Literature Review

Definition

Epilepsy is characterized by recurrent, unprovoked, paroxysmal episodes of brain dysfunction manifesting as a large number of clinical phenomena, like altered levels of consciousness, involuntary movements, abnormal sensory phenomena, autonomic changes and transient disturbances of behaviour. It is a variety of symptoms arising from different kinds of pathologic processes of the brain rather than a specific disease or even a syndrome. During epileptic seizures, there are excessive discharges of electrical activity of the neurons in the brain and the clinical manifestations depend on the origin and the localization of those pathological discharges (Browne & Feldman, 1983; Waltimo et al., 1983; Engel & Pedley, 1997).

Epidemiology

The incidence epilepsy is higher during the first years of life, falls dramatically thereafter and increases again in the elderly. Approximately 50% of cases of epilepsy begin in childhood or adolescence (Hauser et al., 1993). Many studies suggest that males are at a greater risk for unprovoked seizures and epilepsy than female subjects (Hauser et al., 1997). The prevalence of epilepsy is 0.7-0.8% worldwide and the lifetime cumulative incidence is 1-3%.

Etiology

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). At present, more and more genetic factors underlying different types of epileptic syndromes are revealed. It is also known that certain brain areas, i.e. temporal and frontal lobes are more susceptible to produce epileptic seizure activity than the other regions (Larsen & Livanainen, 1994). However, there are also patients with unresolved etiology of epilepsy (Hauser et al., 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.

Classification

The term "epilepsy" encompasses a number of different syndromes whose cardinal feature is a predisposition to recurrent unprovoked seizures. Although specific seizures can be classified according to their clinical features like complex partial seizures and generalized tonic-clonic seizures, (Commission on Classification and Terminology of the International League against Epilepsy, 1981). Epilepsy syndromes also classified according to the type of seizure, the presence or absence of neurological or developmental abnormalities and electroencephalographic (EEG) findings. Epilepsy syndromes fall into two broad categories: generalized and partial syndromes (Benbadis et al., 2001). In generalized epilepsies, the predominant type of seizures begins simultaneously in both cerebral hemispheres. Many forms of generalized epilepsy have a strong genetic component. In partial epilepsies, by contrast, seizures originate in one or more localized foci, although they spread to
involve the entire brain. Most partial epilepsies are believed to be the result of one or more central nervous system insults, but in many cases the nature of the insult is never identified.

**Partial seizures**

Seizures that begin in a focal region of the cerebral cortex, often within one lobe of the brain are termed partial seizures. Partial seizures remain focal throughout the duration of the seizure or propagate *via* neuronal pathways and networks to various regions of the hemisphere (Chabolla *et al.*, 2002). When partial seizure spreads to involve the majority of both cerebral hemispheres, it is said to be secondary generalized (Chabolla *et al.*, 2002). Partial epilepsies comprise slightly over 50% of all epilepsies (Keranen *et al.*, 1988, Hauser *et al.*, 1997, Williamson *et al.*, 1997).

The symptoms associated with a partial seizure depend on the brain regions involved. In theory, any function produced by the cortex (e.g. somatosensory, motor, autonomic, psychic phenomena) can be a symptom of a seizure. The first sign or symptom of a seizure is often, but not always, the best indicator of the site of seizure origin. The most common sites of producing epileptic discharges are temporal and frontal lobes (Chabolla *et al.*, 2002).

**Generalized seizures**

When epileptic seizure involves both cerebral hemispheres at the onset, it is termed as generalized seizure. At the onset of a generalized seizure, patients experience a vague, indescribable warning, although the vast majority of patients lose consciousness without premonitory symptom. With the loss of consciousness, tonic-clonic convulsions occur. A generalized seizure also manifest itself as absence, atonic
or myoclonic seizures. Idiopathic generalized epilepsies are often childhood idiopathic syndromes, some of which have an excellent prognosis, whereas some of them are thought to require lifelong medication (Chabolla et al., 2002).

**Diagnosis**

The procedures needed for the diagnosis of epilepsy include medical history with information on the possible predisposing events, a detailed description of the seizures and clinical evaluation with special respect paid to the cardiovascular and neurological examination. EEG-recording reveals focal or generalized spikes and slow waves or other epileptic phenomena. Magnetic resonance imaging (MRI) is recommended as the first-line imaging method of the brain when seizures are thought to be of focal origin. MRI detects pathologic conditions that cannot be diagnosed with CT.

**Prognosis**

The prognosis of epilepsy depends greatly on the underlying cause. At the beginning of the last century, all epilepsies were considered chronic. Later, however, the prognosis has become markedly better with the better epidemiological studies of less selected populations (Liow et al., 2007). The studies indicate that when treated, more than two-thirds of the patients with newly diagnosed epilepsy soon enter long-term remission. Symptomatic epilepsies are more likely to relapse than idiopathic or cryptogenic epilepsies. An abnormal EEG pattern increases the risk for the recurrence of seizures (Sander & Sillanpaa, 1997).

It is well known that the mortality of the patients with epilepsy exceeds 2-3 times that among the general population. The increased mortality is due to excess
morbidity, accidents during the seizures, status epilepticus and suicides. The most important epilepsy-related death is 'Sudden Unexpected Death in Epilepsy Patients' (SUDEP) that accounts for as much as 10-15% of the deaths among epilepsy patients. Many childhood epilepsies have a better prognosis than epilepsies in adults, but there are also severe childhood epilepsies that eventually lead to increasing neurological deficits and even to death (Sander & Sillanpää, 1997).

