Chapter 3

3. REVIEW OF LITERATURE

In this review, we provide an overview of the key aspects of learning and memory, the structure and connections of hippocampus with an emphasis on its role in learning and memory, and the significance of oxidative stress in memory impairment and dementia. This review also focuses on the current therapeutic options available for cognitive enhancement, particularly in AD treatment, and the behavioral and animal models used to study learning and memory. Further, the physiological and pathological functions of peripheral and central RAS components and its manipulation by pharmacological interventions have been discussed.

3.1. Neurophysiology of learning and memory

Learning and memory are complex higher functions in the brain, and they constitute an area of rapid advances in neurobiology. These interrelated mental processes are presumed to underlie enduring behavioral changes that result from an interaction between the organism and environmental stimuli. The process of memory consolidation involves three main stages, beginning with encoding which includes receiving, processing and combining of received information, followed by storage that involves creation of a permanent record of the encoded information and ends with retrieval or recall (access, selection, reactivation, or reconstruction of stored information in response to some cue for future use in a process or activity) [Dudai 1996; Dudai & Morris 2000; McGaugh 2000]. Initially thought to be a simple concept, memory is now considered to be a constellation of mental abilities using different systems and components within the brain.
Memory can further be categorized into short-term memory (STM) and long-term memory (LTM). Short-term memory has limited capacity and duration and information is not retained indefinitely (Atkinson and Shiffrin 1968). It involves transient patterns of neuronal communications and depends primarily on prefrontal cortex and parietal lobe (Fuster and Alexander 1971; Smith and Jonides 1998). While STM relies mostly on acoustic code for storing information, long-term memory encodes information semantically (Baddeley 1966).

Long-term memory is divided into explicit (declarative) and implicit (non-declarative) memory (Anderson 1976) [Figure 2-1]. Implicit memory affects behavior without awareness. Explicit memory includes recalling of those memories that can be consciously retrieved (Schacter 1996; Markowitsch, 1999, 2000). Explicit memory is further divided into semantic memory, representing general knowledge about the world; and episodic memory representing personal knowledge of one’s own past (Tulving and Markowitsch 1998; Aggleton and Brown 1999).

Working memory, also known as STM, integrates information relevant to cope with the present situation and retains it for a matter of few seconds or minutes after its impact. It has limited information processing capacity, but forms an essential component of many forms of higher mental activity (Baddeley 1986, 1999).

It has been suggested that multiple types of memory are mapped to different anatomical circuits in the brain (Squire 2004). The taxonomic frameworks suggested by different scientists are not mutually exclusive and they share a common generic form. The striatum (basal ganglia) is involved in procedural memory, whereas the neocortex is the centre for priming and perceptual learning (Squire 2004). Simple classical conditioning involving emotional responses are dependent on the amygdala whereas non-associative learning is modulated by reflex pathways in the brain (Figure 2-1).
Finally, declarative memory is associated with medial temporal lobe structures, particularly the hippocampus (Figure 2-1). Episodic memory is most commonly impaired in amnesia while procedural memory systems are believed to be intact in amnesia (Cohen and Squire 1980; Tulving and Schacter 1990). Deficits in episodic memory represent one of the most significant functional problems in individuals with cognitive dysfunction, particularly in AD. The lack of ability to recollect recent events or acquire new information leads to functional decline that are devastating for both the patient and the caregiver.
Figure 0-1: Diagrammatic representation of different types of memory and their anatomical circuits in the brain (Modified from Squire and Knowlton 1994).
3.2. The Hippocampus

3.2.1. Structure of the hippocampus

The hippocampal formation (HF) is a bilateral, archicortical structure within the medial temporal lobe. It consists of the hippocampus proper or Ammon’s horn (CA1, CA2, CA3 and CA4), subiculum, presubiculum, parasubiculum and the dentate gyrus (Figure 2-2). The parahippocampal gyrus, the entorhinal and perirhinal cortices are located inferiorly and laterally to the HF (Rosene and Van Hoesen 1987). The hippocampus projects to all areas of the associative cortex via the perirhinal and parahippocampal cortex which in turn project to the entorhinal cortex, which represents the cortical gate of the hippocampus. Allocortical and subcortical afferents arise from the basal forebrain (medial septum, nucleus basalis of Meynert, nucleus of the diagonal band of Broca) and most limbic structures, including the basal amygdala, the hypothalamus, the limbic thalamic nuclei, the raphe nuclei, the locus ceruleus and the periaqueductal grey matter. Efferent run back to the amygdala, the basal forebrain, the nucleus accumbens and the hypothalamus (Nieuwenhuys et al., 1988) (Figure 0-3).

The HF lies above the subiculum and medial parahippocampal gyrus, with a curved elevation that extends upto 5 cm long, along the floor of the inferior horn of the lateral ventricle. Its anterior end is expanded and its margin presents as two or three rounded elevations, forming the pes hippocampi. The ventricular region is covered by ependyma; fibres of alveus beneath the ependyma converge medially to form the fimbria of the fornix. The neocortex of the parahippocampal gyrus merges with the subiculum by passing medially from the collateral sulcus. The subiculum curves to the inferior surface of the dentate gyrus and passes laterally to the laminae of the hippocampus. The dentate gyrus is a crenated strip of cortex, related
inferiorly to the subiculum, laterally to the hippocampus and medially to the fimbria of the fornix. The dentate gyrus comprises of the following layers:

(i) **The polymorphic layer** is the most superficial layer of the dentate gyrus which consists of several interneurons. The axons of dentate granule cells pass through this layer on the way to CA3

(ii) **The dense granule cell layer** which contains the cell bodies of the dentate granule cells

(iii) **The overlying molecular layer** which receives most of the afferent projections to the dentate gyrus, primarily from the entorhinal cortex. The granule cell and molecular layer are sometimes referred to as the fascia dentata.

The hippocampus is a trilaminar neocortex which consists of a single pyramidal cell layer (hippocampal proper or Ammon’s horn). Three distinct fields, designated CA1-CA3 can be distinguished based on differences in density of pyramidal cell layers. Field CA3 pyramidal cell layers are the largest in hippocampus and is important in neuropathology, as the death of neurons in this area is the first morphological detectable sign of cerebral hypoxia. The CA2 has the most compact layer of pyramidal cells. It is located between CA3 and CA1. Anatomically in the rat, it is best defined as the strip of cells that receive perforant path input from entorhinal cortex. Field CA1 is considered as the most complex of the hippocampal subdivisions. The CA1/CA2 border is not well marked, and at its other end, CA1 overlaps the subiculum for some distance. Approximately 10% of the neurons in this field are interneurons (Standring 2011).

The subicular complex is generally divided into subiculum, presubiculum and parasubiculum. The major subcortical projections of the HF (to the septal nuclei, mammillary nuclei, nucleus accumbens and anterior thalamus), and to the entorhinal cortex, arise from pyramidal neurons
of the subicular complex. Its primary inputs are axons from CA1 and form layer III of entorhinal cortex (Paxinos 1990).

Although the Ammon’s horn is basically a three-layered structure, it is common to describe several strata within the layers of the hippocampus (Figure 0-4):

(i) **Alveus** - consists of subicular and hippocampal pyramidal cell axons passing towards the fimbria

(ii) **Stratum oriens** – includes the basal dendrites of pyramidal cells and some interneurons

(iii) **Stratum pyramidale** – consists of cell bodies of pyramidal neurons which are the principal excitatory neurons of the hippocampus

(iv) **Stratum incidunt** – includes mossy fibres which make contact with proximal dendrites of pyramidal cells in CA3

(v) **Stratum radiatum** – contains Schaffer collateral fibres which are the projections from CA3 to CA1

(vi) **Stratum lacunosum** – also contains Schaffer collaterals and perforant path fibres from the superficial layers of the entorhinal cortex

(vii) **Stratum molecular** – deepest layer in the hippocampus. Here the perforant path fibres form synapses onto distal, apical dendrites of the pyramidal cells (Orhans 2001).
Different subregions (CA1, CA3 and dentate gyrus) of the rat hippocampus

**Figure 0-2:** Diagram showing different subregions of the rat hippocampus; CA1 - Cornu ammonis 1, CA3 - Cornu ammonis 3, DG – dentate gyrus. 
(Adapted from Williams et al., 2011).
Three-dimensional structure of rat brain - Hippocampus and its related structures

**Figure 0-3**: Drawings of rat brain showing the three-dimensional organisation of the hippocampus and related structures. Three coronal sections through the left hippocampus are shown at the bottom right of the figure, with their approximate antero-posterior coordination relative to bregma. CA1, CA2, CA3- cornu ammonis fields 1-3; DG- dentate gyrus; EC- entorhinal cortex; f- fornix; s- septal pole of hippocampus; S- subiculum; t- temporal pole of the hippocampus (Adapted from Cheung and Cardinal 2005).
Figure 0-4: Schematic diagram of the hippocampal formation and its connections. Different layers of the hippocampus are shown; A- alveus, SO- stratum oriens, SP- stratum pyramidale, SL- stratum lacunosum, SR- stratum radiatum, SG- stratum granulosum, SM- stratum molecular, EC- entorhinal cortex, Sub- subiculum, CA - cornu ammonis, DG- dentate gyrus, F- fimbria, LV- lateral ventricle (Modified from O’Keefe and Nadel 1978).
3.2.2. Role of hippocampus in learning and memory

The hippocampus and the amygdala serve a critical role in learning and memory consolidation. The HF and the EPPC in the medial temporal lobe are the major centres for the formation and consolidation of traces of declarative memory, such as familiarity-based recognition and semantic memory (Alvarez and Squire 1994; Aggleton and Brown 1999). The hippocampus is involved in supporting flexible relational associations and cross modal association of stimulus represented in different brain areas (Marr 1971; Mayes et al., 2001). The hippocampus is essential for the maintenance of recent memory and for carrying out normal mental activity. Damage to the hippocampus leads to an inability to convert short-term memories to new long-term memories, although existing long-term memories remain intact and accessible (von Bechterew 1900; Kohnstamm 1917). Perhaps, the most reliable impairments associated with hippocampal damage concern the delayed recall of test stimuli (Spiers et al., 2001). Further, deficits have also been observed for spatial navigation tasks similar to the effects of hippocampal lesions seen in other mammals including humans and monkeys (Burgess et al., 2001).

3.2.2.1. Theories of hippocampal function in memory

The most prominent theories of hippocampal function in memory were advanced by Squire and collaborators (Squire 1992; Squire and Alvarez, 1995).

(i) Spatial learning – It includes the cognitive map theory proposed by O’Keefe and Nadel in 1978. Cognitive mapping refers to learning about the spatial relationships between cues. The authors concluded that there are specific cells in the CA1 and CA3 fields of the rat hippocampus which fire specifically when the animal is located in a certain place in space (O’Keefe and Dostrovsky 1971). They emphasized that hippocampus is essential for spatial navigation in animals such as rats, specifically in situations where approaching a simple
sensory stimulus is not sufficient to solve the task. Such types of behaviour can be
categorized in terms of relying on an allocentric system that provides a record of an object’s
location relative to other objects, features or other landmarks in the environment. The
cognitive map is computed from the animals’ movements and on grounds of visual landmark
information. Spatial information such as memory for specific locations did appear to be one
the several information categories for which learning was poor after bilateral hippocampal
damage (Backer and Squire 1991). This theory has been supported by two remarkable
discoveries – the long-term potentiation (LTP) proposed by Bliss and Gardner (1973) and the
elucidation of hippocampal place unit by O’Keefe and Dostrovsky (1971). Although the
hippocampal involvement in spatial learning tasks is controversial, different authors chose
different interpretations of it and the cognitive map theory still remains controversial
although it laid the foundation for current theories of how hippocampus encodes space
(McNaughton 1996; McNaughton et al., 1996; O’Keefe & Burgess, 1996) and the process of
LTP (Bliss & Lomo 1973; Lomo 2003).

