Chapter 5

Aim 1. To validate time dependent and age dependent existence of cognition deficit in chronic animal models of epilepsy resembling to clinical epilepsy

5.1 Experiment I

Validation of time and age dependent existence of cognition deficit in chronic animal models of epilepsy resembling to clinical epilepsy

5.1.1 Introduction

Age related decline in learning and memory remains distinguishing feature of a variety of mammals, from humans to rodents and generally affects learning, working and reference spatial and long term memory (Frick et al., 2000). In similar way, age of onset of epilepsy is one of the major factors that have wide spread debilitating consequences on learning and memory deficit (Ingram, 1988; Flood and Morley, 1993; Moffat et al., 2001; Sanchetee and Sanchetee, 2007). Juvenile and geriatric onset of epilepsy is more vulnerable to behavioral comorbidities than the patients with adult onset (Haut et al., 2009; Desai, 2010).

Children with epilepsy are at increased risk of developing learning and memory deficit as compared to healthy and children with other chronic illness (Williams, 2003; Rantanen et al., 2009; Vendrame et al. 2009). It negatively affects their quality of life and represents a significant risk factor for academic under achievement, mental slowness, memory difficulties, attention deficits and social incompetency (Williams, 2003; van Rijckevorsel, 2006; Fastenau et al., 2008). Similarly cognitive impairments with epilepsy also increase with advancing age (Haut et al., 2009).

Chemical kindling has been defined as the process whereby repeated applications of subconvulsive chemical stimuli to animals lead to an increase in convulsive activity, resulting in generalized seizures (Gupta et al., 2003). Kindling as chronic epilepsy experimental model associating neuronal plasticity and seizures, is distinctive in providing openings to study progressive cognitive changes with a close resemblance to clinical epilepsy (Gupta et al. 2003; Pourmotabbed et al., 2011).

Age dependent learning deficit has been studied in kindled rats (Grecksch et al., 1997) but till date no such study is available for mice. Moreover, Young et al., (2009) have suggested the baseline behavioral difference in rats and mice while
evaluating cognition. These innate differences necessitate the validation of cognitive performance across the species in the specified models. Swiss Albino mice are preferred because of greater ease, economy of testing potential drugs, wider availability of different age group (Ritzmann et al., 1993) and utility in experimental models for evaluation of anticonvulsants and nootropics (Itoh et al., 1990; Kaur et al., 2010; Khurana et al., 2011). As we are working to reveal pathobiology of learning and memory deficit in kindled mice, it is important to validate existence of such deficit in the different age groups of mice. Therefore, this study was envisaged to find out the correlation between age of mice with feasibility for induction of kindling and associated learning and memory deficit.

5.1.2 Experimental Design

In this experiment 60 male mice of 2, 6 and 12 month age groups were taken. In each age group there were two groups; one naïve and another was PTZ kindled. Naïve animals were untreated while kindled group animals were subjected to kindling (protocol of kindling explained in 4.3). There was following experimental groups:

**Group 1:** Naive (2 month age) (n = 10)

**Group 2:** PTZ-kindled (2 month age) (n = 10)

**Group 3:** Naive (6 month age) (n = 10)

**Group 4:** PTZ-kindled (6 month age) (n = 10)

**Group 5:** Naive (12 month age) (n = 10)

**Group 6:** PTZ-kindled (12 month age) (n = 10)

Successfully kindled animals were included in the study and mortality and resistance to kindling was also recorded. Percentage resistance and mortality in each group was calculated and compared with each other. Naïve and kindled animals in each age group were subjected to elevated plus maze and passive shock avoidance paradigm (protocol explained in 4.5.2) to evaluate the effect of kindling on learning and memory in different age group. Retrieval of learned task was performed on day 1, 5 and 10.

**Statistical Analysis:** The statistical analysis was performed using the Sigma Stat Statistical Software version 3.5. The inter group variation was measured by
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Multivariate analysis of variance (MANOVA) followed by Tukey’s test. Statistical significance was considered at \( p < 0.05 \).

