รายงานวิจัยฉบับสมบูรณ์

การศึกษารูปแบบคลื่นไฟฟ้าสมองและพฤติกรรมการเรียนรู้และจดจำในหนูขาวใหญ่ที่ถูกชักนำให้เกิดการเสื่อมของประสาท

(Studies of EEG patterns, learning and memory behaviors in induced neurodegenerative rats)

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โครงการวิจัยนี้ได้รับทุนสนับสนุนจาก.....เงินรายได้มหาวิทยาลัย มหาวิทยาลัยสงขลานครินทร์
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1. ชื่อชุดโครงการ (ระบุกรณีเป็นโครงการย่อยภายใต้ชุดโครงการ)
2. ชื่อโครงการเดี่ยว : การศึกษารูปแบบคลื่นไฟฟ้าสมองและพฤติกรรมการเรียนรู้และจดจำในหนูขาวใหญ่ที่ถูกชักนำให้เกิดการเสื่อมของประสาท
   Studies of EEF patterns, learning and memory hehaviors in induced neurodegenerative rats
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5. บทคัดย่อภาษาไทยและภาษาอังกฤษ

บทคัดย่อ

พยาธิวิทยาระบบประสาทของภาวะสมองเสื่อมนั้นเกิดขึ้นก่อนการแสดงอาการของโรคนับสิบปี การตรวจพบแต่เนิ่นคือวิธีที่จะทำให้สามารถรักษาก่อนเกิดอาการ การศึกษานี้มีวัตถุประสงค์ในการแยกแยะความแตกต่างของรูปแบบคลื่นไฟฟ้าสมองของหนูปกติและหนูแร็ทที่ได้รับ dexamethasone หนู Wistar โตเต็มวัยเพศผู้ที่ถูกผ่าตัดฝังขั้วไฟฟ้าลงบนกะโหลกเหนือ frontal และ parietal cortexได้รับ dexamethasone (0.5 mg/kg) วันละครั้ง อย่างต่อเนื่องเป็นเวลา 21 วัน มีการบันทึกคลื่นไฟฟ้าสมอง (EEG) ในวันที่ 6, 11,16 และ 21 การวิเคราะห์ด้วย one way ANOVA แสดงให้เห็นการเพิ่มของ parietal power ของคลื่นความถี่ต่ำ (delta, theta และ alpha) โดยเฉพาะอย่างยิ่งคลื่น alpha ซึ่งเห็นได้ชัดตั้งแต่วันที่ 11 การวิเคราะห์ภาวะหลับ-ตื่นในวันที่ 21 ยืนยันการลดลงของ rapid eye movement (REM) sleep การค้นพบนี้ชี้ให้เห็นว่า การวิเคราะห์สัญญาณคลื่นไฟฟ้าสมองนั้นสามารถแสดงรูปแบบและ parameters ที่แตกต่างของภาวะสมองเสื่อมที่ถูกชักนำด้วย dexamethasone นอกจากนี้ สารสกัดเอทานอลของขมิ้น Curcuma longa Linn. (CL) ซึ่งมีสาร curcumin เป็นองค์ประกอบนั้นมีฤทธิ์ต้านอนุมูลอิสระ (antioxidative effects) การศึกษานี้ได้ทำการทดสอบผลของสารสกัดต่อความหนาแน่นของเซลล์สมองที่ย้อมติดด้วยสี cresyl violet ในสมองบริเวณ hippocampus ของหนูแร็ทที่ได้รับ dexamethasone ผลการวิจัยพบว่า การได้รับ dexamethasone มีผลลดความหนาแน่นของเซลล์ที่ติดสีย้อม cresyl violet ที่บริเวณย่อย CA1, CA3 และ dentate gyrus ยกเว้น CA2 ของสมอง hippocampus อย่างมีนัยสำคัญ อย่างไรก็ตาม พบว่าการได้รับสารสกัดล่วงหน้าด้วยความเข้มข้น 100 mg/kg มีผลรักษาระดับความหนาแน่นของเซลล์สมองที่ CA1 และ dentate gyrus จากการทำลายได้อย่างมีนัยสำคัญ โดยสรุปแล้ว การค้นพบนี้ได้ยืนยันฤทธิ์ปกป้องสมองของสารสกัดจากขมิ้น นอกจากนี้ การเฝ้าติดตามคลื่น theta อาจมีข้อดีเป็นพิเศษสำหรับการตรวจหาภาวะสมองเสื่อมแต่เนิ่น

