receptors and the second messengers IP3, cGMP and cAMP. Gene expression studies using Real-time will be done to confirm receptor data. This will be confirmed by confocal microscopic studies using immunofluorescent antibodies to specific receptor subtypes. These molecular level changes will be confirmed with behavioural studies. These studies will help us to elucidate the functional role of 5-HT$_{2c}$ and NMDA receptors in epilepsy and the neuroprotective role of *Bacopa monnieri* through 5-HT$_{2c}$ and NMDA receptors during epilepsy which has immense therapeutic relevance in the management of epilepsy.