Aim 2. To investigate neurochemical mechanisms underlying the induction of cognition deficit in epilepsy by monitoring the biochemical changes in brain of epileptic animals in above validated models

6.1 Experiment II:
Investigation of neurochemical mechanisms underlying the induction of cognition deficit in epilepsy by monitoring the neurochemical changes in brain of postictal and interictal animals

6.1.1 Introduction
Cognitive functions in epileptic patients are as diverse as the epileptic condition itself; origin, topography of epileptogenic foci, pathological mechanisms and various features characterizing the clinical epilepsy, all contribute to the deleterious effect on cognition. Impaired cognitive outcome is generally associated with an early onset and a long duration of the disease and with poor seizure control (Elger et al., 2004).

Management of epilepsy is ineffective with available antiepileptic drugs showing about 70% effectiveness in patients with epilepsy (Elger et al., 2004). This poses a significant risk factor for progressive memory deficit. Further adding to this woe, most of the available antiepileptic drugs, used to manage epilepsy may also cause memory deficit (Arroyo and De, 2001; Lagae, 2006; Vinayan, 2006; Marson et al., 2007; Schmidt, 2009; Eddy et al., 2011). Owing to the risks of seizurogenic potential concerned with management of cognitive comorbidities with conventional memory enhancing drugs (Griffith et al., 2008), these patients often remain untreated. Therefore, clinical interventions to prevent these commodities for the management of patients with epilepsy are still issues of substantial concern.

Disturbances in coordination of neurotransmission in different brain regions during epilepsy may lead to memory deficit. Thus it is hypothesized that examining the effect of neurochemical alterations with learning and memory deficit following pentylenetetrazole-kindling may help to understand the neurobiology of susceptibility to learning and memory deficit in epileptic patients and progressive learning and memory deficit in uncontrolled or untreated patients with epilepsy, to provide novel therapeutic targets for the management of memory deficit in epileptic patients. In chronic epileptic patients, ictal and postictal states are relative momentary than interictal state, which persists for longer time. As the time span of the interictal state
prevails over postictal state, the monitoring of behavioral and neurochemical changes in the interictal state may be crucial to provide valuable insight for the pathology of susceptibility of learning and memory deficit in epilepsy. While for understanding the neurobiology of progressive learning and memory deficit in uncontrolled seizures neurochemical changes may be monitored after multiple convulsive episodes.

Chemical or electrical kindling in rodents are regarded as a model of human temporal lobe epilepsy. Kindling is a phenomenon in which repeated and intermittent subconvulsive stimulus resulting in progressive stimulus-induced convulsions culminating to generalized seizures. Pentylenetetrazole noncompetitively blocks GABA-mediated Cl⁻ influx through allostERIC modulation at Cl⁻ channel, leading to neuronal depolarization and propagation of seizures. Pentylenetetrazole-kindling model also appears distinctive in providing opportunity to study progressive cognitive changes with a close resemblance to clinical epilepsy. Therefore the present study was executed to explore the relationship between memory deficit and neurochemical alterations in discrete brain areas to delineate the possible targets for comprehensive management of this problem in future using pentylenetetrazole-kindling model.

6.1.2 Experimental Design

The group 7: naïve animals, consisted of naïve animals (non kindled, n=10) and rest 30 animals were subjected to pentylenetetrazole-kindling (protocol of kindling explained in 4.3). The animals showing resistance to pentylenetetrazole-kindling were excluded from study and only successfully kindled animals were further incorporated in this study and randomly divided into two groups. Group 8: postictal group animals: consisted of kindled animals (n=10) subjected to frequent convulsive episodes by administering subconvulsive dose of pentylenetetrazole (35 mg/kg; i.p.) on day 5, 10, 15 and 20. Group 9: interictal group animals: consisted of kindled animals (n=10) without frequent convulsive episodes (Figure 6.1.1).

Group 7: Naïve group (n = 10)

Group 8: Postictal PTZ-kindled group (10 ml/kg/day; i.p.; n = 10) [Vehicle Control]

Group 9: Interictal PTZ-Kindled group (10 ml/kg/day; i.p.; n = 10)

In postictal group frequent convulsions were induced (with pentylenetetrazole challenging dose) in order to mimic pathology of uncontrolled seizures (postictal state) in epileptic patient where intermittent seizures occurs. Whereas interictal group
providing insight of interictal state pathology of epileptic patients where next episode of seizure is unpredictable. Effect on learning and memory were analyzed using elevated plus maze and passive shock avoidance paradigm (in postictal group behavioral evaluations were done after 2 h of pentylentetrazole challenging dose, when their locomotor activity becomes normalized) on day 5, 10, 15 and 20 (protocol explained in 4.5.2).

On day 20, after behavioral evaluations, all the animals were decapitated to isolate their different brain parts for neurochemical estimations (protocol explained in 4.6).

Figure 6.1.1 Schematic Presentation of Experimental Protocol

**Statistical Analysis:** The statistical analysis was performed using the Sigma Stat Statistical Software version 3.5. In the behavioral estimations, comprising of two variables (different groups and different days), the intergroup and intra group variation was measured by two-way analysis of variance (ANOVA) followed by Student-Newman-Keuls Test (for multivariate analysis). While for the biochemical estimation (comprising of one variable *i.e.* different treatment) one-way ANOVA followed by Student-Newman-Keuls Test was applied. In the result each value was expressed as mean ± S.E.M. and statistical significance was considered at P < 0.05.

**6.1.3 Results**

Kindling was successfully induced in the mice by the repeated administration of subconvulsive dose of pentylentetrazole in mice. Average number of pentylentetrazole injections required to induce successful kindling state in mice was
found to be 17±3. Animals showing resistance to pentylenetetrazole-kindling were excluded from study and successfully kindled animals were included in the study. Tonic-clonic seizures (stage 5 of modified Racine’s Scale) were observed in animals of postictal group after administration of pentylenetetrazole challenging dose on day 5, 10, 15 and 20.

6.1.3.1 Behavioral Evaluations

Effect on Transfer Latency in Elevated Plus Maze: The significant change in transfer latency was observed on day 0 (F(2,27) = 49.799; P < 0.001), day 5 (F(2,27) = 66.926; P < 0.001), day 10 (F(2,27) = 161.337; P < 0.001), day 15 (F(2,27) = 167.550; P < 0.001) and day 20 (F(2,27) = 151.450; P < 0.001). Naïve animals had shown a tendency to decrease transfer latency on days 5, 10, 15 and 20. There was significant increase in transfer latency of pentylenetetrazole-kindled mice (both postictal and interictal group) as compared to naïve mice on days 0. On day 5, 10, 15 and 20 the transfer latency of post and interictal group mice was significantly higher than that of their transfer latency on day 0 (P < 0.05) and naïve group on respective days (P < 0.001). However transfer latency of the postictal and interictal group was not significantly different than each other on different days (Figure 6.1.2).

Effect on Number of Mistakes and Step Down Latency in Passive Shock Avoidance Paradigm: There was significant change in number of mistakes observed in different groups on day 5 (F(2,27) = 49.457; P < 0.001), day 10 (F(2,27) = 42.106; P < 0.001), day 15 (F(2,27) = 45.275; P < 0.001) and day 20 (F(2,27) = 84.986; P < 0.001). Naïve group animals have shown requirement of average 2.5±0.22 numbers of trials to learn to stay for at least 120s on shock free zone in passive shock avoidance paradigm. While pentylenetetrazole-kindled animals (of both postictal and interictal group) required significantly increased number of trials 8.5±0.6 (P < 0.001) than naïve animals on day 0. On day 5, 10, 15 and 20 there was a significant increase in the number of mistakes in postictal and interictal group than that of the naïve group on the respective days. There was also significant increase in number of mistakes in postictal group (P < 0.001) than that of interictal group on day 5, 10, 15 and 20 (Figure 6.1.3).