Abstract

Neuropathology of brain degeneration precedes the symptoms by decades. Early detection is a new strategy that would allow for early intervention. This study aimed to distinguish between electroencephalographic (EEG) patterns of normal and dexamethasone-treated rats. Adult male

Wistar rats implanted with electrodes on the skull over the frontal and parietal cortices were intraperitoneally injected with either saline of dexamethasone (0.5 mg/kg) once daily for 21 consecutive days. EEG recording was performed on day 6, 11, 16 and 21. One-way ANOVA revealed significant increases in parietal EEG power of slow frequencies (Delta, Theta and Alpha) particularly with the dominant Theta activity seen as early as day 11 of dexamethasone (Dx) treatment. Sleep-wake analysis on day 21 confirmed significant reduction of rapid-eye movement (REM) sleep. These findings indicate that the analysis of EEG signal provides differential patterns and parameters of neurodegeneration induced by dexamethasone treatment. In addition, an ethanolic extract from Curcuma longa Linn. (CL) containing the curcumin constituent has been reported to produce antioxidant effects. The effects of a CL extract on the densities of cresyl violet positive neurons in the hippocampus of Dx treated male rats were also tested. The results showed that Dx treatment significantly reduced the densities of cresyl violet positive neurons in the sub-areas CA1, CA3 and the dentate gyrus, but not in the CA2 area. However, CL pretreatment (100 mg/kg, p.o.) significantly maintained neuronal densities in the CA1 and dentate gyrus. In summary, these data confirmed neuroprotective effects of CL extract. Moreover, monitoring of Theta oscillation might have a special advantage for early detection of neurodegeneration.

6. บทสรุปผู้บริหาร (Executive Summary) ประกอบด้วย

6.1 บทนำ

Neurodegenerative diseases are irreparable conditions that result in a progressive degeneration and destruction of nerve cells. In general, neurodegeneration affects memory processing, thinking abilities, personality and behavior. Etiology of the diseases is complicated with interactions among individuals, genetics and diverse factors such as aging, sex hormones, disorders and stressful life incidents (Esch et al., 2002; Hoenicka, 2006; Winner et al., 2011).

One of the strong candidate factors for neurodegenerative diseases is stress, present in daily life, or from clinical situations. Stress induces the secretion of the corticotropin releasing hormone (CRH) that activates the hypothalamic pituitary adrenal axis (HPA axis) and sympathetic nervous system (O'Connor et al., 2000). Basically, CRH stimulates the release of the adrenocorticotropic hormone (ACTH) from the pituitary gland, which in turn leads to the secretion of glucocorticoids (GCs) from the adrenal cortex as an end point of activation of the HPA axis (Hauger et al., 1987). Chronic psychosocial stress was found to trigger an excessive increase of GC levels (Quax et al., 2013; Srinivasan et al., 2013). The augmented GC levels in blood have been positively correlated with aging in humans and animals (Landfield et al., 1978). A link between the consequences of stress and neurodegeneration has been hypothesized, in particular when hypersecretion of GCs was revealed in neurologic disorders such as Alzheimer's, Parkinson's and Huntington's·diseases (Hartmann et al., 1997; Notarianni, 2013; Vyas and Maatouk, 2013).