**Status Epilepticus**

*Status epilepticus* is defined as a condition characterized by an epileptic seizure frequently repeated and prolonged as to create a fixed and lasting condition (Gastaut et al., 1970). Lowenstein and Alldredge (1998) advocated the use of an operational definition of status epilepticus: either continuous seizures lasting at least 5 minutes or two or more discrete seizures between which there is incomplete recovery of consciousness (Lowenstein & Alldredge, 1998). Any type of seizure develop into status epilepticus, although the form most often seen in adults is tonic-clonic status epilepticus. The morbidity and mortality from status epilepticus are related to three factors: damage to the central nervous system (CNS) caused by acute insult precipitating the status epilepticus, systemic stress from repeated generalized tonic-clonic convulsions and injury from repetitive electrical discharges within the CNS. Systemic effects of repeated generalized seizures influence cardiovascular, respiratory and renal failure (Leppik et al., 1990). In addition, a number of biochemical changes not related to systemic effects of tonic-clonic activity occur in the CNS. Sixty minutes of repeated neuronal discharge results in severe neuronal death (Meldrum & Brierley, 1973). Neuropathological and imaging studies have shown damage in the hippocampus and in the amygdala, piriform cortex, thalamus, cerebellum and cerebral cortex after convulsive and nonconvulsive status epilepticus episodes in patients (Tien
& Felsberg, 1995; Wieshman et al., 1997). In vivo, the measurement of neuron-specific enolase provides further evidence of acute cerebral damage following status epilepticus. The enzyme was elevated in the serum of both generalized convulsive and nonconvulsive status epilepticus patients within 24 to 48 hours after the onset of status (DeGiorgio et al., 1995; Rabinowicz et al., 1995). The therapy for status epilepticus currently consists of agents which stop seizures (benzodiazepines, phenytoin, barbiturates) (Bleck et al., 1991). In a study trial comparing treatments for generalized convulsive status epilepticus, diazepam plus phenytoin, lorazepam or phenobarbital were equally effective therapies (Treiman et al., 1998). Due to the significant morbidity and mortality associated with the insult despite current medical treatment, status epilepticus remains one of the most serious disorders affecting the CNS.

**Structures and Connections of the Medial Temporal Lobe**

The limbic system forms a border around the upper brain stem, diencephalon and corpus callosum. The main components of the limbic system are the hippocampal formation, limbic association cortices including parahippocampal gyrus and the amygdaloid complex (Braak et al., 1996). Both the amygdala and hippocampus are located in the medial part of the temporal lobe, adjacent to the parahippocampal gyrus. This gyrus is a “continuation” of the cingulate gyrus onto the inferior surface of the brain. The cortex of the parahippocampal gyrus includes the subiculum and entorhinal cortex, both of which are functionally related to the hippocampus. The hippocampal formation is a prominent, bulging eminence in the floor of the temporal horn of the lateral ventricle. During development, the hippocampal formation undergoes an enfolding into the temporal lobe. This results in the interdigitation of two c-shaped structures, the hippocampus and the dentate gyrus. There is further subdivision of the
hippocampus into three regions, which are referred to as Cornu Ammonis (CA) fields. The CA3 field borders the hilus of the dentate gyrus; a short CA2 field follows; and a more extensive CA1 merges with the subiculum (Amaral & Insausti, 1990). The major source of cortical inputs to the hippocampal circuit is formed by the entorhinal cortex (Witter & Amaral, 1991). The human entorhinal cortex is made up of six layers, of which layer IV does not appear throughout all subfields of the entorhinal cortex (Insausti et al., 1995). In the most classical hippocampal pathway, cells in layers II and III of the entorhinal cortex give rise to the perforant path that distributes to all subfields of the hippocampal formation, including the dentate gyrus (Witter & Amaral, 1991). From the dentate gyrus, granule cells project to the CA3 field of the hippocampus. The CA3 pyramidal cells in turn send a major projection to the CA1 field. Much of the input from the CA1 field is then sent on to the subiculum. From the subiculum, information conveyed to the deep layers of the entorhinal cortex (Amaral et al., 1984; Witter et al., 1993). The entorhinal cortex receives prominent cortical innervation (Amaral & Insausti, 1990). Two-thirds of this cortical input originates in the perirhinal and parahippocampal cortices (Insausti et al., 1987). The perirhinal cortex areas 35 and 36 (Brodmann et al., 1909) is bounded medially by the entorhinal cortex and laterally by temporal association areas. It also extends anteriorly to include the medial portion of the temporal pole (Suzuki et al., 1996a). The parahippocampal cortex is caudally adjacent to both the entorhinal cortex and the perirhinal cortex, and is made up of a smaller, medially situated area TH and a larger, laterally situated area TF (Suzuki & Amaral, 1994). Direct input to the entorhinal cortex originates in several cortical regions in the frontal and temporal lobes, and in the insular and cingulated cortices, as well as in the adjacent perirhinal and parahippocampal cortices (Insausti et al., 1987). All these projections are reciprocal. There are extensive reciprocal connections with the hippocampus, the entorhinal cortex and the amygdala (Amaral et
The amygdaloid complex is composed of more than ten nuclei and their subdivisions which have different cytoarchitectonic, chemoarchitectonic, and connectional characteristics (Amaral et al., 1992; Pitkänen et al., 1997). The lateral nucleus of the amygdala gives rise to a prominent projection to layer III of the entorhinal cortex (Amaral & Insausti, 1990). There are also additional projections from the amygdaloid complex to the hippocampus and to the subiculum (Aggleton et al., 1986). Conversely, the subiculum and the entorhinal cortex originate return projections to the amygdala (Aggleton et al., 1986; Amaral et al., 1986). In general, the amygdaloid complex projects to a greater number of cortical regions than those from which it receives projections (Amaral et al., 1987). Essentially, all major divisions of the temporal cortex receive a projection from the amygdala. The perirhinal cortex, particularly, has prominent interconnections with the amygdala nuclei (Suzuki et al., 1996b). Moreover, there is evidence of reciprocal connectivity with the amygdala and portions of the frontal and insular cortices. The general conclusion about the functional connectivity is that the amygdaloid complex is directly and reciprocally linked to a wide variety of cortical regions and can influence the sensory information processed to various degrees. In contrast, cortical information is funneled into and out of the hippocampal formation through polysensory border regions and highly processed before reaching the hippocampus (Amaral et al., 1987; Insausti et al., 1987).

Temporal lobe epilepsy (TLE)

Temporal lobe epilepsy is considered the most common epileptic syndrome and it is estimated that approximately 80% of patients with partial seizures have temporal lobe epilepsy (Williamson et al., 1997). TLE can be subclassified into mesial temporal lobe epilepsy (MTLE) and lateral temporal neocortical epilepsy (L.TLE).
MTLE comprises the majority of the cases of epilepsy refractory to pharmacotherapy (Babb & Brown, 1987). However, it may be remediable to surgery because hippocampal sclerosis can often be seen as an underlying pathology in MTLE (Thadani et al., 1995, Benbadis et al., 1996). In fact, surgical treatment can abolish seizures in 80-90% of patients with MTLE (Wieser & Williamson, 1993).