The significant change in step down latency was observed in different groups on day 5 (F(2,27) = 253.490; P < 0.001), day 10 (F(2,27) = 248.199; P < 0.001), day 15 (F(2,27) = 430.948; P < 0.001) and day 20 (F(2,27) = 290.727; P < 0.001). Naïve group
animals had shown average step down latency of 120s on day 5, 10, 15 and 20. Post-hoc analyses indicated a significant decrease in step down latency of the postictal group (P < 0.001) and interictal group (P < 0.001) as compared to the naïve group on day 5, 10, 15 and 20. On day 15 and 20 the step down latency of postictal group was significantly reduced (P < 0.001) than that of interictal group (Figure 6.1.4).

**Figure 6.1.2 Effects on Transfer Latency in Elevated Plus Maze**
Each value is expressed as Mean ± S.E.M. *: as compared to naïve, #: as compared to Postictal group, !: as compared to day 0 or day 5 data. The significance level was considered at P < 0.05 (Student-Newman-Keuls Test)

**Figure 6.1.3 Effects on Number of Mistakes in Passive Shock Avoidance Paradigm**
Each value is expressed as Mean ± S.E.M. *: as compared to naïve, #: as compared to Postictal group, !: as compared to day 0 or day 5 data. The significance level was considered at P< 0.05 (Student-Newman-Keuls Test)
Figure 6.1.4 Effects on Step Down Latency in Passive Shock Avoidance Paradigm
Each value is expressed as Mean ± S.E.M. *: as compared to naïve, #: as compared to Postictal group, !: as compared to day 0 or day 5 data. The significance level was considered at P < 0.05 (Student-Newman-Keuls Test)

6.1.3.2 Neurochemical Changes
While analyzing amino acids using the HPLC-FD method, glutamate and GABA using HPLC-FD method glutamate appeared at 2.72 minutes and GABA at 13.60 minutes of retention time. However in case of monoamine estimation noradrenaline eluted first (retention time = 2.89 minute) followed by dopamine (retention time = 4.68 minute) and serotonin (retention time = 9.16 minute).

Changes in Glutamate/GABA Ratio: The Glutamate and GABA ratio was calculated as the indices of excitatory and inhibitory neurotransmitter ratio in discrete areas of the brain. There was significant difference between the groups in cortical (F(2,27) = 76.740; P < 0.001), hippocampal (F(2,27) = 66.263; P > 0.001), cerebellum (F(2,27) = 27.925; P < 0.001) and brain stem (F(2,27) = 39.836; P > 0.001) glutamate/GABA ratio.

In postictal group, cortical glutamate/GABA ratio was significantly reduced (P < 0.001) however, in interictal group cortical glutamate/GABA ratio was significantly increased (P < 0.001) as compared to naïve animals. There was around two fold increase in cortical glutamate/GABA ratio was observed in interictal group animals as compared to postictal group animals. Hippocampal glutamate/GABA ratio was unchanged in the postictal group while significantly increased (P < 0.001) in interictal group as compared to naïve animals.
Table 6.1.1 Comparative Account of Neurochemical Changes in Naïve, Postictal and Interictal Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Noradrenaline Ψ</th>
<th>Dopamine Ψ</th>
<th>Serotonin Ψ</th>
<th>Glutamate Ψ</th>
<th>GABA Ψ</th>
<th>Glu/GABA</th>
<th>Nitrite Level Ψ</th>
<th>AChE Activity</th>
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<tbody>
<tr>
<td>Cortex</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Naïve</td>
<td>77.25 ± 8.49</td>
<td>551.89 ± 28.63</td>
<td>1070.07 ± 112.82</td>
<td>6276.81 ± 234.55</td>
<td>1743.87 ± 105.08</td>
<td>3.62 ± 0.08</td>
<td>582.08 ± 61.54</td>
<td>153.31 ± 7.26</td>
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<tr>
<td>Postictal</td>
<td>94.62 ± 6.85</td>
<td>129.51 ± 23.96*</td>
<td>255.16 ± 30.86*</td>
<td>4522.37 ± 375.93*</td>
<td>1916.69 ± 124.33</td>
<td>2.35 ± 0.04*</td>
<td>814.23 ± 27.51**</td>
<td>279.95 ± 4.11***</td>
</tr>
<tr>
<td>Interictal</td>
<td>53.83 ± 4.63*</td>
<td>177.38 ± 15.54*</td>
<td>182.63 ± 56.48*</td>
<td>6045.48 ± 22.42*</td>
<td>1385.78 ± 46.05*</td>
<td>4.36 ± 0.18*</td>
<td>770.21 ± 25.95**</td>
<td>185.29 ± 7.26</td>
</tr>
<tr>
<td>Hippocampus</td>
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</tr>
<tr>
<td>Naïve</td>
<td>230.08 ± 4.44</td>
<td>90.27 ± 6.43</td>
<td>826.86 ± 14.52</td>
<td>4306.83 ± 238.80</td>
<td>1790.15 ± 42.30</td>
<td>2.43 ± 0.19</td>
<td>419.58 ± 25.95</td>
<td>162.54 ± 7.03</td>
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<tr>
<td>Postictal</td>
<td>172.18 ± 12.13***</td>
<td>39.66 ± 8.81*</td>
<td>30.87 ± 11.65*</td>
<td>4744.37 ± 67.15</td>
<td>2031.12 ± 203.97</td>
<td>2.44 ± 0.21</td>
<td>745.37 ± 47.04***</td>
<td>210.11 ± 9.56*</td>
</tr>
<tr>
<td>Interictal</td>
<td>71.31 ± 3.91***#</td>
<td>55.73 ± 1.64***#</td>
<td>154.77 ± 48.93***#</td>
<td>6792.23 ± 111.58***#</td>
<td>1230.64 ± 41.09***#</td>
<td>5.51 ± 0.25***#</td>
<td>812.24 ± 67.82***#</td>
<td>198.25 ± 8.82**#</td>
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<tr>
<td>Cerebellum</td>
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<tr>
<td>Naïve</td>
<td>174.34 ± 5.03</td>
<td>403.99 ± 63.11</td>
<td>710.19 ± 72.79</td>
<td>6877.53 ± 183.53</td>
<td>1873.18 ± 76.14</td>
<td>3.68 ± 0.16</td>
<td>482.08 ± 37.83</td>
<td>162.11 ± 9.28</td>
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<tr>
<td>Postictal</td>
<td>71.68 ± 2.35**</td>
<td>26.48 ± 2.98***</td>
<td>271.45 ± 11.62**</td>
<td>4491.10 ± 49.33**</td>
<td>1959.68 ± 25.13</td>
<td>2.29 ± 0.13**</td>
<td>798.21 ± 5.59***</td>
<td>246.67 ± 9.55***</td>
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<tr>
<td>Interictal</td>
<td>155.57 ± 5.27***#</td>
<td>48.49 ± 3.32***#</td>
<td>56.02 ± 4.76***#</td>
<td>5944.92 ± 41.26***#</td>
<td>1822.83 ± 24.46***#</td>
<td>3.26 ± 0.11***#</td>
<td>615.74 ± 25.49***#</td>
<td>210.25 ± 8.22***#</td>
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<tr>
<td>Brainstem</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Naïve</td>
<td>412.82 ± 10.55</td>
<td>1603.59 ± 20.28</td>
<td>1241.77 ± 9.95</td>
<td>6765.27 ± 47.05</td>
<td>2971.05 ± 58.26</td>
<td>2.28 ± 0.13</td>
<td>477.92 ± 27.51</td>
<td>237.67 ± 8.82</td>
</tr>
<tr>
<td>Postictal</td>
<td>109.06 ± 5.58**</td>
<td>602.29 ± 48.09*</td>
<td>1084.63 ± 7.93*</td>
<td>4707.58 ± 213.36*</td>
<td>2705.71 ± 50.13*</td>
<td>1.74 ± 0.19**</td>
<td>485.12 ± 13.98</td>
<td>269.63 ± 6.83**</td>
</tr>
<tr>
<td>Interictal</td>
<td>138.14 ± 9.67***#</td>
<td>89.51 ± 8.51***#</td>
<td>252.57 ± 49.43***#</td>
<td>5796.48 ± 25.13***#</td>
<td>1575.96 ± 48.22***#</td>
<td>3.68 ± 0.15***#</td>
<td>478.92 ± 12.67</td>
<td>275.24 ± 9.55**</td>
</tr>
</tbody>
</table>