(ii) **Novelty detection** – HF is engaged in the novelty assessment of stimulus and this theory
has been derived based on broad experimental evidences from studies in animals and humans.
Novelty assessment is considered an early stage of long-term memory encoding (Tulving et
al., 1996). The probability of long-term storage of items varies with their novelty value.
Novelty assessment involves the limbic system and temporal regions and elaborative
encoding is associated with neuronal activity in the left prefrontal cortex (Kirchoff et al.,
2000). The HF takes part in the selection of to-be-stored material than activity involved with
encoding, storage or retrieval. The hippocampus is essential for detection of novelty of a
stimulus, which is a prerequisite for learning (Jenkins et al., 2004).

(iii) **Consolidation theory** – Consolidation of information from short-term memory to long-
term memory is a typical function of the HF. HF is critical for the retrieval of information
Initially, but after a certain period of time during which consolidation takes place, information becomes independent of hippocampal function (Zola-Morgan and Squire 1990; Alvarez and Squire 1994). Experimental animals with amnesia due to bilateral hippocampal damage are considered as an evidence for a time-limited role of HF in memory consolidation (Scoville and Milner 1957; Squire 1992).

The HF is not considered a repository for permanent memory but stores traces of information held in short-term memory. The HF thus plays an important role in memory storage and retrieval because it is connected with all associative cortices and it exhibits LTP which induces rapid changes in the synaptic circuit (Shors and Matzel 1997; Malenka and Nicoll 1999). The hippocampus forms a temporary memory trace storing patterns of neo-cortical coactivations at the time of encoding of a perceived stimulus. It helps strengthen the corticocortical connections, repeatedly reactivating the cortical and the subcortical sites in the same way that they were activated during encoding. This process is considered the neuronal correlate of consolidation (Wiltgen et al., 2004). The hippocampal formation provides the means for gaining access to the various neocortical representations that together constitute the learned episode and permits access to both the representation of the parts of a learned event and their conjunctions (Farah et al., 1992; McClelland et al., 1992; McClelland 1995).

3.2.2.2. Hippocampus as a binding device

The hippocampus binds together the different components of a learning event by linking neuronal activation in distributed brain regions (Squire and Zola 1991) [Figure 0-5]. Temporary storage of such patterns of coactivations allows the hippocampus to maintain the consistency and relations with the learning event (Cohen and Eichenbaum 1993). The HF is expected to be more involved in the encoding of association between items of an event than
Schematic diagram representing processing and encoding of information by the hippocampus

Figure 0-5: Schematic representation of the processing and encoding of information by the hippocampus from all over the brain, binding it together into an episodic memory. Dorsal (parahippocampal) and Ventral (perirhinal) pathways from the posterior cortex converge into the entorhinal cortex, which then function as the input and output pathway of the hippocampus proper, consisting of the dentate gyrus (DG) and areas of "Ammon's horn" (cornu ammonis CA) - CA3 and CA1. CA3 represents the primary 'engram' for the episodic memory, while CA1 is an invertible encoding of EC, such that subsequent recall of the CA3 engram can activate CA1 and then EC, to reactivate the full episodic memory out into the cortex. (Adapted from Computational Cognitive Neuroscience, O'Reilly and Munakata 2012).
during the encoding of these items in isolations (Henke et al., 1999; Davachi & Wagner 2002). The HF codes spatial and non-spatial relations among events, processing the spatial relations for navigation (Best et al., 2001) and serial relations for solving more abstract, non-spatial relations (Dusek and Eichenbaum 1997).

### 3.2.3. Hippocampus and Alzheimer’s disease

Any event which significantly alters the function of the hippocampus, either temporarily or permanently will cause amnesia in humans and rats. The hippocampus plays a crucial role in learning and memory and damage to the hippocampus and its related systems is crucial to the amnesic syndrome (Scoville and Milner 1957). Spiers et al., (2001) indicated a clear association between amnesia and hippocampal damage, particularly in anterograde episodic memory deficits.

A salient feature of anterograde amnesia after damage to the hippocampus is impairment in learning certain tasks while new learning in other tasks remains unaffected. In addition, hippocampal damage has also been associated with retrograde amnesia, although their nature is less clear (Zola-Morgan and Squire 1990; Astur et al., 1994). An intact hippocampus is required for initial learning and long-term memory retention of some spatial memory tasks, particularly the ones that require finding way to a hidden goal. In AD, it has been seen that semantic memory is consistently disrupted (Tippett et al., 1996, 2007). This is often attributed to the pathology in inferolateral temporal lobes and the frontal lobes which causes a loss of neuronal dendritic trees in these cortical regions (Starr et al., 2005).
Several forms of classical conditioning including fear conditioning and eye blink conditioning that is supported by inputs from entorhinal cortex to hippocampus are shown to be impaired in AD patients (Woodruff-Pak 2001; Hamann et al., 2002). Procedural memory system is however less impaired compared to other forms of memory in patients with AD (Libon et al., 1998). Studies on working memory paradigms indicate that the ability to keep information in mind is more vulnerable to manipulations in AD patients (Belleville et al., 2007).

The hippocampus has long been viewed as one of the sites that is most severely damaged in AD. It has been shown that hippocampal atrophy and alterations in hippocampus shape and structure are more prominent in AD patients than older adults without dementia (Wang et al., 2006; Barnes et al., 2007). MRI findings have shown that fornix and the mammillary bodies in the hippocampus undergo atrophy in AD patients compared to healthy individuals and patients with mild cognitive impairment (Petersen 2003).

Neuropathological studies have also found that regions including the entorhinal cortex and presubiculum contain significantly high levels of Aβ plaques in patients who are diagnosed with AD (Wisniewski and Frackowiak 1997). In AD, the integrity of the hippocampus is compromised by plaques, tangles and eventually the loss of synapses and neurons (Probst et al., 1983). The reduced and altered synapses within the plaque impair intercellular communication and disrupt the essential role of the synapse in learning, memory and cognition. Hippocampal degeneration is thus the basis, at least in part, for the cognitive and behavioural deficits seen in AD.
3.3. Neurotransmitters, neuromodulators and receptor systems involved in learning and memory

Due to considerable research done in the field of cognition, the roles of various neurotransmitters and neuromodulators are much better understood than previously. ACh and glutamate have been most extensively studied in relation to learning and memory processes. However current evidence also links various other neurotransmitters such as GABA, dopamine, serotonin and norepinephrine, as well as various neuropeptides to memory.

1. Cholinergic system

Pharmacological studies using a variety of experimental paradigms have linked ACh to learning and memory. The role of cholinergic pathways is probably best characterized as reflected by use of centrally acting cholinergic in management of AD. Cholinergic agonists such as physostigmine enhance memory, whereas cholinergic antagonists such as scopolamine significantly disrupt acquisition and retention of memory (Bartus et al., 1985; Fibiger 1991). The basal forebrain cholinergic neurons are involved in attentional process and play a critical role in regulating cortical plasticity which is essential for laying down a memory trace (Rasmusson 2000). Thus, ACh may be more concerned with the acquisition and processing of sensory information rather than retention of this information. However, cholinergic modulation of other neurotransmitter systems is likely to affect the memory processes in the brain. Both muscarinic and nicotinic receptors are thought to be involved in mediating these effects (Jones et al., 1999). Nicotine is known to enhance alertness and probably memory through nicotinic receptors (Arnevic et al., 1995). However, involvement of muscarinic receptors has not been ruled out conclusively. Therefore in AD, where there is loss of cholinergic function with age (Bartus et al., 2000), the preferred treatment approach includes cholinergic replacement therapy with anticholinesterases rather than pure nicotine agonists (Buccafasco and Terry 2000; Terry and Buccafusco 2003).
2. **Glutamatergic pathway**

The excitatory amino acid glutamate is the most abundant amino acid transmitter in the CNS involved in learning and memory processes. Glutamate’s role in memory is primarily concerned with LTP, a putative mechanism of memory storage. Among the glutamatergic receptors, NMDA (N-Methyl D-Aspartate) is believed to be the most important receptor playing a role in generation of LTP and therefore in learning and memory. Activation of NMDA receptor requires presence of both glutamate and glycine in response to which it causes profound calcium influx leading to short- and long-term changes into the synapse function. Nitric oxide (NO) acts as a retrograde messenger and plays an adjuvant role in mediating synaptic changes (Rang et al., 2005). Drugs capable of enhancing LTP through action on glutamate or NMDA receptors are being sought with the hope that they will improve learning and memory.

3. **GABAergic system:**

GABA (gamma amino butyric acid) is the primary inhibitory neurotransmitter in the CNS, distributed abundantly in the brain regions involved in learning and memory. GABAergic neurons interact extensively with cholinergic neurons in the medial septum sending projections to and receiving them from the hippocampus. Local release of GABA within the hippocampus can modulate synaptic transmission via activation of presynaptic GABA$_B$ receptors which suppress excitatory synaptic transmission similar to that of cholinergic effects (Hasselmo et al., 1996). GABA is also known to be critically involved in the control of fear and anxiety through the amygdala, and GABAergic system in amygdala has also been shown to be involved in memory consolidation for both positive and negative affective experiences (Salinas et al., 1996; Bergado-Acosta et al, 2008).
4. **Dopaminergic system.**

The major role of dopamine (DA) is as a precursor of noradrenaline but in certain areas of brain it itself acts as a neurotransmitter. However, the distribution of dopamine in brain is more restricted than that of noradrenaline. Recent findings have indicated that dopamine plays a role in higher mental functions including learning. Several studies implicate that reduced DA function is observed in cognitive impairments including several neurodegenerative disorders (Ellis and Nathan 2001). Nigrostriatal system is critical for learning movement sequences or for retrieving them. Dopaminergic neurotransmission in this system is essential for retrieving acquired neuronal activity from long-term storage of activity patterns in the striatum (Matsumoto et al., 1999). Dopamine transmission in prefrontal cortex has also been shown to contribute to regulation of cognitive functions (Thierry et al., 1988). However, it was found that higher activity of dopamine during learning actually interferes with learning. Hence, it implies that dopamine is not normally active during learning but is necessary for memory consolidation (Fibiger and Philips 1976).