5.1.3 Results

Age dependent difference was observed in induction of kindling as endorsed by average requirement of \( 11\pm1 \), \( 15\pm1 \) and \( 18\pm2 \) pentylenetetrazole injections for the induction of kindling in mice belonging to 2, 6 and 12 month age groups respectively (Figure 5.1.1). Further induction of kindling was associated with higher mortality in 2 month age group (20%) as compare to 6 and 12 month age groups. The resistance to kindling was increased with increase in age of animal subjected to kindling, as higher resistance observed in 12 month age group (30%) followed by 6 month and 2 month (10%) age group (Figure 5.1.2).

Kindling significantly affects the transfer latency of mice in different age group animals as compare to their naïve respectively. In the 2 month age group naïve animals (n=10), transfer latency observed on day 1 (27±1.5s) was reduced with subsequent exposure to EPM on day 5 (24±1.13s) and day 10 (17±1.26s), however not significant (Figure 3.3). Whereas, kindled animals in 2 month age group (n=7) showed significant increased the transfer latency in EPM as compare to naïve (\( P < 0.001 \)). The transfer latency in kindled mice (67±1.15s) was significantly increased as compared to their naïve on day 1. With subsequent exposures to EPM, induction of kindling significantly increased the transfer latency on day 5 (85±1.5s) and day 10 (87±1.5s).

In 6 month age group naïve animals (n=10) shown decrease in transfer latency on subsequent exposure to EPM on day 1 (45±1.59s), 5 (43±1.31s) and 10 (37±1.8s), however not significant. Kindled mice have shown significant increased the transfer latency as compared to naïve animals. On subsequent exposures to EPM, the kindled mice (n=7) had shown significant increase in the transfer latency on day 1 (75±1.5s) by 30% (98±1.15s; \( P < 0.001 \)) on day 5 and 69.3% (127±1.18s; \( P < 0.001 \)) on day 10 (Figure 5.1.3).

The 12 month age group naïve animals (n=10) also shown decrease in transfer latency on subsequent exposure to EPM on day 1 (45±1.59s), 5 (41±2.73s) and 10 (39±1.52s) however not significant. Kindled mice in 12 month age group (n=6) have shown significant increase in the transfer latency on subsequent exposure to EPM on
day 1 (79±1.37s; P < 0.001), 5 (105±1.65s; P < 0.001) and 10 (135±1.21s; P < 0.001) in comparison to naïve animals on respective days. With succeeding exposure to EPM the transfer latency observed on day 1 was significantly increased on day 5 and day 10 (P < 0.001).

**Figure 5.1.1 Development of Pentlenetetrazole evoked Kindling in mice at Different Age Groups**

This graph depicts the seizure severity scores following different subconvulsive dose of pentlenetetrazole in different age groups. Each values was expressed as mean seizure severity score ± S.E.M. (kindled animal of 2 month age group n=7; 6 month age group n=7; 12 month age group n=6).

**Figure 5.1.2 Percentage of Animals showing Mortality and Resistance to Pentlenetetrazole Kindling in Different Age Groups**

Percentage mortality due to subconvulsive dose of pentlenetetrazole and resistance to pentlenetetrazole kindling has been expressed. The animals which were not responding to the cumulative administration of subconvulsive dose of pentlenetetrazole they were considered as resistant to pentlenetetrazole kindling and excluded from the study.
Age dependent effect of kindling on transfer latency was also observed in the kindled mice of different age groups. On day 1, transfer latency of 2 month age group was significantly different than that of 6 and 12 month age groups (P < 0.001). The transfer latency on day 5 was also significantly different (P < 0.001) than that of 12 month groups (Figure 5.1.3 and Figure 5.1.4).