Each value is expressed as Mean ± S.E.M. *; as compared to naïve, #: as compared to Postictal group. The significance level was considered at P < 0.05 (Student-Newman-Keuls Test); * significant as compared to naïve; # significant as compared to postictal group (*/#: P < 0.05; **/###: P < 0.01; ***/####: P < 0.001). Ψ: ng/g of tissue; AChE activity was expressed in micro Moles of acetylcholine hydrolyzed per g of tissue.
The hippocampal glutamate/GABA ratio was around two times increased in interictal group animals as compared to postictal animals. Significant reduction in cerebellar glutamate/GABA ratio was observed in postictal and interictal groups as compared to naïve animals. In the brainstem region, significant reduction (P < 0.001) in glutamate/GABA ratio in postictal group while significant elevation (P < 0.001) in glutamate/GABA ratio was recorded in interictal group as compared to naïve animals (Table 6.1.1).

Changes in Noradrenaline Levels: There was a significant difference in concentration of noradrenaline in cortex (F(2,27) = 8.951; P < 0.001), hippocampus (F(2,27) = 106.334; P < 0.001), cerebellum (F(2,27) = 152.987; P < 0.001) and brain stem (F(2,27) = 357.208; P < 0.001) in between the groups. Post-hoc test (Student-Newman-Keuls test) indicated a significant decrease in cortical noradrenaline level of interictal group (P < 0.01) as compared to naïve mice, while no significant change in postictal group was observed. In hippocampus noradrenaline level was significantly decreased in postictal and interictal group (P < 0.001) as compared to naïve group. However, the hippocampal noradrenaline level of postictal group was significantly higher (P < 0.001) than that of interictal group animals. In cerebellum and brain stem region noradrenaline level was significantly decreased (P < 0.001) in postictal and interictal group animals. In cerebellum and brainstem, noradrenaline level of interictal group was significantly higher (P < 0.001) than that of postictal animals (Table 6.1.1).

Changes in Dopamine Levels: There was statistically significant difference in concentration of dopamine in cortex (F(2,27) = 98.136; P < 0.001), hippocampus (F(2,27) = 22.751; P < 0.001), cerebellum (F(2,27) = 33.649; P < 0.001) and brain stem (F(2,27) = 636.184; P < 0.001) in between the groups. The post-hoc test suggested depletion in cortical, hippocampal, cerebellar and brainstem dopamine level of postictal and interictal group (P < 0.001) as compared to naïve group. However, in interictal group animals, dopamine level was significantly higher (P < 0.001) in cortex and hippocampus, unchanged in cerebellum and reduced in brain stem (P < 0.001) as compared to that of postictal animals in respective areas (Table 6.1.1).

Changes in Serotonin Levels: There was significant difference between the groups in the concentration of serotonin in cortex (F(2,27) = 43.178; P < 0.001), hippocampus (F(2,27) = 200.799; P < 0.001), cerebellum (F(2,27) = 61.110; P < 0.001) and brain stem (F(2,27) = 13.847; P < 0.001). The Post-hoc test suggested significant decrease in
cortical, hippocampal, cerebellar and brainstem serotonin level in postictal and interictal group (P < 0.001) as compared to naïve animals. In hippocampus increased level of serotonin was observed in interictal group animals as compared to postictal animals. However, reduced brain stem serotonin level was recorded in interictal animals as compared to postictal animals (Table 6.1.1).

**Changes in Total Nitrite Levels:** There was statistically significant difference in total nitrite level in cortex (F(2,27) = 87.398; P < 0.001), hippocampus (F(2,27) = 176.832; P < 0.001), cerebellum (F(2,27) = 357.639; P < 0.001) in between the groups was observed. However no change in brainstem nitrite level was observed in different groups (F(2,23) = 0.0411; P = 0.960). The Post-hoc test suggested significant enhancement of cortical, hippocampal and cerebellar nitrite level of postictal and interictal animals as compared to naïve animals. However, significant reduction only in cerebellar nitrite level was recorded in interictal group as compared to postictal animals (Table 6.1.1).

**Changes in Acetylcholinesterase Activity:** There was statistically significant difference in acetylcholinesterase activity in cortex (F(2,27) = 83.614; P < 0.001), hippocampus (F(2,27) = 8.409; P < 0.001), cerebellum (F(2,27) = 22.044; P < 0.001) and brain stem (F(2,27) = 5.716; P < 0.05) in between the groups was observed. The Post-hoc test suggested significant enhancement of cortical, hippocampal, cerebellar and brain stem acetylcholinesterase activity of postictal and interictal animals (P < 0.001) as compared to naïve animals. However, acetylcholinesterase activity in cortex and brain stem of interictal group was significantly reduced than that of postictal animals. However no significant difference in hippocampal and brain stem acetylcholinesterase activity was observed between interictal and postictal animals (Table 6.1.1).

**6.1.4 Discussion**

In the present study, first attempt has been made to correlate postictal and interictal learning and memory deficit and associated neurochemical changes in discrete brain parts using pentylenetetrazole-kindling model of epilepsy in search of putative comprehensive or add on target for the treatment of learning and memory deficit associated with epilepsy. Our study has demonstrated susceptibility of learning and memory deficit in interictal group and progressive learning and memory deficit in postictal group along with peculiar neurochemical alterations in discrete brain parts.
Transfer latency in elevated plus maze model corresponds to short term and long term spatial memory; number of trials required during the trial session of passive shock avoidance paradigm suggest the learning behavior; while step down latency indicated contextual fear memory in this study. Interestingly, we found both types of memory impairment in postictal and interictal group. Contextual fear memory was more worsened in postictal group than interictal group and progressive uncontrolled seizures in postictal group further worsened contextual fear memory. These findings are consistent with literature reports suggesting the memory impairment in pentylenetetrazole-kindled animals (Voigt and Morgenstern 1986; Grecksch et al., 1997; Singh et al., 2013; Choudhary et al., 2013) and progressive memory deficit with uncontrolled seizures of epileptic patients (Pitkänen and Sutula, 2002; Stefan and Pauli, 2002). The memory deficit in postictal and interictal group on day 5 was not significantly different than memory deficit on day 20. It may be assumed that molecular changes on 5 could be similar to that of day 20 therefore similar degree of memory deficit was observed in post/interictal group animals on day 5 and day 20.