The brain is a major target for action of GCs with a high lipophilicity for passing the blood brain barrier (BBB) to bind their receptors on neurons and glia (Reul and de Kloet, 1985). Chronic exposure to GCs that accompanied psychosocial stress also resulted in elevation of apoptosis in the hippocampal regions (Arbel et al., 1994). The hippocampus is a major region of the brain for

memory processing. It has a high density of GR receptors that have been hypothesized to underlie memory impairment following long term exposure to stress (Elliott et al., 1993). In an acute study, dexamethasone (synthetic steroid glucocorticoids) induced the death of neuronal cells through the apoptotic process (Mitchell et al., 1998).

Curcuma longa Linn. (CL) or turmeric belongs to the Zingiberaceae family. CL plants are predominantly found in tropical Asia and Africa (Ammon and Wahl, 1991). CL bioactive ingredients in food and their effects on health have been extensively studied. The suppressive effects on reactive oxygen species by CL extracts have generated enormous interest. CL is a dietary food ingredient and is also used as a medicinal herb with antioxidant properties (Maheshwari et al., 2006). Curcumin, the major active component of a CL ethanolic extract, was also known to be an antioxidant with the potential to replace vitamins for neuroprotection of neurological disorders (Kelloff et al., 1996; Garcia-Alloza et al., 2007). In terms of mechanisms, a CL ethanolic extract appeared to reduce oxidative damage and exhibited antioxidative action via potent scavengers of hydroxyl radicals that promoted neurodegenerative processes (Sreejayan and Rao, 1994; Cohly et al., 1998; Barclay et al., 2000; Lim et al., 2001).

The initial phase of research on neuronal death had a major focus on microglial involvement. Microglial activation has been associated with many neurodegenerative mechanisms (Jebelli et al., 2014; Wada et al., 2001). However, in recent decades, additional evidence has also been obtained from astroglial studies. Neuron-astrocyte mal-interaction was found to underlie pathology of Parkinson's disease (Kordower et al., 1999) or Alzheimer's disease (Colombo et al., 2002; Heneka and O'Banion, 2007). A link between astrocytes and neurodegenerative diseases has gained considerable interest. However, both neuroprotective and detrimental roles were reported in terms of critical roles for neuronal survival (Rappold and Tieu, 2010). This suggests that the functional role of the astrocytes remains largely inconclusive.

In the present experiments, long term pretreatment with a C. longa Linn. ethanolic extract was hypothesized to attenuate dexamethasone-induced neuronal and astrocytic damage in the hippocampus of rats. The numbers of neurons and astrocytes in CA1, CA2, CA3 and DG of the hippocampus were counted in histological studies using Nissl staining and glial fibrillary acidic protein immunoreactivity (GFAP-ir), respectively.

6.2 วัตถุประสงค์

- to investigate neurodegenerative parameters from neuronal and glial cell damage induced by prolonged treatment of dexamethasone
- to test the effects of CL extract pretreatment on neuronal and glial cell damage induced by prolonged treatment of dexamethasone
- to investigate EEG pattern of neurodegeneration induced by prolonged treatment of dexamethasone

6.3 สรุป (สรุปผลการทดลองทั้งหมดของงานวิจัยทั้งชุดโครงการ/โครงการ ทั้งตีพิมพ์แล้วและยังไม่ได้ตีพิมพ์)

Conclusion of published data

Long term exposure to dexamethasone decreased the densities of both neurons and astrocytes in the hippocampus. The CL ethanolic extract exhibited a protective effect against the dexamethasone-induced loss of neurons but not the astrocytes. These findings indicate that histological methods can effectively provide scientific evidence of neurodegenerative processes and treatment mechanisms.

Conclusion of the unpublished manuscript

Taken together, the present data demonstrated that change in slow frequency oscillation appeared to be the first warning signal. Therefore, REM suppression and high-voltage sleep spindles may follow at later stages. Monitoring of slow frequency EEG powers especially that of Theta wave at the early stage would offer predictive value for early detection of neurodegenerative diseases. Moreover, these findings also suggest that this animal model of neurodegeneration has high validity that may be useful as a model for testing novel neurodegenerative disease-modifying drugs or substances.