5. **Norepinephrine**

Both central and peripheral adrenergic systems play a role in modulation of learning and memory (Gold et al., 1984; Hemmaty et al., 2008). Studies in animals have suggested that enhanced memory associated with emotional arousal results from an activation of beta-adrenergic stress hormone systems, during and after an emotional experience, which further supports the role of beta receptors in memory formation (Martinez et al., 1980). Although beta receptors in hippocampus have been shown to exert modulatory influence on LTP, the role of alpha receptors cannot be conclusively ruled out. Although the role of noradrenergic system in learning and memory is well established, it is imperative to characterize the receptor subtypes and their functions before this system can be targeted for drug development (Floresco and Jentsch 2011).
It has been shown that norepinephrine (NE) and DA interact in the prefrontal area and affects the prefrontal-dependent working memory tasks. NE has also been found to interact with ACh and both act synergistically to affect some types of memory.

6. **Serotonergic system**

Serotonin (5-HT) has also been shown to be involved in learning and memory (Meneses 1999). Deficiency of 5-HT in certain brain regions, particularly the hippocampus can impair memory (Buhot et al., 2000). 5-HT interacts with 5-HT receptors and plays a role in passive avoidance retention, LTP and long-term depression. It also enhances spatial memory in the Morris water maze task, and it is suggested that some of these changes mediated by 5-HT may also involve modulation of synaptic plasticity and sensory input reorganization, as has been noted in the cholinergic system (Cassel and Jeltsch 1995).

7. **Nitric oxide**

Nitric oxide (NO) plays an important role in hippocampal synaptic plasticity and consequently learning and memory (Harooni et al., 2009). NO is an intracellular retrograde messenger in the brain and its role in LTP and long term depression is well established where it acts to enhance glutamine-NMDA mediated synaptic plasticity (Zhuo and Hawkins 1995). Further, NO and 5-HT have been shown to act synergistically and influence memory processes including spatial learning and memory (Yamada et al., 1995). Considering its potential role in learning and memory, NO is recently being targeted as one of the major therapeutic strategies for AD (Fernandez et al., 2010).
8. Neuroactive peptides

A number of neuroactive peptides coexist with a variety of neurotransmitters such as galanin with ACh, somatostatin with GABA and neurotenin with NE, and together they exert powerful modulatory effects on neurons and neuronal circuits (Feany 1996). Several other peptides including opioids (Decker and McGaugh 1991), angiotensin IV (Gard, 2008), oxytocin, vasopressin (Engelmann et al., 2000) and neurokinin substance P (Huston and Hasenohrl, 1995) also have been shown to play an important modulatory role in learning and memory.

Apart from neurotransmitters discussed above, neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophins have also been shown to affect memory via modulation of cholinergic and glutaminergic systems (Yamada et al., 2002). In addition, steroid receptors in hippocampus and neocortex are also likely to mediate the effects of adrenal steroids on memory.

3.4. Animal models for screening of substances affecting learning and memory

The memory consolidation theory proposed by Muller and Pilzecker in 1900 is considered as a fundamental paradigm in the neuropharmacology of memory (McGaugh 2000). According to this consolidation paradigm, three phases of memory formation can be recognised:

Acquisition/learning \(\rightarrow\) consolidation \(\rightarrow\) recall/retrieval

Acquisition can take place in one or several learning trials, when the animal is exposed to a stimulus. This is followed by consolidation which occurs immediately after this, and subsequently the memory trace will be physically stored over some hours. Recall test is done
with different training test intervals, and LTM retention takes place after a minimum of 6 hours but usually performed with a 24-hour interval (McGaugh 1966; Dudai and Morris 2000).

Any experimental intervention that influences memory processing (e.g. pharmacological treatment) depends on when it is applied: pre-training – any intervention done during this phase may affect both acquisition and/or consolidation, and post-training – since acquisition already takes place, only consolidation may be affected at this stage (Izquierdo et al., 1989; Quillfeldt et al., 1996).

Interpreting data using pre-training or post-training drug manipulations provides a conclusive demonstration on how the test drugs improve learning and memory retention. Pharmacological assessment of learning and memory has been traditionally assessed by exposing experimental rodents to certain behavioural tasks. Depending on the different types of memory, a range of behavioural paradigms can be employed to test the effect of experimental intervention on learning and memory processes.

**i. Open field habituation:** It consists of exposing an animal to an open arena, a new environment in the absence of any aversive or appetitive stimuli, and let the animal explore it freely for a fixed amount of time. This is the classical non-aversive and non-associative task. The memory retention test session is performed after 24 hours of habituation to test for long-term memory and less than 6 hours after habituation for short term memory (Brenes et al., 2009).

**ii. Passive avoidance:** It is a fear aggravated test which involves learning to inhibit a response in order to avoid a noxious event. The term ‘passive avoidance’ is used to describe experiments in which the animal learns to avoid the aversive stimulus by suppressing a particular behaviour. It can be of two types: step-down inhibitory avoidance and step-through
inhibitory avoidance. The step-down inhibitory avoidance is based on negative reinforcement and consists of an apparatus with an elevated platform and an electrified grid floor. In the test session, the animal recalls the aversive experience of the training session, having received a foot shock after stepping down into the grid; the better the retention, the larger the latency to descend into the platform (Netto and Izquierdo 1985).

The step-through avoidance test is used for testing avoidance learning and memory in experimental rodents (Bures et al., 1983). The apparatus used in the present study has been shown in Figure 0-6 and the test employed has been described in details in Section 4 under Materials and Methods.

iii. Shuttle box active avoidance: Avoidance behaviour can be tested by automated shuttle box. Avoidance is induced by conditioned and unconditioned stimuli to the animal. In response, animal must relocate to the adjoining compartment within a present time in order to avert the mild electric shock. The latency of the subject from stimuli onset to escape after pre-training is related to retention of memory task. This task may be classified as operant conditioning where the animal learns the relation between sound and shock and learns how to escape it. This task is also called shuttle avoidance, in a reference to the strategy the animal must learn and perform (Vogel and Vogel 2002).

iv. Elevated plus maze: It serves as a model to evaluate learning and memory wherein the stimulus exists outside the body. The apparatus employed in the present study has been shown in Figure 0-7 and the methodology has been described in Section 4 under Materials and Methods.
Passive avoidance apparatus

Figure 0-6: Passive avoidance apparatus. LC – light compartment; DC – dark compartment; S – stimulator.
Elevated plus maze apparatus

**Figure 0-7**: Elevated plus maze apparatus
v. **Contextual fear conditioning:** The animal learns that certain environmental stimuli predict aversive events. Since there is no possibility of escaping from the stimulus, this task represents classical, Pavlovian conditioning and reflects a defensive behaviour selected by evolution in animals. It provides an interface between memory and emotion (Maren 2001).

vi. **Object recognition:** Recognition is the process wherein a subject is aware that a stimulus has been previously experienced. It requires a series of cognitive operations in order to match the observed event with a memory of the previously experienced one (Steckler et al., 1998).

vii. **Morris water maze:** It is one of the gold standards for test of spatial learning and memory. The animal learns to swim in a water tank guided by external cues, and find its way to a submerged platform. Based upon spatial information, this animal learns how to escape to a platform, so this task represents explicit, associative memory with operant-like spatial learning (Morris 1984). The apparatus has been diagrammatically represented in Figure 0-8 and the detailed methodology is described under Materials and Methods in Section 4.

viii. **8-arms radial maze:** It is also used to assess spatial learning and working memory in rats, designed by Olton and Samuelson in 1976. The apparatus consists of eight arms, all radiating from a small circular platform in the centre. The design ensures that after checking for food at the end of one arm, the animal is forced to return to the central platform before making another choice. As a result, the animal always has 8 possible options. After 8 choices the session is terminated and the animal has to obtain maximum rewards with minimal errors (Peele and Baron 1988).
**Figure 0-8:** A) Morris water maze apparatus B) Schematic representation of the quadrant divisions (Q1-Q4), TQ – target quadrant, P – escape platform
3.5. Animal models of memory disorders

Memory impairment or dementia occurs as a relatively isolated sequel to brain damage. Studies involving human amnesia and use of animal models for investigating those conditions have revealed a wealth of information about the organization of memory in the brain. However despite an extensive literature, the current approaches fail to emulate all of the characteristics of dementia since dementia can result from a wide range of neuropsychiatric disorders including head injuries, brain tumours, cerebrovascular disease, Parkinson’s or AD.

Since AD is the most common cause of dementia, animal models of AD are the ones that have been most thoroughly developed. The knowledge that neurodegeneration occurs at several critical sites in the medial temporal lobe including the hippocampus in AD patients provides a basis for loss of cognitive functions that rely on those structures (Hyman et al. 1984, 1987; West et al., 1994).

Amnesia represents a characteristic feature of AD. Various types of animal model systems have been developed for induction of amnesia. These model systems relate to the neuropathological, neurotransmitter and neuropeptide deficits commonly associated with AD, and attempts to study their consequences for cognitive performance in rodents and primates. With regard to the neuropharmacology of Alzheimer’s dementia, pharmacological modulation of cholinergic (Drachman and Leavitt 1974), noradrenergic (Perry 1981), serotonergic (Chen et al., 1996), GABAergic (Lanctot et al., 2004) and glutaminergic systems (Francis 2003) have been used to model the behavioural and cognitive deficits seen in AD.

With respect to molecular-based models, β-amyloid overproduction and Aβ deposition (Hsiao 1996), exogenous administration of β-amyloid (Cleary et al., 1995), and mutations in apolipoprotein E genes (Raber et al., 1998) have received a great deal of empirical attention. AD-related cognitive deficits can also be induced by lesion of specific brain structures
(hippocampus, striatum and cortex) or pathways in the brain that are important for different aspects of learning and memory (Gray 1983). Chemically induced models have also been developed to mimic or characterise only one specific pathophysiological pathway underlying AD, such as neuroinflammation by use of endotoxins (lipopolysaccharide or proinflammatory cytokines) or alteration of glucose and energy metabolism (Hauss-Wegrzyniak 1998; Wenk 2003).

3.5.1. **Cholinergic blockade as an experimental model of impaired learning and memory**

The vast majority of animal models used to study the effect of cognitive enhancers relate to the cholinergic hypothesis of AD. ACh is the primary neurotransmitter that has been highly implicated in learning and memory, and disruption of the cholinergic system largely contributes to the pathogenesis of cognitive and behavioural disturbances associated with dementia (Terry and Buccafusco 2003). Cholinergic neurotransmission is mediated via ACh and can be mediated through different types of muscarinic or nicotinic receptors. The action of ACh is terminated through action of AChE enzyme (Wessler 2008).

Five different muscarinic receptor subtypes (M1-M5) and seven different forms of the nicotinic receptors have been identified (Golan 2012). The M1 subclass of muscarinic receptors has been studied most extensively because of the availability of a relatively specific antagonist, pirenzipine, which has been found to impair acquisition, retention and indices of working memory (Bymaster et al., 1993). Muscarinic M1 receptor agonists mitigate learning and memory deficits produced by forebrain cholinergic lesions and by use of muscarinic receptor antagonists such as scopolamine or pirenzipine (Dawson et al., 1994). Enhanced ACh release is thought to mediate the cognitive enhancing effects of muscarinic M2 receptor antagonism (Lachowicz et al., 2001).
Cholinergic neurotransmission facilitates neuronal responses and synaptic plasticity associated with AD and is believed to be mediated specifically through the muscarinic receptors. Cholinergic deficits in AD are associated with a loss of cholinergic markers. Degeneration of cholinergic neurons at the nucleus basalis of Meynert (NBM) in the basal forebrain occurs during the early stage of the disease (Davies and Maloney 1976). Post-mortem analysis of AD patients has revealed neuronal loss and neurofibrillary tangle formation, primarily at the pyramidal neurons of the neocortex and the hippocampus. AD also has been shown to affect the serotonergic neurons and the noradrenergic locus coeruleus (DeKosky et al., 1996; Mann 1996).