Concurrent alterations in brain circuitry and chemistry are believed to play a crucial role in epileptogenesis and learning and memory formation. For better understanding of the relationship between the pathophysiology of epilepsy induced learning and memory deficit, the neurochemical interplay in different brain parts was monitored. In our study the pentylenetetrazole-kindling resulted in significant changes in concentrations of different neurotransmitters in discrete brain regions which vary with time function. While discussing, major emphasis have been given to the neurochemical changes in brain regions primarily involved in epileptogenesis, learning behavior and consolidation of memory viz., cortex and hippocampus.

The imbalance between excitatory and inhibitory tone produced by decreased GABAergic and/or increased glutamatergic transmission has been considered as pathological factors for the generation of seizures, both in animal models and in humans (McNamara, 1994; Bradford, 1995). Similarly in our study, the major remarkable finding was a shift in the balance between excitatory and inhibitory amino acid concentrations in the kindled animals. The elevated cortical and hippocampal glutamate/GABA ratio in interictal group suggests the reduced seizure threshold. Although we did not find any spontaneous recurrent seizures in interictal group, but reduced seizure threshold in of pentylenetetrazole-kindled animals can be presumed
through the appearance of tonic-clonic seizures in postictal group animals after administration of subconvulsive dose of pentylenetetrazole. Reduced cortical and hippocampal glutamate/GABA ratio level was decreased and simultaneous enhancement in the GABA level was recorded in postictal group. The reduction in glutamate/GABA ratio, a theoretical marker of the neuronal excitation level, might be due to the activation of compensatory mechanisms in postictal group. This observation is consistent with the hypothesis underlining role of disturbances in the balance between excitatory and inhibitory processes as a major factor leading to epileptogenesis (Morimoto et al., 2004). The evidences suggest that the level of glutamate appears to be enhanced in ictal state leading to precipitation of seizures and exhaustion of glutamate may be responsible for its reduced level in hippocampus (Szyndler et al., 2008) and cortex of postictal group animals.

Glutamate has long been implicated in synaptic plasticity underpinning learning and memory (McEntee and Crook, 1993), via long term potentiation with ionotropic and metabotropic glutamate receptors. During activation of glutamatergic synapse in pyramidal neurons higher level of glutamate at synaps leads to long term potentiation via facilitating translocation new AMPA receptors on synapse (Li and Tsien, 2009). However, in certain physiological conditions like epilepsy and Alzheimer, elevated extrasynaptic glutamate level serves as a neurotoxic agent leading to depolarization of neural membranes and to cell death via extrasynaptic NMDA receptors (Li and Ju, 2012). Therefore excitotoxicity due to elevated glutamate level may be a causative factor both for susceptibility to memory deficit in interictal group as well as progressive memory deficit with uncontrolled seizures as observed in postictal group respectively in our study. However reduced glutamate level in postictal group in comparison to interictal group observed in our study can be justified with exhaustion of glutamate after ictal phase as estimation of glutamate was carried out after cessation of convulsions.

The monoamines (norepinephrine, dopamine and serotonin) represent another group of abundant neuroactive substances in central nervous system that are capable of regulating the initiation and spread of seizure activity (Weinshenker and Szot, 2002) and also play vital role in learning and memory formation (Izquierdo and Medina, 1997). Unanimously, elevated levels of monoamines in the brain have been speculated to exert an anticonvulsant activity (Starr, 1996; Jobe et al., 1999).
However, deficiencies in monoamine systems are implicated in different types of seizures including kindling model of experimental epilepsy (Applegate et al., 1986; Corcoran, 1988; Racine and Coscina, 1979; Zis et al., 1992) possibly via lowering seizure threshold (Chen et al., 1954). The levels of monoamine oxidases, enzymes that catalyze the oxidation of monoamines in the brain, are reported to be elevated in platelets in epileptic patients (Kruk et al., 1980; Kumlien et al., 1995) which may be indirectly responsible for monoamine depletion in our study. Similarly the elevated monoamine oxidase level has been reported in patients with Alzheimer's disease, most common form of dementia (Sherif et al., 1992).

An altered status of noradrenergic neurons has been stated in different psychiatric disorders including epilepsy (Weinshenker and Szot, 2002). Noradrenaline signaling powerfully inhibits seizures, whereas depletion of norepinephrine increases seizure susceptibility (Szot et al., 1999) and accelerates epileptogenesis in various animal models (Weinshenker and Szot, 2002; Giorgi et al., 2004). On the other hand, long lasting hypotheses also state that adrenergic signaling is critical for formation of contextual and spatial memory (Izquierdo and Medina, 1997; Murchison et al., 2004). In our study slight enhancement in noradrenaline level in postictal group and marked depletion in noradrenaline level of interictal animals was recorded as compared to naïve animals. The slight elevation of noradrenaline level in postictal group of pentylenetetrazole-kindled mice might be due to activation of the compensatory inhibitory mechanism of seizures however could not justify associated learning and memory impairment. Persistent depleted level of noradrenaline in hippocampus and cortex of interictal group of pentylenetetrazole-kindled mice can be well correlated with their lowered seizure threshold (Hiramatsu et al., 1982) and learning and memory deficit in these animals. Our observations are in consistent with the previous studies by Szyndler et al., (2002) and Singh et al., (2013) regarding the noradrenaline change in the postictal group and epileptogenic foci of epileptic patients (Goldstein et al., 1988; Devinsky et al., 1992).

The role of dopamine in epilepsy and memory appears intriguing, complex, and unresolved. Dopaminergic neurons play important role in the initiation and spread of seizure activity (Starr, 1996) and helps in consolidation of different forms of memory by inducing long term potentiation in hippocampal pyramidal cells (Wise, 2004). Some report suggests the reduced dopamine content in epileptic foci of the
patients (Hiramatsu et al., 1982; Mori et al., 1987) and might be resulting in inhibition of D<sub>1</sub> receptor mediated GABA release culminating to generalization of seizures (Brozoski et al., 1979). On the other hand reduced dopamine has also been reported to cause profound memory loss in primates (Swanson-Park et al., 1999) possibly via limiting the persistence of D<sub>1</sub> receptor mediated long term potentiation in hippocampus (Dazzi et al., 1997). In our study, decreased cortical and hippocampal dopamine content in interictal group of pentylentetrazole-kindled mice suggests their susceptibility for learning and memory impairment while higher degree of cortical and hippocampal dopamine depletion supports the progressive memory loss in postictal group. The results of dopamine levels are in line with the earlier findings of Szyndler et al. (2002) however contradicted by findings of Dazzi and colleagues suggested elevated extracellular dopamine level in pentyletetrizole-kindled freely moving mice after 4 days of last seizures (Dazzi et al., 1997).

Serotonin, another important monoamine neurotransmitter in central nervous system, is involved in various pharmacological events by virtue of their diverse receptors (Barnes and Sharp, 1999). There is a considerable body of evidence suggesting serotonergic neurotransmission modulates a wide variety of experimentally induced seizures (Yan et al., 1994; Lu and Gean, 1998) and assist memory formation by potentiating acetylcholine release in hippocampus via several receptor subtypes (5-HT<sub>1A</sub>, 5-HT<sub>2A/2B/2C</sub> and 5-HT<sub>4</sub>) (Fink and Götbert, 2007). Reduction in serotonin level tends to worsen the seizure severity, possibly by reducing the seizure threshold (Browning et al., 1978; Statnick et al., 1996) and might be leading to memory deficit. Similarly, in our study marked decrease in hippocampal serotonin level of postictal group animals supports the progressive and uncontrolled seizures and associated memory impairment in these animals. These findings are consistent with earlier experimental reports carried out in pentylentetrazole-kindling (Szyndler et al., 2002), electrical kindling (Kokaia et al., 1989) and pilocarpine model of epilepsy (Trindade-Filho et al., 2008).