Temporal lobe epilepsy is characterized clinically by the progressive development of spontaneous recurrent seizures (SRS) from temporal lobe foci (Engel et al., 1989, 1996). Before exhibiting SRS, patients with TLE usually experience epileptic status early in life, followed by a seizure-free period ranging from months to years (Engel et al., 1989; Lothman & Bertram, 1993). TLE is also characterized pathologically by unique morphological alterations in the hippocampus. The most frequently observed alteration is massive neuronal loss in the hilus of the dentate gyrus and in the CA1 and CA3 pyramidal cell layers (Engel et al., 1989; Lothman & Bertram, 1993; Ben-Ari & Cossart, 2000). Another common morphological alteration is mossy fiber sprouting, the growth of aberrant collaterals of granule cell axons into the inner molecular layer of the dentate gyrus (Sutula et al., 1989; Houser et al., 1990; Babb et al., 1991; Isokawa et al., 1993). A prominent hypothesis states that hippocampal neuronal loss and mossy fiber sprouting play a critical role in the genesis and progression of TLE (Lothman & Bertram, 1993; Wasterlain et al., 1993, 1996; Engel, 1996; Lowenstein et al., 1996; Coulter et al., 1999; Houser et al., 1999; Ben-Ari & Cossart, 2000). This hypothesis is supported by several lines of evidence. First, partial hippocampal removal including the site of neuronal damage results in a seizure-free state or a marked reduction of seizure frequency in many patients (Flaconer & Serafetinides, 1963; Rasmussen et al., 1983; King et al., 1986; Brown & Babb, 1987; Engel et al., 1987, 1989; Bruton et al., 1988; Babb et al., 1990). Second, the sprouted mossy fibers seemingly form a powerful monosynaptic recurrent
excitatory pathway, through which seizures is generated (Sperk et al., 1994; Lowenstein et al., 1996; Lynch & Sutula, 2000). This suggestion is supported by the findings that the intensity and time course of mossy fiber sprouting positively correlate with the severity and time course of SRS (Sutula et al., 1989) and that the sprouted mossy fibers seemingly form recurrent excitatory circuits (Tauck & Nadler, 1985; Cronin et al., 1992; Lynch & Sutula, 2000).

**Cholinomimetics**

The relationship of cholinergic mechanisms to epilepsy was suspected by neurologists by the turn of the 19th century (Langley & Kato, 1915). Decades later it was suggested that acetylcholine (ACh) may be primarily involved in human convulsive disorders (Brenner & Merrit, 1942). In 1945, it was demonstrated experimentally in cats that intracisternal injection of acetylcholine resulted in motor seizures (Forster et al., 1945). An epileptogenic potential of ACh was discovered that chronically isolated monkey cortex demonstrated an increased sensitivity to locally administered Ach (Echlin et al., 1959). In later years, evidence from electrophysiological experiments reinforced the idea that ACh may be involved in the cellular mechanisms of epilepsy (Dichter & Ayala, 1987). In terms of specific mechanisms, it has been shown experimentally that muscarinic cholinergic excitation in the brain occurs as a result of a reduced voltage-dependent and Ca^{2+}-dependent K' conductance and is mediated by voltage dependent Ca^{2+} and K' conductance (Benardo & Prince, 1982). Acetylcholine functions by promoting the inward flow of Ca^{2+} and Na' into neurons which is responsible for the membrane depolarization that leads to epileptic events (Pumain et al., 1983). Muscarinic blockade slows and degrades the
location-specific firing of hippocampal pyramidal were reported early (Brazhnik et al., 2003)

**Pilocarpine**

Pilocarpine is a potent cholinergic agonist originally isolated from the leaflets of *Pilocarpus microphyllus*. It is commonly used in the treatment of acute glaucoma in humans (Hardman et al., 1996). Single systemic high dose (300-400 mg/kg) pilocarpine injection as a novel animal model of TLE was established (Turski et al., 1983). The systemic administration of this muscarinic cholinergic agonist produced electroencephalographic and behavioral seizures, accompanied by widespread brain damage similar to that observed in autopsied brains of human epileptics. The 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. It is generally accepted that the hippocampus is indeed one of the earliest structures affected following pilocarpine treatment. Later 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). Behaviorally, 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., 1957). 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 et al., 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).

**Focal structural lesions in temporal lobe**

It has been established that hippocampal damage is the most common pathology underlying TLE (Babb & Brown, 1987). Neuron loss is usually located in the fields H1 and H3 of the hippocampus and when the neuron loss is restricted to those areas, it is regarded as the classic hippocampal cell loss (Babb & Brown, 1987; Sutula et al., 1989). However, more widespread neuron loss is often seen in resected temporal lobes of patients with TLE (Margerison & Corsellis, 1966; Bruton et al., 1988). The structures suffering from neuron loss in addition to hippocampus include the amygdala, the uncus of the hippocampus and the parahippocampal gyrus. This form of neuron damage is called as mesial temporal lobe sclerosis. (Engel et al., 1992; Wieser et al., 1993; Williamson et al. 1993; Thadani et al., 1995)

The neuronal reorganization continues with recurrent seizures and clinical observations on the development of medical intractability of MTLE also suggest an ongoing process (French et al., 1993; Engel et al., 1997). Recent studies have shown that, at least in some patients there is an association between an initial precipitating injury prior to habitual seizure onset and hippocampal sclerosis (Trenerry et al., 1993; Mathern et al., 2002). However, patients with episodes of generalized tonic-clonic status epilepticus and prolonged partial seizure activity may develop progressive hippocampal neuronal loss in a widespread distribution that is dissimilar to classic Ammon’s horn sclerosis. It is concluded that hippocampal sclerosis is presumably both the cause and effect of seizures (Bruton et al., 1988, Gloor et al., 1991).
**Pathophysiology of Temporal Lobe Epilepsy**

EEG studies show 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 CA 1, 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-endo piriform 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 (DeGiorgio et al., 1997) Recent 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). 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 characterized by early cytoplasmic vacuolization before any nuclear changes occurs and is associated with an inflammatory response (Tomei and 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 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 central nervous system (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.

Impaired Inhibition.

Repeated intense seizures caused an attenuation of gamma-aminobutyric acid (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 al., 1987) 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).