In Passive Shock Avoidance Paradigm naïve animals of 2 month age group required 1.4±0.65 numbers of trials to learn to stay at shock free zone for at least 90 seconds, while naïve animals of 6 and 12 month required 2±1.01 and 2±1.12 number of trials, respectively. Kindled mice in 2, 6 and 12 month age groups have shown significant increase in the number of trials required as compare to their naïve animals. Maximum number of trials was required by 2 month age group (14.3±2.1) followed by 12 month (9.5±1.2) and 6 month (7.8±1.21) age groups. Average number of trials required by 2 month age kindled group was significantly higher than 6 month age group (P < 0.001), but not significantly with 12 month age group (P > 0.05). However the number of trials required by 6 month age group was not significantly different than that of 12 month age group (Figure 5.1.5).

Naïve animals of 2, 6 and 12 month age group stayed at shock free zone for at least 90s in Passive Shock Avoidance Paradigm. Kindling significantly reduced the step down latency in different age groups (Figure 5.1.6 and Figure 5.1.7). In 2 month age group step down latency of naïve animals recorded was 90±0s on day 1, 5 and 10. There was no significant difference in the retrieval performance of naïve animals. But kindling in 2 month age group significantly reduced the step down latency on day 1, 5 and 10 by 45±1.29s, 42±1.13s and 44±1.06s (P < 0.001), respectively. There was no significant difference in the retrieval performance of learned task by 2 month kindled group on subsequent days.

In 6 month age group naïve animals stayed at shock free zone for 90s. While kindling in 6 month age group significantly decreased the step down latency on day 1, 5 and 10 by 52±0.93s, 46±1.06s and 45±1.13s (P < 0.001), respectively. There was no significant difference in the retrieval performance of learned task by 6 month kindled group on subsequent days.

Similarly, in 12 month age group naïve animals step down latency recorded was 90s. While kindling in 12 month age group significantly decreased the step down
latency as compared to naïve on day 1, 5 and 10 by 49±2.08s, 42±1.13s and 38±1.24s (P < 0.001), respectively. The step down latency on day 1 was significantly different than that of on day 5 and day 10 (P < 0.05).

Figure 5.1.3 Time Dependent Changes in Transfer Latency
Each value was expressed as mean ± S.E.M. (all naïve group n=10; kindled animal of 2 month age group n=7; 6 month age group n=7; 12 month age group n=6). *: as compared to naïve; #: as compared to day 0; T: as compared to day 1; Ψ: as compared to day 5; The significance level was considered at P < 0.05 (Tukey’s Test).

Figure 5.1.4 Age Dependent Changes in Transfer Latency
Each value was expressed as mean ± S.E.M. *: as compared to naïve; #: as compared to 2 month; T: as compared to 6 month. The significance level was considered at P < 0.05 (Tukey’s Test).
Figure 5.1.5 Age Dependent Effect on Learning Behavior

Each value was expressed as mean ± S.E.M. *: as compared to naïve; #: as compared to 2 month; The significance level was considered at P < 0.05 (Tukey's Test).

Figure 5.1.6 Time Dependent Changes in Step Down Latency

Each value was expressed as mean ± S.E.M. *: as compared to naïve; #: as compared to day 1; The significance level was considered at P < 0.05 (Tukey's Test).

There was no age dependent decrease in the step down latency observed in the Passive Shock Avoidance Paradigm.
5.1.4 Discussion

This study was first attempt to explore the age dependent effect of pentylentetrazole kindling on male Swiss Albino mice. Cognitive deficits such as learning and memory impairments are the debilitating consequences of aging. Since the mean life span of procured Swiss Albino mice, at our laboratory conditions, ranges 16-20 months. Therefore the 2, 6 and 12 month old mice used in the present study can be admissibly categorized as young, adult and aged.