Biochemical investigations in AD patients have indicated that a selective neurotransmitter pathology, particularly involving the presynaptic markers of the cholinergic system, appears to be uniformly reduced in this disease (Francis et al., 1993). This is elucidated by decrease in choline acetyltransferase (ChAT) activity and increase in AChE activity together resulting in decreased level of ACh, which strongly correlates with the degree of behavioural and cognitive deficits in patients with AD (Perry et al., 1978; Wilcock et al., 1982). A few other studies have reported that a reduction occurs in the number of nicotinic and M2 acetylcholine receptors in brains of AD patients, particularly those localised on the presynaptic cholinergic nerve terminals (Sims et al., 1983). Cholinergic neurotransmission is considered one of the specific targets for Aβ as it has been shown to reduce both choline uptake and ACh releases in vitro (Auld et al., 1998).

Many pharmacological studies have evaluated the effects of cholinomimetic drugs and anticholinergics on learning and memory performance. The most widely used pharmacological model related to AD is scopolamine-induced amnesia which is used to study the role of cholinergic system hypofunction in learning and memory (Sunderland et al., 1996). Scopolamine, a tropane alkaloid drug exhibiting muscarinic receptor antagonistic
effects, induces amnesia in rodents (Bejar et al., 1999) and young healthy subjects (Jones et al., 1991) similar to those in older adults or untreated subjects with dementia. Studies involving the scopolamine-induced amnesia models have hypothesised that the integrity of the cholinergic system is essential to learning and memory, and scopolamine by virtue of its muscarinic receptor antagonistic properties, can disrupt the functional integrity of this system (Drachman and Leavitt 1974). This hypothesis is supported by a series of studies suggesting learning and memory defects in rodents and primate model systems receiving scopolamine either systemically or intracerebroventricularly (Bartus et al., 1985). Compounds that can reverse the scopolamine-induced memory deficits in experimental animals may thus be considered as potential drugs to treat cognitive dysfunction observed in AD.

In both animals and humans, muscarinic receptor blockade by scopolamine or lesions of the cholinergic neurons at basal forebrain impair performance in a variety of attention, learning and memory tasks. Conversely, enhancing the ability of ACh with cholinomimetics such as physostigmine reverses lesions and pharmacologically induced cholinergic deficits, and thus improves cognitive function in AD (Hagan and Morris 1988). Scopolamine-induced cognitive impairments are mediated by motivation deficits or by direct effects on sensorimotor function or by disruption of attentional process (Stanhope et al., 1995). Scopolamine induces significant behavioural deficits and reduces metabolic activity in thalamus (Cohen et al., 1994).

Moreover since systemic administration of scopolamine affects many anatomical sites (reduced activity in cingulate, increased activity in basal ganglia), the learning and memory deficits attributed to it could be due to complex interactions of the drug with multiple CNS structures and different behavioural systems. The memory deficit induced by scopolamine is associated with frontal cortex dysfunction, based on concurrent decrease in regional cerebral blood flow in human subjects (Honer et al., 1988). Reversal of memory deficit and frontal
cortical hypoperfusion by physostigmine adds strong support to this hypothesis (Honer et al., 1988). Cholinergic stimulation or antagonism can change cortical perfusion by affecting glucose metabolism and blood flow since the vascular system is densely innervated with cholinergic fibres and cholinergic receptors (Kalaria et al., 1994). Further, scopolamine may affect brain function and induce memory deficits by increasing oxidative stress in the brain. Scopolamine is known to trigger the induction of reactive oxygen species (ROS) and cause free radical injuries in the brain (Fan et al., 2005). An appropriate dose of scopolamine can increase oxidative stress in the brain, characterised by inactivation of superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity, with consequent inhibition of adenosine triphosphatase (ATPase) in the hippocampus and cortex (Fan et al., 2005). Since oxidative stress plays a major role in the pathogenesis of dementia, the findings from the above studies may substantiate the value of scopolamine induced amnesia in rats as a valid animal model for screening of drugs with potential therapeutic benefit in dementia.

3.6. Oxidative stress and cognitive impairment

Oxidative stress (OS) refers to a state wherein free radicals are produced in excess of body’s antioxidant defence mechanisms (Harman 1981). Excessive generation of free radicals has been critically implicated in several diseases like atherosclerosis, cancer, diabetes, cardiovascular diseases, inflammation, and in pathological manifestations of aging and age-related neurodegenerative disorders including AD, Parkinson’s disease, Huntington disease and amyotrophic lateral sclerosis (Perry et al., 2002; Yun-Zhong et al., 2002; Liu et al., 2003).

The dramatic impairment of learning and memory seen in animals and humans during aging and dementia is also associated with a marked increase in brain oxidative stress (Liu et al.,
OS leads to alterations in cellular activity with an accumulation of oxidation products including advanced glycation end products (Smith et al., 1994), lipid peroxidation products (Sayre et al., 1997), nitration (Smith et al., 1997), free carboxyls and carbonyl modified neurofilament proteins (Smith 1995).

OS significantly contributes to learning and memory deficits seen during aging (Fukui et al., 2001) and AD (Pratico 2008). OS is widely accepted as a contributor to neuronal vulnerability (Lin and Beal 2006; Sayre et al., 2008). A significant loss in learning and memory function associated with dramatic increases in several markers of oxidative stress has long been implicated in several pathological manifestations of aging (Harman 1981; Floyd, 1991). An increase in the level of malondialdehyde (MDA) and isoprostanes which are sensitive and specific markers of in-vivo lipid peroxidation have been reported in cerebrospinal fluid (CSF) and brains of AD patients (Markesbery et al., 1998; Pratico and Sung 2004). In addition, alteration in antioxidant enzymatic activities like SOD and catalase has also been observed in AD patients (Marcus et al., 1998).

In the brain of AD patients, increased lipid peroxidation is most prominent where degenerative changes are seen. The process of peroxidation and overproduction of free radicals in brain leads to depletion of detoxifying endogenous antioxidants such as glutathione (Candelario et al., 2001). Glutathione transferase (GST), the class of enzymes that detoxify the products of oxygen metabolism such as 4-hydroxyalkenals is diminished to a large extent in the CSF and several other brain regions of AD patients, suggesting loss of protection against oxidative stress (Lovell et al., 1998). Besides, the oxidation of proteins and deoxyribonucleic acid (DNA) by free radicals also plays an important role in AD.

Protein oxidation in the brain occurs at the expense of enzymes vital for the functioning of neurons and neuroglia. Oxidation of proteins involves alterations in the three-dimensional structure and physiochemical properties of proteins leading to their fragmentation and
aggregation (Stadtman and Levine 1998). It has been reportedly found that the products of protein oxidation aggregate in the form of fibrils together with increased lipid peroxidation in brains of patients with both AD and mild cognitive impairment (MCI) [Butterfield et al., 2010]. Further DNA oxidation leads to strand breaks, sister chromatid exchange, DNA protein cross linking and modifications of DNA bases (Gabbita et al., 1998). Elevations of 8-hydroxy-29-deoxyguanosine (8-OHdG) levels in intact DNA coupled with increased levels of 5-hydroxyuracil, 5-hydroxycytosine and 8-hydroxyadenine have also been observed in nuclear brain fractions of AD patients. DNA damage thus plays a major role in aging associated changes.

In AD, the increased oxidative stress generated from oxidation of lipids, proteins and DNA coupled with the decline in level of glutathione and other antioxidants have been found to be largely localised to the synapses and correlate with the disease severity (Lovell et al., 1999). The most crucial aspect of the OS in AD pathogenesis is the cytoskeleton modifications of neurons vulnerable to damage in AD, which in turn leads to irreversible cellular dysfunction ultimately leading to neuronal death (Smith et al., 1998). OS has been implicated in Aβ-induced neurotoxicity due to increased hydrogen peroxide and nitric oxide (NO) production as well as elevated oxidative modification of proteins and lipids (Behl et al., 1994; Mattson, 1997). This confirms that β-amyloid accumulation promotes oxidative stress.

Further, in a study by Lim et al., (2001), it was seen that defects in antioxidant defense mechanisms markedly increased Aβ deposition, which could be lowered by the use of dietary antioxidant such as curcumin (Lim et al., 2001). Oxidative stress has been also interlinked with tau pathology which is the chief component of neurofibrillary tangles, another pathological hallmark of AD that tends to correlate with the severity of cognitive decline (Stamer et al., 2002; Goedert and Spillantini, 2006). Cells overexpressing tau proteins have
been found to be more vulnerable to oxidative stress due to depletion of peroxisomes (Stamer et al., 2002).

The mitochondrion is a major site for production of toxic ROS and mitochondrial dysfunction coupled with oxidative metabolism is implicated in the pathogenesis of AD. A number of metabolic and mitochondrial abnormalities have been detected in the hippocampal neurons of AD patients compared to healthy age-matched controls (Grivennikova and Vinogradov 2006; Zhu et al., 2006). Overall, the mechanisms by which mitochondrial dysfunction leads to neuronal changes include ROS generation along with impaired adenosine triphosphate (ATP) production, changes in mitochondrial permeability and excitotoxicity (Parker and Davis 1997).

Additionally, transition metals such as copper (Schlief and Gitlin 2006), zinc (Smart et al., 2004) and iron (Munoz and Humeres 2012) catalyse many enzymatic reactions and are essential for brain functions. An imbalance in the homeostasis of these metals occurs in presence of oxidative stress such as lipid peroxidation and leads to a series of biochemical and metabolic alterations that eventually leads to neuronal cell death. Abnormal levels of copper, zinc and iron have also been observed in the AD hippocampus and amygdala, the areas that are characterised by severe histopathologic alterations during AD (Deibel et al., 1996). Thus, OS is linked to several pathological manifestations in AD including neurotoxicity induced by Aβ, tau pathology and mitochondrial dysfunction. ROS may also be generated from abnormal accumulation of transition metals, caused due to combined effects of Aβ accumulation and tau pathology, ultimately leading to OS. Thus strategies to inhibit OS and glycation can prove effective in reducing the cognitive dysfunction seen in dementia.
3.7. Current therapeutic options for treatment of cognitive dysfunction

Impairment of memory and cognitive dysfunction are the earliest features of AD. Certain pharmacological compounds have been shown to exert nootropic effects, enhancing cognition and memory in experimental rodents and humans. Considering the multifactorial pathology of AD, and with advances in understanding the biology of cognition, development of strategies that may alter the underlying pathology and progression of the disease is of the highest priority to alleviate or prevent AD. Several drugs with putative neuroprotective effects are thoroughly being investigated and many of them are in different phases of clinical development. The current therapeutic options for AD include cholinesterase inhibitors, glutamate receptor modulator, antioxidants and anti-inflammatory agents (Lleo 2007).