ROLE OF NEUROTRANSMITTERS IN EPILEPSY

Epinephrine and Norepinephrine (NE)

The modification 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 et al., 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 (Libet et al., 1977; 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; Browning et al., 1989; Lauterborn & Ribak, 1989; Dailey et al., 1991) (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 et al., 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, 1986;
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 et al., 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 favor 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., 2000; 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
D1 receptor activation. In addition, changes in excitability mediated by D2 receptors
have been reported (Gulledge & Jaffe 1998; 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 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 may lead 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 1992; McCormick & Pape, 1990) and neocortical circuits (McCormick et al., 1993). Dopamine is known to modulate epileptiform discharges both in vivo (Alam & Starr, 1992, 1993b, 1994a; George & Kulkarni, 1997) and in vitro (Alam & Starr 1993b, 1994b; 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 D₁ receptors and an anti-convulsant effect via D₂ receptors (Starr et al., 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 D₁ and D₂ receptors (Bo et al., 1995). Seizure enhancement is presumed to be a specific feature of D₁ receptor stimulation, whereas D₂ receptor stimulation is anticonvulsant (Alam & Starr, 1992, 1993). Decreased D₂ receptor binding in the brainstem were reported in other neurological diseases like diabetes (Shankar et al., 2006)
Serotonin

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, may be 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 hyperpolarization 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). Blockade of a number of receptors- 5-HT₃ and 5-HT₂A/C was reported to not alter the reduction in seizure severity and increase in the threshold produced by fluoxetine (Watanabe et al., 1998) 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 behaviors. Interactions between serotonergic and other neurotransmitters contribute to the behavioral phenotype (Samyai 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-HT$_{2C}$ receptors potentates cocaine induced seizures (O’Dell et al., 2000). The up-regulation of 5-HT$_{2C}$ receptors were reported in the brain stem which induce sympathetic stimulation were reported (Pyroja et al., 2007)

GABA

$\gamma$-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 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 GABA$_A$ 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 $\alpha4$ 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 GABAA receptor \( a_5 \) subunit in CA1/2 (Houser et al., 1995). Loss of \( a_5 \) and \( a_2 \) 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.
Glutamate

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 behavioral 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.

Metabotropic glutamate receptors are classified into three functional groups on the bases of their sequence homology, second messenger effectors and pharmacology (Dingledine & Conn 2000, Meldrum, 2000). Group I comprises mGluR1 and mGluR5, which are linked via G proteins to activation of phospholipase C. Group II (mGluR2 and mGluR3) and Group III (mGluR4, mGluR6, mGluR7, mGluR8) are both negatively linked to adenylyl cyclase activation. Activation of Group I mGluR enhances neuronal excitability by several mechanisms (blockade of accommodation to a steady current, potentiation of the effects of NMDA and AMPA and depolarization); accordingly, agonists acting on Group I receptors have convulsant activity (Ghauri et al., 1996, Tizzano et al., 1995). Conversely, Group I antagonists selective for mGluR1 have anticonvulsant activity in several experimental seizure models (Chapman et al. 1999 & 2000, Thomsen et al., 1994). Activation of Group II and Group III receptors by reasonably selective agonists appears to have mixed convulsant/anticonvulsant action, although a prolonged anticonvulsant action
seems to predominate (Tang et al., 1997, Tizzano et al., 1995). Down-regulation of mGluR8 in pilocarpine epileptic rats was reported early (Kral et al., 2003). The anticonvulsant effect of metabotrophic glutamate 8 receptor agonist in the pilocarpine model of epilepsy was reported. (Jiang et al., 2007)

L-Glutamate is the major excitatory neurotransmitter in the brain and serves a number of functions in the CNS (Nicholls & Attwell, 1990). This dicarboxylic amino acid is a precursor to the inhibitory amino acid neurotransmitter γ-aminobutyric acid (GABA) for the Krebs cycle intermediate α-ketoglutarate, and for the amino acid glutamine. Glutamate also functions as a detoxification agent for ammonia products in the brain. In addition to the many metabolic roles of glutamate, the most significant function of glutamate in the brain is its function as the primary excitatory neurotransmitter (Mayer & Westbrook, 1987). As a neurotransmitter, extracellular glutamate levels must be maintained at controlled levels. Although transporters exist to move glutamate into the brain across the blood-brain-barrier, the majority of glutamate is synthesized de novo from glucose, glutamine or aspartate (Lattera et al., 1999). Glutamate is stored in synaptic vesicles. The signaling actions of glutamate are mediated at the neuronal membrane through specialized receptor macromolecules. The binding of glutamate to specific sites on its receptor molecule causes a conformational change that initiates signal transduction cascades in the neuron. Glutamate receptors are broadly categorized based on the signaling cascade they trigger. Ionotrophic glutamate receptors are coupled to ion permeable channels which, under physiological conditions, depolarize neurons. In contrast, metabotrophic receptors are coupled are coupled to guanosine triphosphate binding proteins (G proteins) and second messenger systems that modulate synaptic transmission (Dingledine et al., 1999).
The ionotropic glutamate receptors are post-synaptic, ligand-gated ion channels. Three types of ionotropic glutamate receptors have been categorized and named according to selective ability of N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) or kainate (KA) to activate them (Dingledine et al., 1999). The AMPA receptor contributes to the early, fast component of the excitatory post-synaptic potential (EPSP). As a low affinity glutamate receptor, the AMPA receptor is typically permeable to the monovalent cations, sodium (Na') and potassium (K'). However, AMPA receptors that lack a GluR2 subunit are also permeable to the divalent cation, Ca²⁺ (Wisden & Seeburg, 1993). This ligand-gated channel demonstrates little voltage dependence, and currents are very brief (a few milliseconds) due to the low glutamate affinity and a high rate of desensitization (Boulter et al., 1990; Dingledine et al., 1999). KA receptors are very similar in function to AMPA receptors. Like AMPA receptors, KA receptors are voltage-independent, monovalent cation permeable channels with low affinity and fast kinetics (Michaelis, 1998). KA receptor-mediated EPSPs have smaller peak amplitudes and slower decay kinetics than those derived from AMPA receptors (Frerking & Nicoll, 2000). The NMDA receptor is quite different from the AMPA and KA subtypes of glutamate receptor. First, in addition to their permeability to Na' and K', NMDA receptors have high permeability to Ca²⁺ (Dingledine et al., 1999). NMDA receptors also have slower kinetics attributed to a much higher affinity for glutamate (Conti & Weinberg, 1999). The conductance through NMDA receptors can last several hundred milliseconds and constitutes a slower, later phase of the EPSP (Conti & Weinberg, 1999). Metabotropic receptors (mGluRs) are the other major category of glutamate receptors which is G-protein coupled. There are eight types of metabotropic glutamate receptors that are further classified according to the second messenger systems to which they are linked (Conn & Pin, 1997). These receptors are found both on the pre-
synaptic and post-synaptic membranes. Pre-synaptic mGluRs decrease neurotransmitter release, while mGluRs on the post-synaptic membrane regulate the function of ligand-gated ion channels including all three subtypes of ionotropic glutamate receptors (Anwyl, 1999). Thus, metabotropic glutamate receptors can act to modulate synaptic transmission in the CNS.