In the central nervous system, nitric oxide could behave as a secondary and retrograde messenger, neuromodulator, and neurotransmitter, which may suggest it’s involvement in many physiological (synaptic plasticity and long term potentiation) and pathological processes (Böhme et al., 1991; O’Dell et al., 1991; Huang, 1997; Hou et al., 1999). Several evidences demonstrated that excessive production of nitric
oxide causes neurodegeneration culminating to the pathogenesis of epilepsy, Alzheimer disease and other neurodegenerative disorders (Hou et al., 1999; Penix et al., 1994; Murphy, 1999; Ghasemi and Dehpour, 2011). In our study elevated total nitrite content in the cortex and hippocampus of pentylenetetrazole-kindled mice suggest elevated nitrosative stress in these areas. This elevated nitrosative stress level can be deemed as one of the pathogenic factors for epileptogenesis and associated learning and memory deficit in pentylenetetrazole-kindled mice. However no significant change in cortical and hippocampal nitrosative stress level in between postictal and interictal group, suggests no significant difference in nitrosative stress level with progressive and uncontrolled seizures.

Hippocampus and cortex are enriched with cholinergic afferents which, under normal conditions, play a pivotal role in the control of neuronal excitability (Friedman et al., 2007) and in cognitive processes (Weckesser et al., 1997; Niewiadomska et al., 2002) and upto some extent modulation of neuronal excitability which may initiate seizures in epileptic patients (Turski et al., 1989; Gloveli et al., 1999). Chronic pentylenetetrazole treatment in rodents has been reported to decrease the basal ACh release in hippocampus which was worsened with pentylenetetrazole treatment but not with concomitant GABA_A agonist treatment (Serra et al., 1997). One of the reasons for decreased basal ACh level in pentylenetetrazole-kindled mice might be the constitutive overexpression of AChE, linked with elevated intracellular Ca^{2+} level mediated excitotoxicity and altered gene expression during seizures. The long lasting changes in AChE level appease due to splicing of AChE pre-mRNA to produce unique AChE-R mRNA followed by reduction in ACh level (Friedman et al., 2007). The constitutive overexpression of AChE has been reported to be involved in reduced dendritic branching and cognitive deterioration (Beeri et al., 1997). The results of our study can be advocated in light of above as uncontrolled seizures in postictal group resulted in increased cortical and hippocampal acetylcholinesterase activity, suggesting the progressive memory loss via ACh mediated intracellular Ca^{2+} augmentation (Egorov and Müller, 1999) which might be leading to excitotoxicity and long lasting AChE upregulation as evident in cortex and hippocampus of interictal group as well. Although increased ACh level has been reported to improve memory deficit but in these kindled animals elevated ACh level might be indulged into ACh dependent excitotoxicity.
The results of the present study suggest reduction in GABAergic, dopaminergic, serotonergic and enhanced nitrosative stress and glutamatergic neurotransmission may play an interesting role in the learning and memory deficit associated with pentylenetetrazole-kindling. Most of the AEDs working through GABAergic mechanisms control seizures, but fail to cure the associated memory deficit in epileptic patients (Marson et al., 2007). Therefore GABAergic approach do not appears as a probable target for management of memory deficit in epilepsy. Indeed iGluR receptor antagonist will exert anticonvulsant effect (Czuczwar, 2000) but may cause inhibition of iGluRs dependent LTP formation culminating to worsening of memory deficit in epileptic patients, however some studies suggest the use of NMDA antagonist for the improvement of memory as well (Parsons et al., 2007). Therefore use of selective glutamate receptor can be explored further to evaluate its beneficial effect on learning and memory deficit in epilepsy. Further nitrosative stress levels can be reduced using NOS inhibitors but their use appears to be inappropriate due to their controversial role as proconvulsant and anticonvulsant (Del-Bel et al., 1997). Cholinergic modulation using acetylcholinesterase inhibitors has long been practiced for memory complications but it should be cautiously used in epileptic patients due to their seizurogenic potentials (Griffith et al., 2008). Therefore use of selective cholinergic modulation in hippocampus and cortex might be useful as adjuvant therapy for the management of memory deficit in epilepsy. Dopaminergic and serotonergic modulation appears suitable as their elevated level may improves memory and epilepsy. Therefore use of selective dopaminergic and serotonergic analogues can be further explored for their effectiveness in epilepsy as well as memory deficit associated with it.

In conclusion, selective dopaminergic, serotonergic and glutamatergic facilitation appears to be comprehensive targets whereas selective cholinergic modulation may be useful as adjuvant target for the management of memory deficit in epilepsy. These speculations warrant further experimental validation, which is underway.
6.2 Experiment III:

Comparative analysis of phenytoin and valproate in epilepsy associated learning and memory deficit and associated neurochemical changes

6.2.1 Introduction

Despite of wide recognition of epidemiological aspects of cognitive comorbidities of epilepsy, clinical interventions for management of these comorbidities in epileptic patients are issues of substantial concern. Moreover, current treatment strategies also appear to be ineffective in terms of their efficacy and associated cognitive side effects (Marson et al., 2007).

Most commonly prescribed antiepileptic has been reported to be associated with cognitive decline in epileptic patients (Thompson et al., 1981; Aldenkamp et al., 1994; Meador et al., 1995). Phenytoin has long been suggested to produce worst cognitive performance as compared to other antiepileptic drugs (Meador et al., 1995). Another choice of drug for management of epilepsy, sodium valproate, has been reported to have lesser cognitive side effects as compared to other antiepileptic drugs (Trimble and Thompson, 1984; Marson et al., 2007; Sun et al., 2008). However some study suggests no clinical significant difference in phenytoin and sodium valproate (Meador et al., 1995) and some suggest higher risk of Parkinson related cognitive side effect of valproate (Ristić et al., 2006). Till date no study has been carried out to validate cognitive effects of these drugs in experimental animals along with their effect on neurochemical status of discrete brain parts.

Therefore it is imperative to establish the effect of these drugs on cognitive changes in experimental model of chronic epilepsy. Effect of these drugs on cognitive behavior and associated neurochemical changes in discrete brain parts would help revealing correlative neurochemical changes responsible for their effect on cognition and would also help in development of newer and safer strategies for the restoration of cognition in epileptic patients.

Chemical kindling in rodents has long been serving as a better tool for understanding epilepsy and associated cognitive deficit. Moreover it is regarded as a model of human temporal lobe epilepsy which appears distinctive in providing opportunity to study progressive cognitive changes with a close resemblance to
clinical epilepsy. Thus, in continuation to our previous study to find a comprehensive or add on target for treatment of epilepsy and associated memory deficit this study was envisaged to explore protective effect of phenytoin and sodium valproate on pentylenetetrazole-kindling induced memory deficit in mice and associated neurochemical changes in discrete brain parts.

6.2.2 Experimental Design

The group 7: naïve animals, consisted of untreated animals (n=10) and rest 30 animals were subjected to pentylenetetrazole-kindling. Excluding resistant and mortality, only successfully kindled animals were further randomly divided into 3 groups. Group 8 (vehicle control group) consisted of kindled animals receiving normal saline (10 ml/kg/day; i.p.; n=10), group 10 (phenytoin per se group) consisted of kindled animals treated with phenytoin (30 mg/kg/day; i.p.; n=7) and group 11: consisted of kindled animals receiving sodium valproate (300 mg/kg/day; i.p.; n=8).