i. **Cholinesterase inhibitors:** The majority of therapeutic approaches in AD focus on the cholinergic system and acetyl cholinesterase inhibitors (AChEIs) have become part of standard care in the management of AD. Currently, four AChEIs have been approved by the U.S. Food and Drug Administration for the treatment of AD and they include donepezil, rivastigmine, galantamine and tacrine (Doody et al., 2001). Tacrine is now rarely used because it causes hepatotoxicity in approximately 40% of patients. AChEIs increase the brain availability of ACh by inhibiting the enzyme AChE. ACh is synthesised in cholinergic neurons by the action of ChAT and its action is terminated by AChE. AChE inhibition enhances cholinergic neurotransmission by prolonging the duration of ACh molecules remaining in the synaptic cleft and thus enabling it to combine with muscarinic receptors (Giacobini 2003). However, the benefits of AChEIs are relatively modest, with evident improvement in cognitive function observed in less than 10% of subjects (Courtney et al., 2004). The likelihood of cognitive or behavioural improvements with AChEIs is small compared to the overall course of the disease.
ii. **Glutamate receptor modulator:** Glutamate is the major excitatory neurotransmitter in the brain. Excessive glutaminergic neurotransmission is associated with neuronal dysfunction and death (Michaelis 1998). NMDA, a glutamate receptor, has been implicated in long-term potentiation, one of the important neuronal mechanisms responsible for learning and memory. An antagonist of NMDA receptor, memantine, has been found to be effective in treatment of moderate-to-severe AD (Lipton and Chen 2004). However similar to AChEIs, memantine appears to offer only modest symptomatic benefits to AD patients and its use has been limited to patients with mild AD (Reisberg et al., 2003).

iii. **Antioxidants:** As discussed, oxidative stress is one of the factors implicated in pathogenesis of AD. The antioxidant largely employed for treatment of AD has been vitamin E (Sano et al., 1997). A 3-year clinical trial assessing the effects of vitamin E in patients with mild cognitive impairment however failed to show a significant effect on slowing the progression of AD (Petersen et al., 2005). A naturally occurring compound, *Ginkgo biloba*, which has antioxidant properties, is also widely used by elderly individuals and patients with dementia to enhance cognition (Luo et al., 2002). Although the efficacy of *Ginkgo biloba* in treating dementia appears very small, most of their formulations appear safe.

iv. **Anti-inflammatory agents:** Another strategy aimed in treatment of AD is reducing the brain inflammation with nonsteroidal anti-inflammatory drugs (NSAIDs). Although NSAIDs were claimed to have a neuroprotective effect against the development of AD (Int’ Veld et al., 2001), this effect does not extend to efficacy once symptoms of AD have appeared. Trials with NSAIDs reported little or no benefit in patients with established AD (Rogers et al., 1993; Aisen et al., 2002). Currently, NSAIDs are not recommended as treatment for patients with AD.
Abnormalities of glucose metabolism and insulin resistance also contribute to the learning and memory deficits as seen in dementia (Craft 2005). The thiazolidinediones have been shown to increase peripheral insulin sensitivity through its effects on Peroxisome Proliferator-Activated Receptor (PPAR-\(\gamma\)) agonists and are currently being investigated for their cognitive enhancing properties (Pederson et al., 2006).

Drugs currently available for cognitive enhancement in AD include cholinesterase inhibitors and the NMDA-receptor antagonist, memantine. However, at present, there is no treatment available that can halt or delay the progressive deterioration of cognitive functions in AD patients. It is forecasted that by 2050, the number of demented persons would rise to 114 million (Wimo et al., 2003). The development of novel drugs or drug targets with strong disease-modifying or cognitive enhancing potential therefore represents one of the biggest unmet medical needs today.

### 3.8. Renin Angiotensin System (RAS)

#### 3.8.1. Physiological and pharmacological effects of RAS

The primary role of RAS is regulation of blood pressure and maintenance of body fluid homeostasis (Hall 1991). Renin, the first component of the RAS, was isolated from the kidney extracts more than 100 years ago (Tigerstedt et al., 1898). Renin is an aspartyl protease released from the juxtaglomerular cells of the kidney in response to changes in sodium chloride absorption. The substrate for renin is the inactive precursor, angiotensinogen, which converts Ang-I to Ang-II in presence of ACE (Johnston 1990). The major substrates for Ang-II formation in brain is regulated by angiotensinogen synthesis in the astrocytes and its release into the CSF.
Angiotensinogen constitutes one of the most abundant proteins in the CSF (Sandor and Jong 1987). Angiotensinogen is also found in neurons at regions such as the subfornical organ, paraventricular nucleus, nucleus of the solitary tract, and rostroventrolateral medulla, which are the brain centres involved in cardiovascular regulation (Diz 2006). ACE is a zinc metalloproteinase, which in addition to kidney is also found in the endothelial cells of the blood vessels. In the endothelial cells, it mediates the degradation of bradykinin, which is a potent vasodilator (Pipili et al., 1989).

Ang-II, a potent vasoconstrictor, exhibits peripheral actions as well as produces a centrally mediated hypertensive response (Philips and de Oliviera 1987). The brain RAS is well known for its involvement in controlling systemic BP, including the regulation of cerebral blood flow (Gasparo 2000). Ang-II administered centrally was found to strongly stimulate drinking behaviour in rats (Epsstein 1970). Reduction of central Ang-II has been found to be associated with antidepressant (Martin 1990; Vijayapandi and Nagappa 2005), and anxiolytic actions (Costal 1990). Ang-II also governs the regulation of neurotransmitters such as norepinephrine and serotonin (Philips and de Oliviera 1987), while inhibiting the release of ACh (Barnes et al., 1990). Today, it is well established that Ang-II in the brain can exert a number of actions including modulation of autonomic nervous system (ANS) activity, hypothalamo-pituitary adrenal (HPA) axis activity and vasopressin release (Aguilera and Kiss 1996; Fink 1997), and also regulates the processing of sensory information, emotional responses and learning and memory activities (Haulica et al., 1999; Llorens and Mendelsohn 2002).

Ang-II that is generated is further converted to 2-8 hexapeptide Ang-III by the enzyme aminopeptidase A (Reaux et al., 1999). This Ang-III is then cleaved by aminopeptidase A or N to form the neuroactive 3-8 hexapeptide fragment of Ang-II called angiotensin-IV (Ang-IV) [Lavoie and Sigmund 2003]. Ang-IV is degraded by aminopeptidase into amino-terminal
deleted peptides. Besides regulating blood flow, Ang-IV has been shown to be primarily implicated in modulating exploratory behaviour and processes involving learning and memory (von Bohlen 2003) [Figure 0-9].

Ang-II can also be converted to Ang (1-7) by ACE-2 (Ohishi et al., 2013) which is more active in the brain than ACE. Ang 1-7 binds to a G-protein coupled receptor, Mas, which is encoded by the Mas protooncogene (Bunnemann et al., 1990). Mas is expressed in the brain, primarily in regions such as the hippocampus, amygdala, dentate gyrus, and piriform cortex (Martin et al., 1992; Ohishi et al., 2013). High amounts of Mas transcripts are present in the hippocampus and cerebral cortex of rat brain (Ohishi et al., 2013). This Ang (1-7)/Mas receptor system is essential for counteracting organ inflammation and fibrosis (Passos-Silva et al., 2013). It also increases utilization of glucose, reduces insulin resistance, and has significant importance in learning and memory processes (Simões et al., 2013).
Diagram depicting the RAS pathway in the brain

**Figure 0-9:** Diagram of enzymatic pathways proposed for production of angiotensin peptides from angiotensinogen within the brain (Adapted from McKinley et al., 2003).
3.8.2. Angiotensin receptors

Angiotensins exert their function through AT\textsubscript{1}, AT\textsubscript{2} and AT\textsubscript{4} receptor subtypes (Baltatu and Bader 2003; McKinley 2003; Gard 2002). AT\textsubscript{1} receptor subtype is a 7-transmembrane receptor with molecular weight of 40-42 kDa coupled to second messenger systems including phospholipase C, Ca\textsuperscript{2+} and cyclic AMP (cAMP) [DeGasparo et al., 2000]. AT\textsubscript{1} receptors are localized in the kidney, heart, vascular smooth muscle cells, adrenal gland, brain, adipocytes, platelets and placenta (Timmermans et al., 1993). The brain contains high density of AT\textsubscript{1} receptors. Most of the physiological effects of Ang-II are mediated through AT\textsubscript{1} receptor and they include the classic angiotensin functions such as maintenance of BP, fluid homeostasis, and sexual behaviour (Wright and Harding 1992). The AT\textsubscript{2} receptors are present in the uterus, adrenal gland, CNS, heart and kidney (Ohishi et al., 2013). Most reports concerning this receptor subtype suggest its role in brain differentiation and development, apoptosis, regulation of cerebral blood flow (CBF) and NMDA receptor modulation (Wright and Harding 1992). However, all the known physiological and clinical effects of Ang-II are mediated largely by the AT\textsubscript{1} receptors.

Ang-IV has low affinity for AT\textsubscript{1} and AT\textsubscript{2} receptors but has high affinity for the AT\textsubscript{4} receptor subtype (Allen et al., 1998). AT\textsubscript{4} is an insulin-regulated aminopeptidase and mediates actions of Ang-IV on central motor activities, on sensory processing and on learning and memory (von Bohlen and Halbach 2003).

3.8.3. Renin angiotensin system and the central nervous system

Within the brain, Ang-II controls BP, fluid balance and hormone secretion. However beyond the actions mediated by peripheral RAS components in the CNS, an intrinsic RAS exists in the brain. Several components of RAS, viz., angiotensinogen, ACE, Ang-II and angiotensin
receptors are found in the CNS indicating the existence of localised RAS in the brain. Immunohistochemical studies revealed the presence and distribution of renin, angiotensinogen, Ang-I and Ang-II in a number of regions of the rat brain (Changaris et al., 1978). Immunoreactivity to angiotensinogen and Ang-I were found in the hypothalamus whereas immunoreactivity to Ang-II was localised in neurons and vessels in the brainstem, hypothalamus, hippocampus, cerebellum, and cortex (Healy and Printz 1984; Simoese - Silva 2013). In the human brain, Ang-II immunoreactivity has been reported in regions such as basal ganglia, brainstem, hypothalamus, cortex and cerebellum (Dzau and Pratt 1986) [Figure 0-10].

Binding sites for Ang-II in the human brain have been shown to be localised in the forebrain, basal ganglia, substantia nigra, cerebellum, cortex and hippocampus (Barnes 1993; MacGregor 1995). AT$_1$ receptor distribution has been found to be present in the circumventricular organs, hindbrain, substantia nigra, periaqueductal grey, nucleus tractus solitarius (NTS), cortex and hippocampus (Allen et al., 2000) [Figure 0-10]. AT$_1$ is localized to the presynaptic neurons and to the glial cells in the brain (Barth and Gerstberger 1999). AT$_2$ receptor is localised primarily in the thalamus and cerebellum (Lenkei et al., 1997; Allen et al., 2000). AT$_4$ receptor expression has been found in the hippocampus, cortex, amygdala, thalamus, hypothalamus, substantia nigra, cerebellum and periaqueductual grey (von Bohlen 2003). The expression of AT$_4$ receptor in brain areas relevant to memory function indicates a potential role for Ang-IV in memory acquisition and cognitive processing (von Bohlen 2003).