**Calcium ion homeostasis**

Calcium (Ca\(^{2+}\)) plays a fundamental role in the cell as a second messenger governing cellular functions such as differentiation and growth, membrane excitability, exocytosis, and synaptic activity. Neurons possess specialized homeostatic mechanisms to ensure tight control of cytosolic Ca\(^{2+}\) levels so that multiple independent Ca\(^{2+}\)-mediated signaling pathways can exist in the normal cell (Arundine and Tymianski, 2003). In excitotoxicity, excessive stimulation of glutamate receptors and an increase in extracellular glutamate concentration can lead to the disregulation of Ca\(^{2+}\) homeostasis (Arundine & Tymianski, 2003). An overwhelming increase in free intracellular calcium concentration can activate a self-destructive cellular cascade involving many calcium-dependent enzymes, such as phosphatases (e.g., calcineurin), proteases and lipases. Lipid peroxidation can also cause production of free radicals which damage vital cellular proteins and lead to neuronal death (Choi, 1988; Michaels & Rothrnan, 1990; Tymianski & Tator, 1996; Delorenzo et al., 2005).

The NMDA receptor mediates the vast majority of Ca\(^{2+}\) influx during excitatory neurotransmission (Ozawa, 1993). In addition, AMPA and KA receptors of certain subunit composition are permeable to Ca\(^{2+}\) (Jonas & Bumashev, 1995). Calcium extrusion across the plasma membrane. Two transport systems exist to pump
free intracellular Ca\(^{2+}\) out of the neuron into the extracellular space. Because Ca\(^{2+}\) extrusion acts against a large Ca\(^{2+}\) concentration gradient, these systems are energy-dependent and are, therefore highly susceptible to ischemic injury (Tymianski & Tator, 1996). The ATP-driven Ca\(^{2+}\) pump (Ca\(^{2+}\) - ATPase) expends one molecule of ATP for each Ca\(^{2+}\) ion extruded and is modulated by calmodulin, fatty acids, and protein kinases (Carafoli, 1992). The second transport system, the Na\(^+\) -Ca\(^{2+}\) exchanger, is indirectly coupled to ATP utilization in that it utilizes the Na\(^+\) concentration gradient maintained by the ATP-driven Na\(^+\) - K\(^+\) exchanger. This electrogenic exchange system is triggered by increases in Ca\(^{2+}\) and extrudes one Ca\(^{2+}\) for every two or three Na\(^+\) that enters the neuron (Tymianski & Tator, 1996). Calcium buffering and sequestration can also reduce free intracellular Ca\(^{2+}\) levels. The endoplasmic reticulum (ER) functions as a Ca\(^{2+}\) store. Calbindin D-28k, one of the major CaBPs, is present at high cytosolic concentrations in neurons such as purkinje cells and hippocampal granule cells. Together with its high cytosolic concentration, the ability of calbindin to bind up to four Ca\(^{2+}\) ions at a time suggests that it plays an important role in Ca\(^{2+}\) buffering (Mattson et al., 1995). The hippocampal formation is a locus of epileptic seizure activity (Lothman et al., 1991). Recent research suggests that the absence of calcium buffer proteins results in marked abnormalities in cell firing (Bastianelli et al., 2003). The calcium-binding proteins are present mainly in GABAergic interneurons, thus their disturbance could result in an alteration of inhibitory mechanisms (Krsek et al., 2004). Hippocampal neurons rich in the main Ca\(^{2+}\)-binding protein, calbindin D-28k, appear to be relatively resistant to degeneration in a variety of acute and chronic disorders (Sloviter, 1989; Hauser & Annegers, 1991; Magloczky et al., 1997). Calbindin-like immunoreactivity is present in all dentate granule cells and some, but not all, CA1 and CA2 pyramidal cells in rat hippocampi. In area dentata, calbindin immunoreactivity is normally present in a
suggest that there is a loss of calbindin from granule cells of the dentate gyrus and select CA1 neuron populations in mouse models and in rat kindling models of epilepsy (Kohr et al., 1991). Thus, the possible role of Ca\(^{2+}\) as a second messenger mediating some of these changes in hippocampal CA neurons, dentate granule neurons and interneurons is an important area of investigation.

**Acetylcholine**

The cholinergic system plays a crucial role in modulating cortical and in particular hippocampal functions including processes such as learning and memory (Ashe & Weinberger, 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 (Krnjevic' 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, 1990). For instance, subicular cells recorded from freely moving animals generate "location specific" firing patterns; this indicates a possible contribution of this region to spatial learning (Barnes et al.,
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 et al., 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; Insausti et al., 1987; Reep et al., 1987) that is then conveyed to the hippocampal formation via the perforant path (Steward and 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; Insausti et al., 1997). In addition, the deep layers of the EC also project massively on the EC superficial layers (Kohler, 1986b; Dolorfo & Amaral, 1997) thereby closing an EC-hippocampal loop. Thus, by virtue of its extensive projection systems, the EC network may act 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 & Garciá-Austi, 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.

**Muscarinic receptors**

Muscarinic receptors are a family of G protein-coupled receptors that have a primary role in central cholinergic neurotransmission. Specific agonists, which activate postsynaptic muscarinic receptors, stimulate cholinergic signaling (Valentin et al., 2006). The muscarinic acetylcholine receptors are widely distributed throughout the body and subserve numerous vital functions in both the brain and autonomic nervous system (Hassal et al., 1993). Activation of muscarinic receptors in the periphery causes decrease in heart rate, relaxation of blood vessels, constriction in the airways of the lung, increase in the secretions and motility of the various organs of the gastrointestinal tract, increase in the secretions of the lacrimal and sweat glands, and constriction in the iris sphincter and ciliary muscles of the eye (Wess, 1993). In the brain, muscarinic receptors participate in many important functions such as learning, memory and the control of posture.

Muscarinic receptors are members of a large family of plasma membrane receptors that transduce the intracellular signals via coupling to guanine nucleotide binding regulatory proteins. (Hulme et al., 1990; Bonner, 1989; Nathanson, 1987). Molecular cloning studies have revealed the existence of five molecularly distinct mammalian muscarinic receptor proteins (Hulme et al., 1990; Bonner, 1989).