6.4 เอกสารอ้างอิง (กรณีที่ไม่มีใน Reprint หรือ Proceeding ตามที่แนบในภาคผนวกข้อ 7.1)

ไม่มี

7. ภาคผนวก

7.1 แนบสำเนาบทความที่ได้รับการตีพิมพ์แล้ว (Reprint)

- สำเนาบทความที่ตีพิมพ์ใน Acta Histochemica ซึ่งเป็นวารสารในฐาน ISI ที่มี impact factor 1.360 (ดังเอกสารแนบ)

7.2 ผลการวิจัยส่วนที่ยังไม่ได้ตีพิมพ์หรือตีพิมพ์ไม่ได้ แต่อยู่ในวัตถุประสงค์ของโครงการวิจัย ประกอบด้วย

7.2.1 Materials and methods

Animal model

Adult male Wistar rats (weighing 300-350 g) were obtained from Southern Laboratory Animal Facility, Prince of Songkla University, Thailand. The rats were maintained at 22 °C with a 12/12 dark/light cycle (light on at 07:00 am). Standard commercial food pellets and filtered tap water were available ad libitum. The experimental protocols described in this study were approved and guided by the Animal Ethics Committee of the Prince of Songkla University. Surgical procedure was done by following the previous study (Cheaha et al., 2014). Briefly, animals were anesthetized with intramuscular injection of 60 mg/kg Zoletil® 100 (Virbac, Thailand Co. Ltd.). Stainless steel screw electrodes were implanted on the skull over the frontal cortex (AP; +3, ML; 3) and the parietal cortex (AP; -4, ML; 4) on the left side skull. The reference and ground electrodes were placed at midline above the cerebellum. Bipolar wire electrodes were inserted into the dorsal neck muscles for electromyography (EMG). All electrodes were secured in place with acrylic resin (Unifast trad, Japan). Rats were randomly divided as control and dexamethasone groups for 21-day treatment. Control rats received 0.9% saline (i.p.) whereas treated rats received 0.5 mg/kg dexamethasone (i.p.).

Dexamethasone preparation

Dexon® (General Drugs House co., Ltd.) was diluted in 0.9% saline for the final concentration (0.5 mg/ml). Both control and dexamethasone groups were injected with a volume of 1ml/kg.

EEG recording

The process of recording was performed as previously described (Cheaha, Sawangjaroen, and Kumarnsit, 2014). EEG signals of individual rats in a recording chamber were recorded for 2-h period through recording cables. EEG signals were amplified with a low-pass 60 Hz, high-pass 0.1s and digitized at 400 Hz by a PowerLab/4SP system (AD Instruments) with 12-bit A/D, and stored in a PC through the Chart program software. The EEG signals were processed through 1.25 – 45 Hz band pass filter. The digitized EEG data were segmented into 1024-point (50% overlap) and the signals were converted to power spectra by the FFT algorithm (Hanning window cosine transform). Then, the power spectra of 2.56-sec sweeps of selected period were averaged to create the power spectra of the period. In each power spectrum, values were divided into 5 frequency bands: Delta, 1.25-4.5 Hz; Theta, 4.75-6.75 Hz; Alpha, 7-12.5 Hz; Beta, 12.75-30 Hz; and Gamma, 30.25-45 Hz. EEG powers in each frequency bands of each group were averaged and expressed as power (μV2) and power density (μV2/Hz).

Analysis of sleep-wake cycle

EEG signals from the frontal and parietal cortices and EMG signal from the dorsal neck muscles were used to determine sleep-wake states of animals. Awake periods (AW) were identified with fast wave, low amplitude EEG and the presence of high EMG activity (Fig. 1A). The interchange among different sleep-wake periods can be displayed in spectrograms for visual inspection (Fig. 1B). Slow wave sleeps (SWS) or non-rapid eye movement sleeps (non-REM sleep) were identified with slow wave and high amplitude EEG. REM sleeps were detected with fast wave, low amplitude EEG and the absence EMG activity. Specific characteristics of EEG power spectrum were examined by using FFT for quantitative data (Fig. 1C). Awake signals were identified with peaks at theta wave for frontal and parietal EEG. On the other hand, SWSs were recognized with peaks specifically within delta range for both frontal and parietal EEG. The most unique characteristic was detected during REM sleep when parietal EEG exhibited dominant theta peak whereas frontal EEG had relatively overall low power.