**Group 7:** Naive group (n = 10)

**Group 8:** Postictal PTZ-kindled group (10 ml/kg/day; i.p.; n = 10) [Vehicle Control]

**Group 10:** Phenytoin per se treatment group (30 mg/kg/day; i.p.; n = 7)

**Group 11:** Sodium valproate per se treatment group (300 mg/kg/day; i.p.; n=8)

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**Figure 6.2.1 Schematic Presentation of Experimental Protocol**

The treatment schedule was followed up to 20 days. All the kindled animals (group 8, 10 and 11; elaborated in Figure 6.2.1) were challenged with pentylenetetrazole subconvulsive dose (35 mg/kg; i.p.) on day 5, 10, 15 and 20 and corresponding seizure severity score was recorded using modified Racine’s scale.
(protocol explained in 4.5.1). After 2 h of pentylenetetrazole challenging dose, once their locomotor activity become normalized, animals were evaluated for their performance in elevated plus maze and passive shock paradigm (protocol explained in 4.5.2) on days 5, 10, 15 and 20. After behavioral assessments on day 20, all the animals were sacrificed after 4h of last pentylenetetrazole injection for neurochemical analysis (protocol explained in 4.6).

**Statistical Analysis**

The statistical analysis was performed using the Sigma Stat Statistical Software version 3.5. Statistical significance was calculated using One-way ANOVA followed by Student-Newman-Keuls Test. Each value was expressed as mean ± S.E.M. and statistical significance was considered at P < 0.05.

**6.2.3 Results**

Successfully kindled animals were included in the study and the animals showing resistance to pentylenetetrazole kindling were excluded from study.

**6.2.3.1 Behavioral Evaluations**

**Effect on Seizure Severity Score:** The significant change in seizure severity score was observed on day 5 ($F_{(3,29)} = 141.323; P < 0.001$), day 10 ($F_{(3,29)} = 202.554; P < 0.001$), day 15 ($F_{(3,29)} = 568.625; P < 0.001$) and day 20 ($F_{(3,29)} = 319.095; P < 0.001$) in different groups. On days 5, 10, 15 and 20, after administration of pentylenetetrazole challenging doses in vehicle treated animals have shown significant increase ($P < 0.001$) in seizure severity score as compared to naïve animals. The treatment with phenytoina (30 mg/kg/day, i.p.) and sodium valproate (300 mg/kg/day, i.p.) significantly ($P < 0.001$) reduced the seizure severity score as compared to vehicle treated group after pentylenetetrazole challenging doses on different days (Figure 6.2.2).

**Effect on Transfer Latency:** The significant change in transfer latency was observed on day 0 ($F_{(3,29)} = 6.740; P = 0.001$), day 5 ($F_{(3,29)} = 25.618; P < 0.001$), day 10 ($F_{(3,29)} = 22.305; P < 0.001$), day 15 ($F_{(3,29)} = 34.483; P < 0.001$) and day 20 ($F_{(3,29)} = 64.67; P < 0.001$) in different groups. Naïve animals had shown a tendency to decrease transfer latency in elevated plus maze on day 10, 15 and 20. The significant increase ($P < 0.001$) in transfer latency was observed in vehicle treated group as compared
naïve group on different days. Phenytoin treatment *per se* did not show any significant change in transfer latency as compared to vehicle treated animals on different days. However, sodium valproate treatment significantly reduced the transfer latency in elevated plus maze model on day 10 (P = 0.002), day 15 (P < 0.001) and day 20 (P < 0.001) as compared to vehicle treated animals (Figure 6.2.3).

**Figure 6.2.2 Effect on Seizure Severity Score**
Naïve animals were not challenged with pentylenetetrazole challenging dose therefore no response (stage 0) has been mentioned. Each value is expressed as Mean ± S.E.M. *: as compared to naïve, #: as compared to vehicle treated group. The significance level was considered at P < 0.05 (Student-Newman-Keuls Test)

**Figure 6.2.3 Effect on Transfer Latency in Elevated Plus Maze**
Each value is expressed as Mean ± S.E.M. *: as compared to naïve, #: as compared to vehicle treated group. The significance level was considered at P < 0.05 (Student-Newman-Keuls Test)
Figure 6.2.4 Effect on Number of Mistakes in Passive Shock Avoidance Paradigm
Each value is expressed as Mean ± S.E.M. *: as compared to naïve, #: as compared to vehicle treated group. The significance level was considered at P < 0.05 (Student-Newman-Keuls Test)

Figure 6.2.5 Effect on Step Down Latency in Passive Shock Avoidance Paradigm
Each value is expressed as Mean ± S.E.M. *: as compared to naïve, #: as compared to vehicle treated group. The significance level was considered at P < 0.05 (Student-Newman-Keuls Test)

Effect on number of mistakes and step down latency: The significant change in number of mistakes was observed on day 5 ($F_{(3,29)} = 19.209$; $P = 0.001$), day 10 ($F_{(3,29)} = 16.452$; $P < 0.001$), day 15 ($F_{(3,29)} = 19.559$; $P < 0.001$) and day 20 ($F_{(3,29)} = 26.607$; $P < 0.001$) in different groups. Naïve group animals have shown requirement of
average 2.83 ± 0.21 numbers of trials to learn to stay for at least 120s on shock free zone in passive shock avoidance paradigm. Pentylentetrazole-kindled animal required significantly increased number of trials 8.1 ±0.7 (P < 0.001) than naïve animals on day 0. The number of mistakes in vehicle treated animals was significantly (P < 0.001) higher than number of mistakes of naïve animals on day 5, 10, 15 and 20. However no significant change in number of mistakes was observed in phenytoin treated animals as compared to vehicle treated animals on different days. However sodium valproate treatment significantly (P < 0.001) reduced the number of mistakes on day 5, 10, 15 and 20 as compared to vehicle treated animals (Figure 6.2.4).

The significant change in step down latency was observed on day 5 (F(3,29) = 15.358; P = 0.001), day 10 (F(3,29) = 25.505; P < 0.001), day 15 (F(3,29) = 12.401; P < 0.001) and day 20 (F(3,29) = 84.406; P < 0.001) in different groups. Naïve group animals had shown average step down the latency of 120s on day 5, 10, 15 and 20. The step down latency in vehicle treated animals was significantly (P < 0.001) reduced than step down latency of naïve animals on day 5, 10, 15 and 20. However no significant change in step down latency of phenytoin treated animals was recorded on different days, as compared to vehicle treated animals. However sodium valproate treatment significantly (P < 0.001) increased the step down latency on day 10, 15 and 20 as compared to vehicle treated animals (Figure 6.2.5).