All mammalian muscarinic receptor genes share one common feature with several other members of G-protein receptor gene family i.e., their open reading frame
contained within a single exon (Bonner et al., 1987). Like all other G protein coupled receptors, the muscarinic receptors are predicted to confirm to a generic protein fold consisting of seven hydrophobic transmembrane helices joined by alternating intracellular and extracellular amino-terminal domain and a cytoplasmic carboxy-terminal domain. The five mammalian muscarinic receptors display a high degree of sequence identity sharing about 145 amino acids. Characteristically all muscarinic receptors contain a very large third cytoplasmic loop, which, except for the proximal portions, displays virtually no sequence identity among the different subtypes (Bonner, 1989). Agonist binding to muscarinic receptors is thought to trigger conformational changes within the helical bundle, which are then transmitted to the cytoplasmic face where the interaction with specific G proteins occur. Site directed mutagenesis and receptor-modeling studies suggest that a conserved Asp residue present in TM II of almost all G protein coupled receptors plays a pivotal role in mediating the conformational changes associated with receptor activation (Wess, 1993).

The ligand binding to muscarinic receptors is predicted to occur in a pocket formed by the ring like arrangement of the seven transmembrane domains (Wess et al., 1991; Hulme et al., 1990). Ligand binding appears to be initiated by ion-ion interaction between positively charged amino head present in virtually all muscarinic receptor ligands and a conserved Asp residue located in TM III. In addition a previous mutagenesis study has shown that replacement of the conserved TM III Asp residue in the rat muscarinic M1 receptor with Asn results in a receptor unable to bind to [3H] QNB.
Sequence analysis shows that the hydrophobic core of all muscarinic receptors contains a series of conserved Ser, Thr and Tyr residues, most of which do not occur in other G protein coupled receptors. Pharmacological analysis of mutant M3 muscarinic receptors showed that two Thr residues (Thr231 and Thr234) and four Tyr residues (Tyr148, Tyr506, Tyr529 and Tyr533) are important for high affinity acetylcholine binding (Wess et al., 1991). It has been shown that a Pro 201 to Ala mutant M3 muscarinic receptor exhibits affinities for both muscarinic agonists and antagonists 80-450 times less than those of the wild type (Wess et al., 1993).

In the periphery, among other effects, muscarinic receptors mediate smooth muscle contraction, glandular secretion and modulation of cardiac rate and force. In the central nervous system there is evidence that muscarinic receptors are involved in motor control, temperature regulation, cardiovascular regulation and memory. Interest in the classification of muscarinic receptors involved in functions at different locations has been heightened by the potential therapeutic application agents in areas such as Alzheimer's disease, Parkinson's disease, asthma, analgesia, and disorders of intestinal motility and cardiac and urinary bladder function (Caulfield & Birdsall, 1998).

**Classification**

Muscarinic receptors are widely distributed throughout the central and peripheral nervous system. They have critical functions in learning and memory, attention and motor activity (Levey, 1993; Weiner et al., 1990; Bonner, 1989). The five muscarinic receptor subtypes are designated as M1 - M5. The odd-numbered receptors (M1, M3, and M5) couple to Gq/11, and thus activate phospholipase C,
which initiates the phosphatidyl inositol trisphosphate cascade. This leads to the dissocation of phosphatidyl 4, 5- bisphosphates (PIP2) into two components, i.e., IP₃ and DAG. IP₃ mediates Ca²⁺ release from the intracellular pool (endoplasmic reticulum), whereas DAG is responsible for activation of protein kinase C. On the other hand, PIP2 is required for the activation of several membrane protein, such as the "M current" channel and Na⁺/Ca²⁺ exchangers and muscarinic receptor- dependent depletion of PIP2 inhibits the function of these proteins (Suh & Hille, 2005; Winks et al., 2005; Fuster et al., 2004; Meyer et al., 2001; Caulfield & Birdsall, 1998; Bonner et al., 1988; Bonner et al., 1987;). The M1, M2 and M4 subtypes of mAChRs are the predominant receptors in the CNS. These receptors activate a multitude of signaling pathways important for modulating neuronal excitability, synaptic plasticity and feedback regulation of ACh release (Volpivelli et al., 2004)

**Muscarinic M1 receptor**

M1 receptors are predominantly expressed in the forebrain, including the cerebral cortex, hippocampus and corpus striatum, where this sub-type contributes by 50-60% to the total of the muscarinic receptors (Gerber et al., 2001; Miyakawa et al., 2001; Hamilton et al., 1997). The M1 receptor subtype, which is also expressed in peripheral tissues, has been implicated in stress adaptive cardiovascular reflexes and central blood pressure control. Studies have shown that central administration of the M1 specific antagonist pirenzepine lowered the blood pressure (Buccafusco, 1996; Brezenoff & Xiao, 1986). A putative overexpression of the M1 subtype in selected brain areas of spontaneously hypertensive rats has been reported (Scheucher et al., 1991). Muscarinic agonist depolarization of rat isolated superior cervical ganglion is mediated by M1 receptors (Brown et al., 1980). M1 is one of the predominant
Muscarinic receptor subtypes expressed in pancreatic islets (Gilon & Henquin., 2001). Studies in pancreatic islets revealed that activation of muscarinic receptors is pertussis toxin insensitive and Gq mediated. Muscarinic M1 receptor number decreased in the brainstem at time of pancreatic regeneration without any change in the affinity (Renuka et al., 2006).

**Muscarinic M2 receptor**

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

**Muscarinic M3 receptor**
M3 muscarinic receptors are broadly expressed in the brain, although the expression level is not high, compared to those of the M1 and M2 receptors (Levey, 1993). Muscarinic M3 receptor is widely distributed in the peripheral autonomic organs with the highest expression found in the exocrine glands (Candell et al., 1990; Pedder et al., 1991; Kashihara et al., 1992; Matsui et al., 2000). Expression of the M3 receptor in the rat pancreatic islets and insulin secreting cell lines has been established (Lismaa, 2000). M3 receptor also triggers direct contractions of smooth muscle, however, it only represents a minor fraction of total muscarinic receptor population in smooth muscle. It is expressed in relatively low density throughout the brain. Studies using knock out mice for M3 receptors gave evidences for the primary importance of these receptors in the peripheral cholinergic system. In urinary bladder, pupillary muscles and intestinal smooth muscles the cholinergic contractions are mediated predominately by M3 receptors (Matsui et al., 2000). Central Muscarinic M3 receptor subtypes functional balance is suggested to regulate sympathetic and parasympathetic activity (Renuka et al., 2004).