3.8.4. Brain renin angiotensin system in cognitive function

Ang-II is the dominant effector molecule regulating RAS. Basic experiments suggest a role of brain Ang-II in cognitive function, neural injury and neural inflammation (Min et al., 2011). However, the actual role of Ang-II on learning and memory has been difficult to comprehend.
Initial studies have shown that central injection of Ang-II in rodents improves aversive memory (Braszko 2002) and facilitates learning and retention in conditioned avoidance test (Georgiev and Yonkov 1985). Similarly, Ang-II was found to exert diverse effects on central AT₁ receptor to enhance learning and memory with differential effects on learning, storage and recall (Figure 0-11). Administration of an AT₁ receptor blocker, losartan, was shown to ameliorate the Ang-II induced improvement in object recognition but had no significant effect on Ang-II induced cognitive responses (Kulakowska 1996).

Paradoxically, another subsequent finding showed that losartan could facilitate spatial and short-term working memory, and reverse scopolamine-induced cognitive deficits (Raghavendra 1998). Similarly, Ang-II injection directly into the dorsal neostriatum was found to impair the step-down shock avoidance response in rats while a similar study showed that intracerebroventricular (i.c.v.) injection of Ang-II improved memory retention in a similar conditioned avoidance test (von Bohlen and Albrecht 2006), thus presenting differential effects of Ang-II on memory.

Several contradictory findings suggest that Ang-II decreases cognition. Investigation using ACEIs such as captopril suggested that Ang-II suppression may enhance cognitive functions. Daily administration of captopril was shown to improve learning and retention behavioural deficits in aged mice (Barnes 1989). Centrally active ACEI such as perindopril was also reported to prevent chronic central hypoperfusion in rats and mice induced with AD (Yamada 2010). In rats, injection of Ang-II above the CA1 region was shown to block the induction of LTP, and this blockade could be reversed by administration of ARBs (von Bohlen and Albrecht 1998, 2006).
Figure 0-10: A diagrammatic sketch of the mammalian brain showing Ang-II-sensitive areas and inaccessible areas. Areas shown in red contain AT1 receptors that are accessible to circulating Ang-II. These include the subfornical organ (SFO), the organum vasculosum of the lamina terminals (OVLT), and the area postrema (AP). Areas marked in blue also contain AT1 receptors that cannot be reached by systemic Ang-II owing to the blood-brain barrier. These include the supraoptic (SON) and the paraventricular nuclei (PVN) of the hypothalamus, the rostral (R) and caudal (C) ventrolateral medullae (VLM), and the nucleus tractus solitarius (NTS). These regions are only accessible to Ang-II synthesized locally in the brain (Adapted from Bodiga and Bodiga 2013).
Diagram showing RAS pathway and involved receptors in the mammalian brain

**Figure 0-11:** Schematic representation showing the formation of functionally active components of RAS and involved receptors in the mammalian brain. Ang-II acts on AT$_1$ and AT$_2$ receptors. Essential functions in the brain are also mentioned. Underlined: Examples of receptor antagonists (Adapted from Albrecht 2010).
To further investigate the paradoxical effects mediated by Ang-II on cognition, cognitive tests were performed in human renin and human angiotensinogen gene transgenic mice (hRN/hANG–Tg). Continuous activation of Ang-II showed that the avoidance rate in hRN/hANG –Tg mice did not increase from 14 weeks of age. But it was higher from 8-13 weeks of age when compared to wild-type mice (Inaba et al., 2009). From these findings, it was concluded that acute or subacute Ang-II administration may enhance cognitive function but chronic treatment with Ang-II may result in cognitive impairment by exhausting neural function.

Besides inhibiting release of Ach, Ang-II has been shown to participate in cerebrovascular remodelling, promoting vascular inflammation, mediating OS and causing impairment of CBF (Kazama et al., 2004; Marchasi et al., 2008). It has been implicated in cognitive processing, arousal and attention in the brain (Barnes et al., 1992). It suppresses LTP in the hippocampus and amygdala of rats by acting through the AT1 receptor (Karczmar 1993). Ang-II induces astrocytes ageing in humans, which is implicated in neurodegenerative diseases via generation of superoxide radicals (Liu et al., 2011). Endothelial function in cerebral blood vessels was shown to be disrupted in a genetic model of Ang-II dependent hypertension (Didion et al., 2000; Faraci et al., 2006).

Findings from various studies have suggested that RAS besides mediating memory and learning is also involved in AD, stroke, depression and emotional stress (Philips and de Oliveira 2008). Accumulating evidences have revealed that RAS alteration in the brain have important implications in the pathogenesis of AD. ACE activity was increased in the cortex, medial hippocampal, parahippocampal gyrus and caudate nucleus of AD patients, and these changes correlated with parenchymal Aβ load (Areegui et al., 1982). A similar study showed that the activity of ACE and the concentration of ACE correlate directly with the severity of AD (Miners et al., 2009). Increased Ang-II, AT₁ (Savaskan et al., 2001) and AT₂ (Barnes
1996) receptor binding have been shown to be increased in cortex of AD subjects, suggesting an increased brain RAS activity during the disease process. Ang-II suppresses the release of ACh and since ACh is the primary neurotransmitter involved in mediating learning and memory functions, the augmented RAS activity may represent an important factor contributing to the cognitive dysfunction in AD.

Furthermore, Ang-II containing neurons found in the striatal and hippocampal regions of AD patients were found to correlate with Aβ plaque load (Savaskan et al., 1991). Elevated ACE and AT₁ receptor activity might also contribute to the abnormalities in cerebral blood flow that underlies one of the important aspects of cognitive decline. Vascular Aβ deposits are also partly mediated by an increased RAS in the brain probably by allowing the diffusion of RAS components through the damaged blood-brain barrier in the vessel walls (Faraci et al., 2006; Miners et al., 2009). Cerebrovascular dysfunction has been associated with cognitive disorders including AD, and vascular risk factors not only increase the risk of developing vascular dementia but also contribute to the progression of AD (Skoog et al., 1999). RAS alterations thus seem to represent a pathological link common to different types of dementia.

Taken together, these findings suggest that an increased level of octapeptide Ang-II can disrupt cognitive function by causing neuronal degeneration mediated through CNS inflammation, decreased cerebral blood flow, cholinergic dysfunction, oxidative stress and cellular senescence in the brain.

3.8.5. Role of Angiotensin-IV and Ang (1-7) on cognition

While Ang-II disrupts memory and learning, the hexapeptide Ang-IV can facilitate memory and acquisition [Figure 0-11]. Ang-IV binds specifically to the enzyme insulin regulated aminopeptidase (IRAP) in the brain and mediates the memory enhancing effect of this
peptide (Skoog et al., 1999; Chai et al., 2008). Ang-IV administered intracerebroventricularly facilitates exploratory behaviour and improves memory retention in rodents (Braszko 2004; Lee et al., 2004). Treatment with AT₄ agonists has been shown to reverse scopolamine-induced memory deficits in rats during passive avoidance and spatial learning tasks (Albiston 2004). Ang-IV has been shown to facilitate LTP in the CA1 and dentate gyrus regions in the hippocampus of rats (Wayner et al., 2001). Since LTP represents a correlation of processes involved in memory, the above findings suggest that Ang-IV indeed has a behavioural effect particularly on cognition.

Ang (1-7) that is formed by ACE-2 mediated degradation of Ang-II by also holds importance in memory processing. Ang (1-7) acts through Mas receptor and the Ang (1-7)/Mas receptor system has been identified in the brain with high concentration in the dentate gyrus and piriform cortex. Besides its role in increasing glucose utilization and reversing insulin resistance, it also facilitates LTP and plays a neuroprotective role in response to cerebral ischemia (Jiang et al., 2012) [Figure 3-11].

3.8.6. Effects of angiotensin on cholinergic system and Aβ metabolism

Two prominent theories have been proposed to explain the pathological changes seen in AD - the amyloid cascade hypothesis (van Nostrand et al., 1996) and the cholinergic hypothesis (Drachman and Leavitt 1974). Aβ peptide is a 39-42 amino acid peptide produced by the conversion of APP. Aβ accumulation produces the neurodegenerative characteristics seen in AD (Albiston 2004). ACE of the RAS has been shown to form an important link with respect to AD. Initial findings suggested that ACE can degrade Aβ and treatment with an ACEI lisinopril could interfere with the ACE’s ability to inhibit the aggregation of Aβ (Hu et al., 2001). However subsequent findings contradicted the data and suggested that blockade of
RAS may affect Aβ metabolism and prevent Aβ deposition. Treatment with ARB, valsartan, was able to improve spatial learning and attenuated oligomerization of Aβ into high molecular weight oligomeric peptides (Wang et al., 2007). Another ARB, telmisartan, was able to decrease the Aβ concentration in the brain of mice receiving i.c.v. injection of Aβ (1-40) and this effect was attributed to its antioxidant property and hippocampal astrocyte activation (Tsukuda et al., 2009). The brain penetrating ACEI ceranopril was also shown to produce delayed but long-lasting inhibition of ACE activity in the rat brain (Cushman et al., 1989).

A randomised, prospective clinical trial conducted over a period of 12 months on 150 patients reported that the so-called brain penetrating ACEIs captopril and perindopril could reduce the cognitive decline in patients with mild-to-moderate AD (Kehoe and Wilcock 2007). Thus the potential benefits of ACEIs and ARBs on Aβ-mediated cognitive function need further evaluation.

The cholinergic hypothesis of AD posits that AD is characterised by a loss of cholinergic neurons resulting in decreased level of neurotransmitter ACh. This loss of cholinergic neurons is associated with disruption of ACh-receptor signalling and a resultant decrease in the levels of these receptors on the brains of AD patients (Wayner et al., 2001; Pakaski and Kalman 2008). Based on this hypothesis that the ACh level is decreased in AD, certain predominantly centrally acting AChEIs such as rivastigmine and donepezil were developed (Wang et al., 2007). AChEIs can inhibit central and peripheral AChE and thereby increase the levels of ACh. AChE binds to the non-amyloidogenic form of Aβ and appears to induce a conformational shift to the amyloidogenic form (Inestrosa et al., 1996; DeFerrari et al., 2001; Rees and Brimijoin 2003). Treatment with AChEIs thus neutralizes the catalytic site of the enzyme and thereby reduces Aβ peptide aggregation. However, the AChEIs only partially
delay the progressive symptoms of AD and do not appear to impact the underlying pathology of the disease (Zhu et al., 2009).

Although there are few reports about the correlation between Ang-II and cholinergic transmission, it has been strongly suggested that Ang-II inhibits release of ACh from rat entorhinal and human temporal cortex. This demonstrates a specific interaction between angiotensin signalling and cholinergic system (Barnes et al., 1989).

Further, it has been shown that Ang-II blocks nicotine-induced neuroprotection against Aβ (1-42) via activation of tyrosine phosphatase-Src homology region 2 domain-containing phosphatase-1 (SHP-1) [Wang et al., 2000; Shaw et al., 2003]. Ang-II has also been shown to disrupt the nicotinic ACh-receptors that have a role in memory processing probably through AT₂ receptor induced SHP-1 activation (Seguin et al., 2012). Also, Aβ triggers AT₂ receptor oligomerization in the hippocampus (AbdAlla et al., 2009) and impairs coupling of the muscarinic ACh receptor to the GTP binding proteins (Gx q/11) [Tienari et al., 1996]. Thus, Ang-II and AT₂ receptor may interact with the cholinergic system and mediate some of the cognitive and behavioural deficits seen in AD.