Our results, concerning induction of kindling, demonstrated age-dependent variations. The kindling was developed at faster rate in young as compared to adult and aged mice. With the advancement in age kindling rate was further decreased. Increased resistance to pentylentetrazole kindling was observed in aged mice while higher mortality was recorded in young kindled mice. Similar age related differences in the susceptibility to kindling were illustrated in literature for amygdala kindling (Welsh and Gold, 1983; Michelson and Butterbough, 1985), hippocampal kindling (deToledo-Morrell et al., 1984; deToledo-Morrell et al. 1988). The similar age
dependent variations in kindling rates have been reported in kindled rats (Grecksch et
al., 1997). The increase mortality in lower age was well suggested by a clinical study
supporting the long term mortality in childhood-onset epilepsy which reflects the
considerable risk of epilepsy-related death, including sudden and unexplained death
associated with childhood-onset epilepsy (Sillanpää and Shinnar, 2010).

Chemical kindling, in this study, showed negative effects on the learning in
different age group more pronounced in young group followed by elderly. Whereas
adult kindled mice showed least learning deficit. The “U” shape response on decline
in learning behavior had been observed in our study and suggests the young and aged
having higher learning deficit while adults prompted with lesser learning deficits. The
similar observations have been recorded in number of mammalian species (Voigt and
Morgenstern, 1986; Grecksch et al., 1997; Moffat et al., 2001) and in children with
epilepsy (Williams 2003; Rantanen et al., 2009).

EPM is a well establish model to evaluate spatial memory deficit in epileptic
and different CNS pathology of rodents (Itoh et al., 1990; Kumaran et al., 2008; Kaur
et al., 2010; Khurana et al., 2011). Therefore EPM was used in this study to evaluate
short term (working) and long term (reference) spatial memory in mice. Kindling
significantly altered the retrieval of learned task in EPM as function of age. As the age
of animal progresses the working spatial and reference memory of the kindled mice
worsened and thus defined worsening effect of age on spatial memory in kindled
mice. The similar observations have been suggested by the several studies performed
on the rats (Rauca et al., 2000; Moffat et al., 2001; Gupta et al., 2003). Similarly,
decline in spatial memory has been reported in kindled rats (Lopes da Silva et al.,
1986) and the negative correlation of age with working memory formation has been
reported in non epileptic humans (Morris et al., 1988; Daffner et al., 2011). As
epilepsy triggers the neurodegenration (Schroeder et al., 1993; Uchida et al., 1999;
Liu et al., 2003; Noebels, 2002) therefore as the time span of epilepsy increases (from
day 1 to day 10) the associated spatial memory worsens in this study. While
measuring short term and long term contextual memory in Passive Shock Avoidance
Paradigm, there was memory decline observed in kindled mice. But age dependent
negative effect on contextual memory was not found in kindled mice. Thus suggesting
the loss in working and reference contextual memory with kindling in mice but this
impairment did not worsened with the different onset of age.
Normally aging process affects cognition by slow modulations in place cells (Wilson et al., 2005), dopamine level in prefrontal cortex (Mizoguchi et al., 2009), NMDA binding capacity to its receptor (Grecksch et al., 1997), \( \text{Ca}^{2+} \) channelopathy (Takahashi et al., 2009) and nitric oxide level in the brain (Lores-Arnaiz and Bustamante, 2011). It was speculated that these effects might be synergized by the epileptic activity of brain (Schroeder et al., 1993; Uchida et al., 1999; Liu et al., 2003; Noebels, 2002) leading to enhanced working and reference memory deficit in different age groups (Barnes et al., 1997). These mechanisms can be investigated further to explore the possible pathogenic mechanism of cognitive deficit associated with epilepsy.

Thus the present study validated the existence of onset of age dependent learning and memory deficit in kindled mice and suggested the suitability of 6 month old mice for long term experimental studies to evaluate epilepsy induced cognitive deficit. As this age group have optimal requirement of pentylenetetrazole injections, insignificant mortality and resistance to kindling and significant learning and memory deficit. At the end of the study it was presumed that, if the kindling could have been sustained in the mice of 2 month age up to 6 or 12 month age, more worsening effect on learning memory deficit would be expected.