6.2.3.2 Neurochemical Estimations

Changes in glutamate and GABA levels: The significant difference was observed in glutamate level of cortex (F(3,29) = 17.138; P <0.001) and hippocampus (F(3,29) = 9.238; P < 0.001) with different treatments in this study. Vehicle treatment significantly depleted the glutamate level in cortex (P < 0.001) and hippocampus (P = 0.008) as compared to that of naïve animals. Phenytoin and sodium valproate treatment significantly increased (P < 0.001) the cortical glutamate level as compared to that of vehicle treated animals (Table 6.2.1). The significant difference was observed in GABA level of cortex (F(3,29) = 214.378; P <0.001) and hippocampus (F(3,29) = 11.910; P < 0.001) with different treatments. Vehicle treatment significantly elevated the hippocampal GABA level (P = 0.008) as compared to that of naïve animals. Phenytoin treatment did not change the cortical (P = 0.398) and hippocampal (P = 0.988) GABA level as compared to that of vehicle treated animals.
Table 6.1.2 Effect of Different AEDs on Neurochemical Changes in Discrete Brain Parts

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glutamate $\Psi$</th>
<th>GABA $\Psi$</th>
<th>Noradrenaline $\Psi$</th>
<th>Dopamine $\Psi$</th>
<th>Serotonin $\Psi$</th>
<th>Nitrite Level $\Psi$</th>
<th>AChE Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortex</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Naïve</td>
<td>6372.71 ± 184.35</td>
<td>1743.87 ± 115.38</td>
<td>77.25 ± 8.49</td>
<td>551.89 ± 28.63</td>
<td>1070.07 ± 112.82</td>
<td>582.08 ± 61.54</td>
<td>153.31 ± 7.26</td>
</tr>
<tr>
<td>Vehicle</td>
<td>4627.47 ± 271.63 $^*$</td>
<td>1936.39 ± 104.83</td>
<td>94.62 ± 6.85</td>
<td>129.51 ± 23.96 $^*$</td>
<td>255.16 ± 30.86 $^*$</td>
<td>814.23 ± 27.51 $^*$</td>
<td>279.95 ± 4.11 $^*$</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>6069.34 ± 274.35 $^*$</td>
<td>1719.74 ± 118.75</td>
<td>93.18 ± 2.54</td>
<td>741.21 ± 9.67 $^<em>$</em></td>
<td>922.98 ± 68.72 $^<em>$</em></td>
<td>645.22 ± 11.41 $^*$</td>
<td>290.14 ± 10.12 $^*$</td>
</tr>
<tr>
<td>Sod. Valproate</td>
<td>6487.29 ± 137.27 $^*$</td>
<td>2499.52 ± 96.81 $^*$</td>
<td>58.62 ± 8.28 $^*$</td>
<td>204.46 ± 30.29 $^*$</td>
<td>624.19 ± 22.21 $^*$</td>
<td>494.58 ± 19.01 $^*$</td>
<td>160.59 ± 12.51 $^*$</td>
</tr>
<tr>
<td><strong>Hippocampus</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Naïve</td>
<td>4286.43 ± 181.36</td>
<td>1811.61 ± 57.23</td>
<td>230.08 ± 4.44</td>
<td>90.27 ± 6.43</td>
<td>826.86 ± 14.52</td>
<td>419.58 ± 25.95</td>
<td>162.54 ± 7.03</td>
</tr>
<tr>
<td>Vehicle</td>
<td>4801.28 ± 72.37 $^*$</td>
<td>2072.41 ± 82.74 $^*$</td>
<td>172.18 ± 12.13 $^*$</td>
<td>39.66 ± 8.81 $^*$</td>
<td>30.87 ± 11.65 $^*$</td>
<td>745.37 ± 47.04 $^*$</td>
<td>210.11 ± 9.56 $^*$</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>5151.11 ± 114.74 $^*$</td>
<td>2073.97 ± 37.21 $^*$</td>
<td>274.79 ± 4.43 $^*$</td>
<td>75.11 ± 11.20 $^*$</td>
<td>486.23 ± 1.92 $^*$</td>
<td>563.14 ± 15.23 $^*$</td>
<td>205.61 ± 11.21 $^*$</td>
</tr>
<tr>
<td>Sod. Valproate</td>
<td>5067.76 ± 89.41 $^*$</td>
<td>2357.11 ± 77.87 $^*$</td>
<td>56.76 ± 17.47 $^*$</td>
<td>151.13 ± 33.23 $^*$</td>
<td>468.86 ± 17.07 $^*$</td>
<td>830.00 ± 2.79 $^*$</td>
<td>225.35 ± 16.20 $^*$</td>
</tr>
</tbody>
</table>

Each value is expressed as Mean ± S.E.M. The significance level was considered at $P < 0.05$ (Student-Newman-Keuls Test; $^*$: significant as compared to naïve and $^*$: significant as compared to vehicle). $\Psi$: ng/g of wet tissue; AChE activity was expressed in micro Moles of acetylcholine hydrolyzed per g of wet tissue.
However, sodium valproate treatment significantly elevated cortical (P < 0.001) and hippocampal (P = 0.016) GABA level as compared to vehicle treated animals (Table 6.2.1).

**Changes in monoamine levels:** The significant difference was observed in noradrenaline level of cortex ($F_{(3,29)} = 4.789; P = 0.008$) and hippocampus ($F_{(3,29)} = 72.53; P < 0.001$) with different treatments in this study. Vehicle treatment significantly depleted (P < 0.001) the noradrenaline store in hippocampus as compared to that of naïve animals. Phenytoin treatment significantly increased in hippocampal (P < 0.001) noradrenaline level as compared to that of vehicle treated animals. However sodium valproate treatment significantly reduced noradrenaline level in cortex (P = 0.012) and hippocampus (P < 0.001) noradrenaline level as compared to that of vehicle treated animals (Table 6.2.1).

The significant difference was also observed in dopamine levels of cortex ($F_{(3,29)} = 116.072; P < 0.001$) and hippocampus ($F_{(3,29)} = 6.650; P < 0.001$) with different treatments. Vehicle treatment causes significant reduction (P < 0.001) in cortical dopamine level as compared to naïve animals. Treatment with phenytoin significantly enhanced the dopamine level in cortex (P < 0.001) as compared to that of vehicle treated animals. However sodium valproate treatment significantly increased hippocampal dopamine level as compared to that of vehicle treated animals (Table 6.2.1).

The significant difference was also observed in serotonin level of cortex ($F_{(3,29)} = 22.457; P < 0.001$) and hippocampus ($F_{(3,29)} = 632.635; P < 0.001$) with different treatments. Vehicle treatment significantly reduced (P < 0.001) the cortical and hippocampal serotonin level. Phenytoin treatment significantly elevated the serotonin level in cortex (P < 0.001) and hippocampus (P < 0.001) as compared to vehicle treated animals. Sodium valproate treatment also significantly increased the serotonin level in cortex (P = 0.003) and hippocampus (P < 0.001) serotonin level as compared to that of vehicle treated animals (Table 6.2.1).

**Changes in total nitrite level:** Significant changes in nitrite level was recorded in cortex ($F_{(3,29)} = 10.464; P < 0.001$) and hippocampus ($F_{(3,29)} = 44.708; P < 0.001$) with different treatments. Vehicle treatment in this study caused significant elevation (P < 0.001) of cortical and hippocampal nitrite level as compared to naïve animals.
Treatment with phenytoin significantly reduced total nitrite level in cortex (P = 0.01) and hippocampus (P < 0.001) as compared to that of vehicle treated animals. While treatment with sodium valproate significantly reduced (P < 0.001) cortical total nitrite level and increased in hippocampal (P = 0.048) total nitrite level as compared to that of vehicle treated animals (Table 6.2.1).

**Changes in acetylcholinesterase activity:** The significant change in acetylcholinesterase activity has also been observed in cortex (F(3,29) = 69.586; P < 0.001) and hippocampus (F(3,29) = 6.587; P = 0.002) with different treatments. Vehicle treatment significantly elevated (P < 0.001) the cortical and hippocampal acetylcholinesterase activity as compared to naïve animals. Treatment with phenytoin did not induce any significant change as compared to vehicle treated animals. Sodium valproate treatment significantly reduced (P < 0.001) the cortical acetylcholinesterase activity as compared to vehicle treated animal (Table 6.2.1).