3.8.7. Role of Ang-II on neurovascular unit

Neurovascular unit consists of vascular cells (non-neuronal cells) and glial cells (astrocytes, microglia and oligodendroglia) and plays an important role in the pathogenesis of neurodegenerative and cerebrovascular disorders (Iadecola 2010). Ang-II increases production of free radicals in cerebral microvessels and impairs the cerebral blood flow which is critical to maintain homeostatic balance of the cerebral microenvironment (Ando et al., 2004; Girouard et al., 2008). Slow injection of Ang-II was shown to reduce the neural activity by decreasing the CBF without prominent effect on BP (Capone et al., 2011). Ang-II
also increases the inflammation of cerebral microvessels via induction of OS and causes immune-endothelial cell interactions, thereby increasing the permeability of BBB (Zhang et al., 2010). Further, Ang-II coupled with aldosterone secretion can induce astrocyte senescence via superoxide production, neuroinflammation and neuronal degeneration, all of which increases the susceptibility to AD (Dzau 1986; Mogi et al., 2012).

3.9. Inhibitors of renin angiotensin system

Ang-II has limited therapeutic utility; clinical interest thus focuses on inhibitors of the RAS, including ACEIs, ARBs and direct renin inhibitors. These RAS inhibitors have been developed as therapeutic agents targeted for the treatment of hypertension (Brunner et al., 1979; Materson and Preston 1994) and its related complications including congestive heart failure (CONSENSUS Study Investigators 1987), acute myocardial infarction (Pfeffer et al., 1992) and chronic renal disease (Kunz et al., 2008).

3.9.1. Angiotensin Converting Enzyme Inhibitors (ACEIs)

Competitive inhibition of angiotensin I (inactive) to angiotensin II (active) is the common pharmacological property of all ACEIs and this effect results in the reduction or absence of synthesis of Ang-II (Brunner et al., 1979). The ACEIs are classified into three broad groups based on the chemical structure of their active moiety: sulfhydryl-containing ACEIs such as captopril and zofenopril; dicarboxylic-containing ACEIs which include benazepril, enalapril, lisinopril, moexipril, quinapril, ramipril, spirapril, trandolapril, and perindopril; and phosphorous-containing ACEIs such as fosinopril (Harrold 2002). Currently, only 11 of these ACEIs are available for clinical use.
Captopril is a sulfhydryl containing ACEI and the first to be marketed. Enalapril is a dicarboxylate, non-thiol containing ACE that acts as a prodrug. Lisinopril is a lysine derivative of enalaprilat, the active metabolite of enalapril. Among the dicarboxylate ACEIs, lisinopril has the most unique chemical feature. It contains the basic amino acid lysine (R1=CH2CH2CH2CH2NH2) instead of the standard non-polar alanine (R=CH3) residue and it does not require bioactivation since its carboxylic acid groups are not esterified. Among the remaining ACEIs, the major structural difference exists in the ring of the C-terminal amino acid. Lisinopril, like enalapril, contains pyrrolidone ring of proline, whereas other compounds including ramipril contains larger bicyclic or spiro ring systems (Figure 0-12). This variation seen in the ring structure of ACEIs like ramipril, benazepril or trandolapril facilitates enhanced binding to tissue ACE and increases their potency. Such a variation also accounts for differences in absorption, plasma protein binding, onset of action and duration of action among these ACEIs. In contrast, fosinopril is a phosphinic acid containing ester prodrug. It contains an acyloxy alkyl group that account for its better lipid solubility and improved bioavailability (Harrold 2002).

Thus, although grouped into a common therapeutic class based on their common mechanism of action, ACEIs differ in their chemical structure and hence each ACEI probably has some “not-so-common” actions. There are well-documented differences between ACEIs in terms of potency, physicochemical properties, pharmacokinetics (lipophilicity, bioavailability, plasma half-life, routes of elimination) and if they are administered as prodrugs. ACEIs also differ markedly in their distribution and affinity for tissue-bound ACE, which might be useful in inhibiting some local RAS while leaving others intact. Owing to these considerable differences exhibited by different members within the class, specific ACEIs are approved for the treatment of hypertension, heart failure, myocardial infarction, asymptomatic left ventricular dysfunction, and diabetic nephropathy (Brown and Vaughan 1998).
Furthermore, based on their ability to cross the BBB, ACEIs have been classified as centrally acting ACEIs and non-centrally acting ACEIs (Gao et al., 2013). The ability to cross the BBB was determined by analysis of tissue-specific ACE activity following oral or subcutaneous administration of ACE inhibitors and, by tissue-specific imaging of a radio-labelled ACE inhibitor following administration of different ACE inhibitors (which compete for binding with the radio-labelled ACE inhibitor).

Captopril, lisinopril, fosinopril, ramipril, perindopril, and trandolapril were classified as crossing the BBB, while enalapril, benazepril, moexapril, and quinapril were classified as not (Johnston et al., 1988; Cushman et al., 1989; Gohlke et al., 1989; Johnston et al., 1989; Chai et al., 1992; Chrysant et al., 2004; Jouquey et al., 1995; Tan et al., 2005).

The pharmacology of clinically used ACEIs (Brown and Vaughan 1998; Opie 1999) has been described below in Table 0-1:
Figure 0-12: Chemical structure of some of the clinically used ACEIs.

(Adapted from Harrold 2002).
**Table 0-1 : Pharmacology of clinically used ACEIs**

<table>
<thead>
<tr>
<th>ACEI</th>
<th>Zinc ligand</th>
<th>Prodrug</th>
<th>t½ (h)</th>
<th>Bioavailability (%)</th>
<th>Protein Binding (%)</th>
<th>Lipophilicity</th>
<th>Elimination route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Captopril</td>
<td>Sulphydryl</td>
<td>No</td>
<td>2</td>
<td>75–91</td>
<td>25-30</td>
<td>+</td>
<td>Kidney</td>
</tr>
<tr>
<td>Benazepril</td>
<td>Carboxyl</td>
<td>Yes</td>
<td>10-11</td>
<td>37</td>
<td>96</td>
<td>+</td>
<td>Kidney</td>
</tr>
<tr>
<td>Enalapril</td>
<td>Carboxyl</td>
<td>Yes</td>
<td>11</td>
<td>60</td>
<td>50-60</td>
<td>++</td>
<td>Kidney</td>
</tr>
<tr>
<td>Ramipril</td>
<td>Carboxyl</td>
<td>Yes</td>
<td>Triphasic 4, 9–18, &gt;50</td>
<td>50-60</td>
<td>73</td>
<td>+</td>
<td>Kidney</td>
</tr>
<tr>
<td>Perindopril</td>
<td>Carboxyl</td>
<td>Yes</td>
<td>9</td>
<td>75</td>
<td>60</td>
<td>+</td>
<td>Kidney</td>
</tr>
<tr>
<td>Fosinopril</td>
<td>Phosphinyl</td>
<td>Yes</td>
<td>12</td>
<td>36</td>
<td>89-96</td>
<td>+++</td>
<td>Kidney</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>Carboxyl</td>
<td>No</td>
<td>12</td>
<td>6-60</td>
<td>minimal</td>
<td>0</td>
<td>Kidney</td>
</tr>
<tr>
<td>Moexipril</td>
<td>Carboxyl</td>
<td>Yes</td>
<td>2-12</td>
<td>13</td>
<td>90</td>
<td>+</td>
<td>Kidney</td>
</tr>
<tr>
<td>Quinapril</td>
<td>Carboxyl</td>
<td>Yes</td>
<td>1.9–2.5, 25 terminal</td>
<td>&gt; 60</td>
<td>97</td>
<td>++</td>
<td>Liver, kidney</td>
</tr>
<tr>
<td>Spirapril</td>
<td>Carboxyl</td>
<td>Yes</td>
<td>30</td>
<td>50</td>
<td>Not available</td>
<td>+</td>
<td>Liver, kidney</td>
</tr>
<tr>
<td>Trandolapril</td>
<td>Carboxyl</td>
<td>Yes</td>
<td>16-24</td>
<td>10</td>
<td>80</td>
<td>++</td>
<td>Liver, kidney</td>
</tr>
</tbody>
</table>

Drug lipophilicity as mentioned above is however not the sole predictor of drug efficacy. Lipophilicity of the most effective ACEIs like ramipril or perindopril is related to a relatively narrow interval of log P (0.6–0.8). Ramipril and perindopril are prodrugs with the longest terminal t½ that can be given once daily to treat hypertension (Opie 1999). The other ACE inhibitors are either too hydrophilic (enalapril and lisinopril, log P from −1.2 to 0.1) or highly
lipophilic (captopril, quinapril and fosinopril, log P from 1 to 4.8) and therefore may be less effective (Pilote et al., 2004).

The differential pharmacokinetic features among different ACEIs also affect their dosing schedule. The duration of action of a drug determines dosing frequency; drugs with a longer duration of action require fewer daily doses. With the notable exception of captopril, all ACEIs can be given once daily which generally improves patient compliance (Leonetti et al., 1995).

### 3.9.2. Angiotensin Receptor Blockers (ARBs)

The ARBs available for therapeutic use bind with high affinity to the AT$_1$ receptor and have almost little or no affinity for the AT$_2$ receptor. At present, there are eight different ARBs available in the market: losartan, candesartan, irbesartan, valsartan, olmesartan, eprosartan, telmisartan and azilsartan. Losartan, the first orally active ARB available on the market represents the prototype of AT$_1$ receptor antagonist (Timmermans et al., 1993). The other clinically used ARBs share common molecular structures with the first marketed ARB losartan (Figure 0-13). Losartan, valsartan and olmesartan contains the biphenyl moiety with an attached, acidic tetrazole (imidazole) group but each has structural feature unique from that of losartan. The tetrazole group plays a role in binding to the acidic groups of Ang-II. Telmisartan does not appear to bear any structural relationship to this class, the most characteristic difference being the replacement of tetrazole with a carboxyl group that plays a role in receptor binding. Telmisartan also lacks a carboxylic acid near the imidazole nitrogen that further contributes to receptor binding. The second imidazole ring can form hydrogen bond with the AT$_1$ receptor. Olmesartan contains a hydroxyl group in addition to an $\alpha$-carboxyl group in the imidazole ring. Valsartan named for the valine portion of the
compound has a unique feature as it does not contain nitrogen containing heterocyclic ring in its structure (Figure 0-13). Valsartan represents a nonheterocyclic AT\textsubscript{1} receptor selective antagonist in which the imidazole of losartan has been replaced by an acrylate amino acid (Buhlmayer et al., 1994), and it is slightly more potent than losartan. The relatively newer ARB, azilsartan, exhibits another modification, biphenyl-5-oxo-1, 2, 4-oxadiazole which is believed to increase its lipophilicity and bioavailability compared with candesartan (Kohara et al., 1996). Eprosartan has a large substituent on the imidazole ring, whereas olmesartan is more closely related to losartan. As a consequence of these differences in structure, each of the ARBs can bind to the AT\textsubscript{1} receptor in slightly different ways (Ohno et al., 2011).