Muscarinic M4 receptor

Muscarinic M4 receptor is known to be abundantly expressed in the striatum (Levey, 1993). Muscarinic M4 receptors act as inhibitory muscarinic autoreceptors in the mouse (Zhang et al., 2002). The neuroblastoma-glioma hybrid cell line NG108–15 expresses M4 mRNA and M4 receptors can be detected readily in radioligand binding assays (Lazareno et al., 1990). Inhibition of adenylyl cyclase activity by muscarinic agonists in rat corpus striatum is mediated by M4 receptors (Caulfield, 1993; Olianas et al., 1996). Muscarinic M1 and M3 receptors function differently regulate glucose induced insulin secretion were reported (Renuka et al., 2006).
**Muscarinic M5 receptor**

The M5 receptor was the last muscarinic acetylcholine receptor cloned. Localization studies have revealed that the M5R is abundantly expressed in dopamine-containing neurons of the substantia nigra par compacta, an area of the midbrain providing dopaminergic innervation to the striatum. Concordantly, oxotremorine-mediated dopamine release in the striatum was markedly decreased in M5R-deficient mice. More intriguingly, in M5R-deficient mice, acetylcholine induced dilation of cerebral arteries and arterioles was greatly attenuated (Yamada et al., 2001), suggesting that the M5 receptor might be a suitable target for the treatment of cerebrovascular ischemia. Muscarinic M5 receptor subtype expressed at low levels in the brain (Hulme et al., 1990; Hosey, 1992).

Studies of the M5 receptor have been hampered both by the lack of selective ligands and of tissues or cell lines that endogenously express the native receptor protein. Immunoprecipitation and RT-PCR studies have shown that the M5 receptor is expressed at very low densities in the mammalian brain. However, *in situ* hybridization studies have demonstrated that M5 transcripts are highly concentrated in the basal ganglia and are the only muscarinic receptor transcripts expressed on dopaminergic neurons in the substantia nigra pars compacta (SNC) and ventral tegmental area (VTA) (Reever et al., 1997). Another potentially useful system is the eosinophilic leukemia cell line (EoL-1) where M5 receptors can be induced on differentiation with interferon-γ (Mita et al., 1996).
Signal transduction by muscarinic activation

Gq-protein-coupled receptors (GqPCRs) are widely distributed in the CNS and play fundamental roles in a variety of neuronal processes. Their activation results in phosphatidyl inositol 4,5-bisphosphate (PIP2) hydrolysis and Ca\(^{2+}\) release from intracellular stores via the phospholipase C (PLC)-inositol 1,4,5-trisphosphate (IP\(_3\)) signaling pathway. Because early GqPCR signaling events occur at the plasma membrane of neurons, they might be influenced by changes in membrane potential (Billups et al., 2006). Muscarinic receptors, which are G protein coupled, stimulate signaling by first binding to G protein complex (αβγ) which provides specificity for coupling to an appropriate effector. The α subunit interacts with an effector protein or ion channel to stimulate or inhibit release of intracellular second messengers. Mutation analysis showed that the G protein is primarily but not exclusively acts through interaction with the third cytoplasmic loop. It is suggested that the short sequences, N terminal 16-21 and C terminal 19 amino acids of the loop play a key role in determining the specificity (Wess et al., 1989).

Cyclic adenosine monophosphate

Adenylate cyclase can be either positively or negatively regulated by G protein coupled receptors resulting in an increase or decrease in the generation of the second messenger, Cyclic adenosine monophosphate (cAMP). The stimulation of muscarinic M2 and M4 receptors endogenously expressed in cell lines, results in the inhibition of adenylate cyclase. G protein reconstitution experiments have shown that M2 receptors inhibit adenylate cyclase through Gi and possibly through the pertussis toxin insensitive Gz. In neuroblastoma SK-N-SH cells which express endogenous

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muscarinic M3 receptors stimulate adenylate cyclase activity (Baumgold & Fishman, 1988). The muscarinic M1 receptor which ectopically expressed at physiological levels in A9L cells, was shown to stimulate adenylate cyclase through an IP$_3$ and Ca$^{2+}$ dependent mechanism (Felder et al., 1989). In contrast, M1 receptors stimulate adenylate cyclase in CHO cells predominantly through an IP$_3$ and Ca$^{2+}$ independent mechanism that also contained a small Ca$^{2+}$ dependent component (Gurwitz et al., 1994).

**Phospholipase C**

The family of phospholipase C (PLC) enzymes has been grouped into three classes, β, γ and δ (Rhee & Choi, 1992). PLC serves as the primary effector for the muscarinic M1 receptor that is coupled through Gq α subunits (Berstein et al., 1992). Muscarinic M1, M3 and M5 receptors can stimulate the production of IP$_3$, independent of direct PLCβ and G protein interaction (Gusovsky, 1993). This alternate route for the generation of IP$_3$ involves the tyrosine kinase dependent phosphorylation of PLCγ, a mechanism normally stimulated by growth factors and their receptors (Meisenhelder et al., 1989). Expression studies revealed that the cloned muscarinic M2 receptor stimulates PLC through a pertusis toxin-sensitive G protein although with lower efficiency than M1 or M3 receptors (Ashkenazi et al., 1987). Inhibition of PLC by an endogenously expressed M2 receptor has been reported in FRTL5 cells suggesting that negative regulation may also occur in some cells (Bizzarri et al., 1990).
**Phospholipase A2**

Phospholipase A2 catalyze the hydrolysis of membrane phospholipids to generate free arachidonic acid and the corresponding lysophospholipid. Muscarinic receptors have been shown to stimulate the release of arachidonic acid and its eicosanoid metabolites in a variety of tissues including heart, brain and muscle (Abdel-Latif, 1986). Ectopic transfection experiments indicates that the muscarinic M1, M3 or M5 receptors, but not M2 or M4 receptors are linked to phospholipase A2 activation (Conklin *et al.*, 1988; Felder *et al.*, 1990; Liao *et al.*, 1990). Muscarinic receptor stimulated release of arachidonic acid occurs predominantly through the activation of phospholipase A2 and phosphatidylcholine serves as the primary substrate. Studies suggested that calcium influx, through voltage independent calcium channel activation and diacylglycerol, through PLC activation were essential for phospholipase A2 activation (Felder *et al.*, 1990; Brooks *et al.*, 1989). In ileal smooth muscle cells, carbachol stimulated phospholipase A2 itself caused calcium influx, implicating an amplification mechanism in phospholipase A2 regulation (Wang *et al.*, 1993).