Data analysis

Power density (μV2/Hz) of frontal and parietal EEG during baseline and post-treatment period were calculated. Baseline values were set to 100% and changes of post-treatment value were documented in percent of baseline values. For spindle investigation, frontal EEG was scanned through 6-10 and 10-14 Hz filters for high-voltage spindles and regular sleep spindles, respectively. Numbers of the occurrence of spindles were counted from loops or peaks of EEG power density of filtered signal.

All data were expressed as mean ± S.E.M.). Effects of treatment were determined by using one-way ANOVA and followed by student Newman Keul for multiple comparison. t-test was used

for the analysis of two-set data. *, ** and *** indicate p<0.05, 0.01 and 0.001, respectively, for statistically significant differences.

7.2.2 ผลการทดลองและวิจารณ์

Effects of dexamethasone on overall EEG power spectrum EEG powers of animals were analyzed from 2-hr records on day 1, 6, 11, 16 and 21 following the start of dexamethasone treatment. Broad spectrum of EEG power was divided into 5 frequency bands: Delta, Theta, Alpha, Beta and Gamma. Therefore, values of different time points were analyzed for each frequency range. All data were normalized with baseline values obtained before the start of dexamethasone treatment to express as percent baseline (% baseline). Data were shown in comparison to that of controls. Frontal EEG analysis revealed no significant change induced by the dexamethasone in any duration of any frequency band (Fig 2A). However, the analysis of parietal EEG signals indicated that dexamethasone significantly increased powers of slow waves which included Delta, Theta and Alpha bands (Fig. 2B). Multiple comparisons indicated that significant changes were observed only on day 21 for delta band. In theta band, significant changes were confirmed as early as day 11 and until day 21. Alpha powers were also significantly increased on day 16 until day 21.

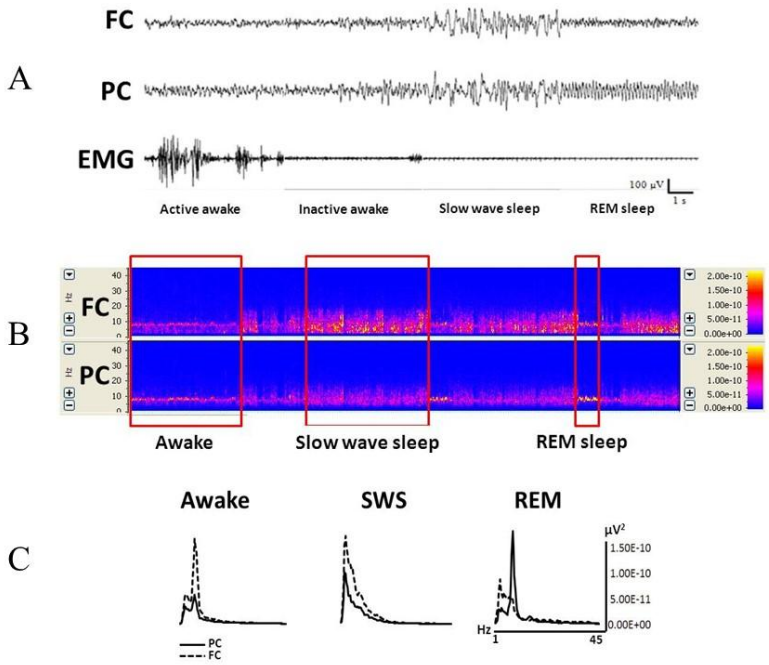

Fig. 1 Determination of sleep-wake states from EEG signals. Raw EEG and EMG signals were used to identify different brain states; active awake, inactive awake, slow wave sleep and REM sleep periods (A). The spectrograms displaying spectral power are expressed and referenced with color codes of frequency (Y axis) in time domain (X axis) (B). Each sleep-wake state was confirmed with specific characteristic of EEG power densities (C).