Despite belonging to the same drug class, ARBs thus differ in certain aspects of their chemical structure which account for their important differences in pharmacokinetic and pharmacodynamic characteristics, in terms of active metabolite, bioavailability, volume of distribution, plasma half-life, renal or hepatic elimination and protein binding. Telmisartan is the longest-acting among AT1 receptor blocker currently available. It binds to the AT1 receptor with the strongest affinity of currently available ARBs. The dissociation time for telmisartan is 213 minutes, followed by 166 minutes with olmesartan to 67 minutes with losartan (Figure 3-14).

Differences among ARBs also exist with respect to the pharmacokinetic features (Michel et al., 2013) which are described in Table 0-2:
Figure 0-13: Chemical structure of some of the clinically used ARBs.
(Adapted from Harrold 2002).

Figure 0-14: Dissociation half-life of ARBs from AT₁ receptor
(Adapted from Kakuta et al., 2005)
Table 0-2: Pharmacokinetic features of ARBs

<table>
<thead>
<tr>
<th>ARB</th>
<th>Active metabolite</th>
<th>Bioavailability (%)</th>
<th>Terminal t½ (h)</th>
<th>Protein binding (%)</th>
<th>Routes of elimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telmisartan</td>
<td>No</td>
<td>40-60</td>
<td>24</td>
<td>&gt;99.5</td>
<td>Kidney</td>
</tr>
<tr>
<td>Losartan</td>
<td>EXP3174</td>
<td>30</td>
<td>6-9</td>
<td>99.8</td>
<td>Liver, kidney</td>
</tr>
<tr>
<td>Valsartan</td>
<td>No</td>
<td>25</td>
<td>6-9</td>
<td>94-97</td>
<td>Kidney, liver</td>
</tr>
<tr>
<td>Olmesartan medoxomil</td>
<td>Olmesartan</td>
<td>29</td>
<td>10-15</td>
<td>99</td>
<td>Kidney, liver</td>
</tr>
<tr>
<td>Candesartan cilexetil</td>
<td>Candesartan</td>
<td>15</td>
<td>5-9</td>
<td>&gt;99</td>
<td>Kidney, liver</td>
</tr>
<tr>
<td>Eprosartan</td>
<td>No</td>
<td>13</td>
<td>5-7</td>
<td>98</td>
<td>Kidney, liver</td>
</tr>
</tbody>
</table>

All ARBs are lipophilic to a certain extent; their lipophilicity varies markedly with telmisartan being the most lipophilic (Wienen et al., 2010) and losartan being the least (Ribadeneira et al., 1996). The high lipophilicity exhibited by telmisartan facilitates its oral absorption and permits better tissue and cell penetration. Telmisartan is slowly eliminated after oral administration, with an elimination half-life of approximately 20 – 30 hours while eprosartan has the shortest half-life of 5-7 hours (Michel et al., 2013; Israel 2000) [Table 3-2].
A number of pre-clinical studies have demonstrated the central effects of several ARBs, suggesting that ARBs can cross the blood brain barrier. Penetration of ARBs into the brain may also have beneficial effects on cognitive function as well as on the development of Alzheimer’s disease beyond those of blood pressure reduction (Duron and Hanon 2010). ARBs thus exhibit a wide range of differences in their physicochemical, pharmacological, and pharmacokinetic properties. Although ARBs are differentiated by receptor affinity to a limited extent only, their dissociation time from the receptor varies substantially, and this may hold importance with regards to the surmountability of their effects and may also contribute to their duration of action.

3.10. Link between hypertension and cognitive impairment

Hypertension has been well recognised as a major risk factor for the development of cardiovascular and cerebrovascular diseases, and more importantly for the development of all types of dementia including AD and vascular dementia (Launer et al., 2000; Forette et al., 2002). Hypertension, like dementia, advances with age. Several studies have examined the association between hypertension and dementia suggesting that an elevated mid-life BP precedes the onset of dementia, but in the years preceding dementia onset, the BP appears to decline (Skoog and Gustafson 2006).

This observation has been considered to be secondary phenomenon possibly due to hypertension-induced pathological changes that are interestingly also relevant to AD. Neurofibrillary tangles and senile plaques which occur as neuropathological features of AD were found to be elevated in patients with hypertension (Skoog and Gustafson 2003). A post-mortem analysis of 243 patients showed that elevated mid-life BP was associated with changes in vascular tissues, a lower brain weight and increased number of amyloid-β plaques.
in hippocampus and neocortex (Petrovitch et al., 2000). Hippocampal atrophy, which is one of the radiological hallmarks of AD, was also observed in patients with untreated hypertension (Korf et al., 2004).

Hypertension increases the risk of cognitive impairment and dementia by increasing the risk for infarcts and stroke. Untreated hypertension leads to thickening of cerebral blood vessels and thickening of atheromatous plaques that in turn leads to narrowing of vessel lumens (Skoog 1997). It is well established that the CA1 region of the hippocampus is extremely vulnerable to ischemia (Pulsinelli 1985). In the CA1 region, a global ischemic episode induces selective neuronal death of pyramidal cells resulting in anterograde amnesia and a significant impairment of the cognitive functions (Schmidt 1991). The CA1 region is also very vulnerable to neurofibrillary degeneration, which in turn leads to loss of hippocampal volume (Schuff et al., 2003).

Given the association between development of neurofibrillary tangles and Aβ plaques with the progression of AD, it is hypothesised that vascular risk factors probably could contribute to increased Aβ accumulation ultimately leading to AD (Kalaria 2010). The expression of APP is also elevated in the brain post-ischemia and cleavage of APP leading to Aβ may be increased by ischemic episodes (Badan et al., 2004). Taken together, these findings suggest that cerebral ischemia arising from lacunar infarcts and diminished cerebral perfusion due to narrowing of the vessel lumen may contribute to cognitive deficits and dementia.

Considering the link between hypertension and cognitive decline, and the association between dementia and RAS, it is reasonable to hypothesise that antihypertensive treatment acting on RAS might protect against the development of dementia. Generally, ACEIs and ARBs seem to be effective in improving cognitive function in hypertensive patients (Fogari et al., 2003). Secondary analysis of data between two large-scale stroke prevention trials
reported a marked reduction in the incidence of dementia with ACEIs viz., enalapril and perindopril (Kehoe and Wilcock 2007). But a similar association has not been demonstrated in another large-scale clinical trial with perindopril (Peters et al., 2008). Similarly, ACEIs did not reveal any neuroprotective effect in another prospective study which analysed the impact of antihypertensive treatment with ACEIs on the incidence of dementia (de Boer and van Veldhuisen 2008.).

In contrast, the ARBs which are “ACE-sparing” were found to exert greater neuroprotective effects than ACEIs against cognitive impairment and dementia. ARBs have been shown to protect against both cognitive decline and Aβ-related pathology in animal models of AD (Fogari et al., 2003; Kehoe et al., 2009). More recent observational studies also showed a positive association between ARBs and slower progression of AD compared to lisinopril and other antihypertensive medications (Li et al., 2010).

Perturbation of RAS has thus been observed in various studies of AD and drugs acting on RAS have been shown to exert beneficial cognitive effects in animals and humans. Given the harmful effects mediated by an overactive RAS, it is thus postulated that drugs which inhibit their activity may offer a new therapeutic strategy in slowing or preventing the cognitive deterioration seen in dementia and AD.

3.11. Need for the study

Although a growing body of evidence indicates that antihypertensive treatment with RAS blockers could be of potential use to reduce the rate of cognitive decline in AD, they need further validation. How treatment with ACEIs or ARBs might affect cognition is not answered and the exact mechanism of action of these compounds in improving memory
function remains unclear. ACEIs and ARBs have been found to be effective in maintaining and improving cognitive function through unidentified mechanisms independent of blood pressure control (Fogari and Zoppi 2004) which needs to be identified.

Considering the complexities in the pathophysiology of AD, therapies that target different mechanisms such as amyloid β, oxidative stress, inflammatory processes in the brain or cholinergic pathway could be the next best approach. Since the brain RAS is involved in the pathological changes seen in AD including inhibition of ACh release, generation of oxidative stress, hippocampal degeneration and impairment of memory, it is believed that antihypertensive RAS medications such as ACEIs and ARBs may exert neuroprotective properties and benefit in mitigating the cognitive decline commonly seen in dementia.

As populations age worldwide, the incidence of dementia will increase. Until now, no agents have been identified that can prevent or reverse dementia, and currently available treatments for improving cognitive function in demented patients are predominantly symptomatic (Reichman 2003). The evidence for a significant role of cardiovascular risk factors and cardiovascular disease in dementia and Alzheimer’s disease is very strong. Individuals with high cardiovascular risk scores (such as those with hypertension, hypercholesterolemia, diabetes and smoking) are at an increased risk for dementia incidence whether exposure is measured in midlife or a few years before dementia onset (Whitmer et al., 2005). BP control, in particular, has been associated with both a reduced incidence of cognitive impairment and rate of cognitive decline (Gasecki et al., 2013).

It is well recognised that pharmacotherapy targeting the RAS is one of the most effective means of reducing hypertension and cardiovascular morbidity. Studies in both animals and humans have found that ACEIs and ARBs help to preserve cognitive function through a mechanism that is independent of their antihypertensive effects, which needs to be identified.
Since polypharmacy is a common problem seen in elderly patients, if the same class of drugs can be used to treat multiple conditions, compliance to treatment can be improved. The paucity of effective treatment for dementia, the ready availability and safety of these drugs in clinical settings, and the evidence that they could be of therapeutic potential in halting the progression of cognitive decline suggest a strong need for further preclinical and clinical evaluation of ACEIs and ARBs as cognitive enhancing agents.

In addition, as drugs that interfere with RAS are being increasingly developed, it is imperative to elucidate the precise mechanisms through which various components of RAS act on the brain and exert their various effects, including those on cognition. More importantly, if these drugs are found beneficial, they provide the advantage of being immediately available for accelerated testing and progressing, while avoiding the rigorous and lengthy drug discovery pipeline from initial compound discovery to eventual implementation.

Although evidences from preclinical and observational studies suggest that RAS inhibitors may have beneficial effects on cognitive function, the underlying mechanisms are still unknown. It needs to be established if Ang-II mediated inhibition of ACh is the primary mechanism by which ACEIs and ARBs improve cognitive function or there are other undetermined properties or mechanisms of RAS inhibitors that might affect cognition. Also, it is important to identify if the benefits they confer on cognitive function show an intraclass or interclass variation because all drugs within a class may not be interchangeable. Since all drugs have unique chemical structures, it may be wrong to assume that all drugs belonging to ACEIs and ARBs share the same pharmacological effect.

Members of a drug class share qualitative mechanism of action that defines the class, but since they are not identical, the assumption that they are clinically interchangeable for every indication requires further validation.
The present study was thus undertaken to:

- Evaluate the effects of structurally heterogeneous ACEIs and ARBs on learning and memory
- Provide an insight into the probable mechanisms by which these drugs mediate their effects on cognition
- Compare and identify if any difference exists between the drug groups and among various agents within the group in terms of memory and cognition.