**Phospholipase D**

Muscarinic receptor stimulated phospholipase D has been reported in a number of cell types including canine synaptosomes (Qian & Drewes, 1989), rat astrocytoma cells (Martinson, 1990), human neuroblastoma cells (Sandmann & Wurtman, 1991) and rat parotid cells (Guillemain & Rossignol, 1992). Association of muscarinic subtypes with phospholipase D has been shown in human embryonic kidney cells transfected with the M1-M4 receptors. In most cells studied,
phospholipase C and D are usually stimulated simultaneously following receptor activation (Liscovitch, 1991).

**Calcium influx and release from intracellular stores**

Muscarinic receptors typically stimulate biphasic increases in intracellular calcium in most cells. The transient phase represents the release of calcium from IP$_3$ sensitive intracellular calcium stores. Calcium influx through calcium channels play a central role in the regulation of multiple signaling pathways activated by muscarinic receptors. In excitable cells such as neurons and muscle cells calcium passes predominantly through voltage sensitive calcium channels (VOCC). In non-excitable cells, such as fibroblasts and epithelial cells, calcium passes through a family of poorly characterised voltage - insensitive calcium channels. VICCs open in response to receptor activation and have been classified into (1) receptor operated calcium channels which are second messenger independent, (2) second messenger - operated calcium channels (SMOCCs) and (3) depletion operated calcium channels which open following IP$_3$ mediated depletion of intracellular stores and provide a source of calcium for refilling the stores.

*Bacopa monnieri* (Linn.) Pennell

**Family: Scrophulariaceae**

*Bacopa monnieri* commonly called as ‘Brahmi’ in Malayalam and Hindi is a small creeping, glabrous, punctuate herb numerous ascending branches, commonly growing in most parts of India. It is being cultivated as a commercial crop. 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 fruits throughout the year, though mostly during February to April. Drug consists of fresh or dried whole plant.

The plant elaborates several triterpenoids 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 triterpene 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 (luteolin and its glycosides), sugars (D-mannitol) usual sterols (β-sitosterol, stigmasterol and its esters) and paraffins (heptacosane, 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) developed based on the hydrolysis of Bacosides to an aglycone that has an absorption maximum at 278

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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).

Due to the importance of *Bacopa monnieri* in the indigenous system of medicine, systematic chemical examinations of the plant have been carried out by several groups of researchers. The major chemical entity shown to be responsible for the memory-facilitating action of *Bacopa monnieri*, Bacoside A, was assigned as 3-(α-L-arabinopyranosyl)-O-β-D-glucopyranoside-10, 20-dihydroxy-16-keto-dammar-24-ene (Chatterji et al., 1965). Bacoside A usually co-occurs with bacoside B; the latter differing only in optical rotation and probably an artifact produced during the process of isolating Bacoside A (Rastogi et al., 1990). The chemical composition of bacosides, contained in the polar fraction, has been established on the basis of chemical and physical degradation studies. On acid hydrolysis, bacosides yield a mixture of aglycones, Bacogenin A1, A2, A3 (Kulshreshtha & Rastogi, 1973, 1974; Chandel et al., 1977) and two genuine sapogenins, jujubogenin and pseudojujubogenin (Rastogi et al., 1994).

*Bacopa monnieri* extracts and isolated bacosides have been extensively investigated in several studies for their neuropharmacological effects and a number of reports are available confirming their nootropic action. Preliminary studies established that the treatment with crude extract (Malhotra & Das, 1959) and with the alcoholic extract of *Bacopa monnieri* plant (Singh & Dhawan, 1982) enhanced learning ability in rats. Subsequent studies indicated that the cognition-facilitating effect was due to
two active saponins, Bacosides A and B, present in the ethanol extract (Singh & Dhawan, 1992). These active principles, apart from facilitating learning and memory in normal rats, inhibited the amnesic effects of scopolamine, electroshock and immobilization stress (Dhawan & Singh, 1996). It has been suggested that the Bacosides induce membrane dephosphorylation with a concomitant increase in protein and RNA turnover in specific brain areas (Singh et al., 1990). Further, Bacopa monnieri has been shown to enhance protein kinase activity in the hippocampus which could also contribute to its nootropic action (Singh & Dhawan, 1997). A study of Bhattacharya et al., (1999) reported that a standardized Bacoside-rich extract of Bacopa monnieri, administered for 2 weeks in rats, reversed cognitive deficits induced by intracerebroventricularly administered colchicines and by injection of ibotenic acid into the nucleus basalis magnocellularis. The central cholinergic system is considered the most important neurotransmitter involved in the regulation of cognitive functions. Cholinergic neuronal loss in hippocampal area is the major feature of Alzheimer’s disease (AD) and enhancement of central cholinergic activity by anticholinesterase is presently the mainstay of the pharmacotherapy of AD-type senile dementia. Administration of Bacopa monnieri for two weeks reversed the depletion of acetylcholine, the reduction in choline acetylase activity and the decrease in muscarinic cholinergic receptor binding in the frontal cortex and hippocampus induced by neurotoxin, colchicines (Bhattacharya et al., 1999). It has been suggested that the behavioral effects of cholinergic degeneration can be alleviated by a reduction in noradrenergic function (Sara et al., 1989). Bacopa monnieri is known to lower norepinephrine and increase 5-hydroxytryptamine levels in the hippocampus, hypothalamus and cerebral cortex (Singh & Dhawan, 1997). Bacopa monnieri is suggested to indirectly modify Ach concentrations through its influence on other neurotransmitter systems. In a recent study, standardized extract of Bacopa monnieri

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used to evaluate the antidementic and anticholinesterase activities in adult male Swiss mice (Das et al., 2002). Antidementic activity was tested against scopolamine (3 mg/kg ip)-induced deficits in passive avoidance (PA) test.

The present work is to understand the alterations of total muscarinic, muscarinic M1 and glutamate 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*, Bacoside A and Carbamazepine in vivo. The molecular changes in the muscarinic M1 receptors in the pre- and post-treated epileptic model with *Bacopa monnieri*, Bacoside A and Carbamazepine were also studied. These studies will help us to elucidate the functional role of muscarinic and glutamate receptors in epilepsy.