**6.2.4 Discussion**

In the present study, first attempt has been made to correlate the effect of clinically used antiepileptic drugs, phenytoin and sodium valproate, on learning and memory deficit and associated neurochemical changes in discrete brain parts using pentylenetetrazole-kindling model of epilepsy to find appropriate add on therapy. Our study has demonstrated that phenytoin and sodium valproate treatment provided significant seizure control but phenytoin has worst effect on memory as compared to sodium valproate in pentylenetetrazole-kindled mice along with different neurochemical changes in discrete brain parts.

Pentylenetetrazole-kindled animals, treated with vehicle, have shown reduced seizure threshold as increased seizure severity score after pentylenetetrazole challenging dose on different days. These animals were also recorded with impaired spatial as well as contextual fear memory. These findings are in accordance with earlier documented evidences suggesting memory loss in pentylenetetrazole-kindled animals (Grecksch et al., 1997; Takechi et al., 2012; Choudhary et al., 2013; Singh et al., 2013). Abnormality in the neurochemical status of pentylenetetrazole-kindled animals includes reduction in dopaminergic, serotonergic, cholinergic and enhanced nitrite level in hippocampus and cortex. The decreased glutamate/GABA ratio in kindled animals suggested the activation of feedback inhibition in cortex and
hippocampus which limits the spread of convulsions. These abnormalities in neurochemical status are also congruent with reported evidences (Singh et al., 2013).

Treatment with phenytoin elicited their standard antiepileptic effect by reducing the seizure severity score after challenging dose of pentylenetetrazole on different days. Suppression of the sodium channel could be one the major anticonvulsant mechanism of phenytoin (Yaari et al., 1986). Phenytoin also reduces extracellular Ca\(^{2+}\) concentration by blocking potassium stimulated uptake of Ca\(^{2+}\) (Pincus and Lee, 1973), which is generally elevated during seizure (Stringer and Lothman, 1989). On the other hand blockade of sodium channel and reduction in extracellular Ca\(^{2+}\) concentration leads to abolition of long term potentiation (LTP) (Komatsu and Yoshimura, 2000; Hardingham et al., 2006) and justify memory loss in phenytoin treated animals.

Apart from these, phenytoin treatment resulted in elevation of hippocampal norepinephrine and serotonin levels which has been suggested to limit the spread of seizure threshold. Similar observations on monoamine levels have been reported with chronic phenytoin treatment and suggested as its possible anticonvulsant mechanism (Meshkibaf et al., 1995; Choudhary et al., 2013). However elevated monoamine levels with phenytoin could not justify associated memory loss.

Elevated nitrite level has been considerably reported in epileptic animals (Choudhary et al., 2013; Singh et al., 2013) therefore reduction in cortical and hippocampal nitrite level can also be considered as one of anticonvulsant mechanism of phenytoin in this study. On the other hand, nitric oxide is an important component for LTP induction (Izumi et al., 2008) and low level nitric oxide (as observed by reduced nitrite levels with phenytoin treatment) might reduce LTP formation and could contribute to memory loss.

While analyzing cholinergic innervations, by estimating acetylcholinesterase activity, it was observed that phenytoin treatment could not reduce the cortical and hippocampal acetylcholinesterase activity and it remained same as that of vehicle treated animals. Persistent increase in acetylcholinesterase activity may lead to reduced acetylcholine and thus may reduce synaptic plasticity and culminate to memory loss (Beeri et al., 1997; Friedman et al., 2007). This might be one of the reasons that despite of seizure control phenytoin treatment could not improve memory.
in these animals. Increased acetylcholinesterase activity with phenytoin treatment has been documented factor leading to phenytoin induced memory loss in different experimental conditions (Choudhary et al., 2013; Sudha et al., 1995).

Treatment with sodium valproate significantly reduced the seizure severity. While correlating behavioral findings with neurochemical status it was apparent that sodium valproate treatment enhanced cortical and hippocampal GABA levels and thus leading anticonvulsant effect. Elevation in GABA level with sodium valproate treatment has long been reported as its most promising anticonvulsant mechanism (Balding and Geller, 1981; Owens and Nemeroff, 2003). There might be four possible mechanisms for this GABA enhancement: 1) inhibition of GABA degradation by inhibiting GABA transaminase, 2) increase of GABA synthesis by increased activity of glutamic acid decarboxylase, 3) decrease in GABA turnover, and 4) reduction in the reuptake of GABA (Owens and Nemeroff, 2003). This elevated GABA level may also lead to inhibition of LTP in CA1 region (Ji et al., 1995). However, activation of presynaptic GABA_{A} receptor may facilitate LTP in hippocampal mossy fiber synapses (Ruiz et al., 2010) which could be considered as one of the protective mechanism of sodium valproate against pentylenetetrazole-kindling induced memory deficit in mice.

Simultaneously sodium valproate per se treatment elevated dopamine and serotonin level in cortex and hippocampus. Indeed, behavioural and neurochemical data have indicated that the activation of hippocampal dopamine system with sodium valproate treatment might be engaged in curbing neuronal hyperexcitability by exerting profound inhibitory effect on epileptogenesis mediated via dopamine D_{2} receptors (Suppes et al., 1985; Alam and Starr, 1993) and D_{1} receptor dependent GABA release (Mori et al., 1987). Simultaneous facilitation of D_{1}/D_{5} receptors mediated LTP (Lemon and Manahan-Vaughan, 2006) with elevated level of dopamine in hippocampus might be resulting in memory improvement with sodium valproate treatment in our study. Similar effect on hippocampal dopamine and serotonin level has been reported after chronic sodium valproate treatment in normal animals (Baf et al., 1994).

Sodium valproate treatment increased hippocampal nitrite level and thus might be facilitating LTP in hippocampus and improving the memory functions.
Figure 6.3 Possible Involvements of Different Neurotransmitters in Epilepsy and Memory Deficit
Clinically sodium valproate has been reported to elevate serum nitrite level in epileptic children (Karabiber et al., 2004). Simultaneous reduction in cortical nitrite level could reduce the cortical synaptic plasticity and may alleviate seizures.

On other side reduction of cortical acetylcholinesterase activity by sodium valproate, might augment acetylcholine level in cortex and restore the memory formation process. Simultaneous elevation hippocampal nitrite level can also contribute to memory facilitation in sodium valproate treated animals and depletion of cortical nitrite level might be contributing to its neuroprotectant effect on cortical neurons.

Comparative analysis of phenytoin and sodium valproate suggests that sodium valproate controls seizures and associated memory loss more efficiently than phenytoin. However sodium valproate may be associated with some other psychiatric side effects therefore there is need to develop a comprehensive or adjuvant target for the appropriate management of epilepsy and associated memory deficit. This study suggests that phenytoin along with appropriate anticholinesterase can be explored for their efficacy for management of epilepsy and associated memory deficit. Further suggestions may include exploration of dopaminergic and serotonergic receptors as comprehensive target for their protective effect in epilepsy induced learning and memory deficit.

**Conclusion:**

The results of experiments carried out to achieve our second aim suggest the following targets as comprehensive and add on targets:

1. Selective iGluR antagonists, which do not alter LTP, can be useful for comprehensive management of epilepsy associated comorbidities
2. Selective 5-HT R analogs which facilitate LTP and reduce EPSP
3. Selective DA_{1} R agonist or DA_{2} R antagonist may be useful
4. Selective α_{1/2} R analogs can be useful; peripheral side effects may arise
5. GABA antagonist may reduce epilepsy but further worsen memory
6. AChE inhibitors, selective mAChR antagonist with no effect on hippocampal LTP, selective nAChR analogs may be useful
7. nNOS inhibitors may be useful as antiepileptic but may worsen memory