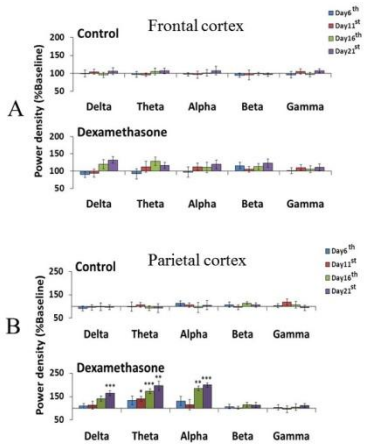

Fig.2 Long term effects of dexamethasone on EEG spectral powers of the frontal **(A)** and parietal **(B)** cortices. Baseline activities were evaluated from 2-hr recordings on the first day before drug administration. After the administration, EEG was recorded for 2 hrs. in each assigned day. EEG power spectra were divided into 5 frequency ranges: Delta, Theta, Alpha, Beta and Gamma. Data are expressed as mean ± S.E.M. of power density (%baseline). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared with control values.

Effects of dexamethasone on sleep-wake patterns

Sleep-wake patterns were analyzed from EEG signals recorded following a 21-day period of dexamethasone treatment. Hypnograms were created to display a visual presentation of sleep-wake cycles in time domain (Fig. 3A). Time fragments of each parameter during 2-hr period recording were summed. Total times spent for awake periods, SWS and REM sleep were analyzed and expressed in percent total. The results showed that prolonged exposure to dexamethasone significantly decreased percentage of REM sleep but not AW or SWS (Fig. 3B). The analysis of sleep latency also revealed no significant change induced by dexamethasone.

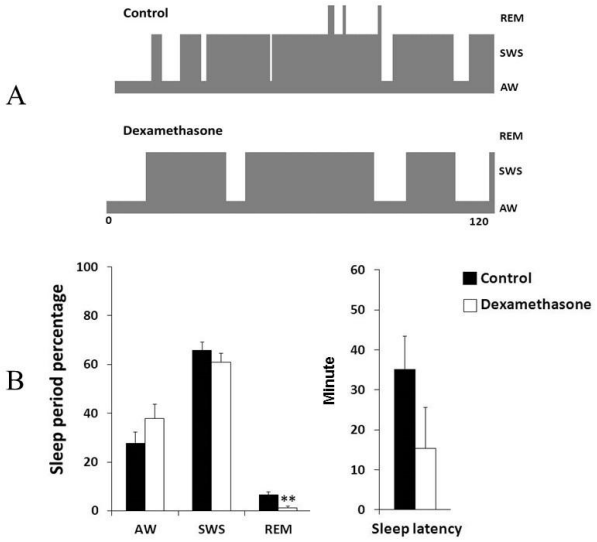

Fig. 3 Effects of long term dexamethasone on sleep-wake cycle. Hypnograms of representative rats from control and dexamethasone groups were created to show the dynamic presentation of sleep-wake states **(A)**. Mean of time spent in each brain states and sleep latency are shown **(B)**. Sleep-wake data were analyzed from EEG signals recorded for 2 hrs. on day 21 of the treatment. ** $p<0.01$ compared with control levels.

Effects of dexamethasone on EEG power spectrum during awake and slow wave sleep periods

EEG power was separately analyzed with regard of specific awake and SWS periods. Signals were taken from the first 5-min recording of the first appearance of each brain states after dexamethasone treatment for 21 days. EEG power analysis was performed for 5 frequency ranges (Fig 4A-E). Awake EEG analysis confirmed that dexamethasone significantly induced increases in power of slow frequency bands which included Delta, Theta and Alpha activity for parietal EEG and only Alpha activity for frontal EEG (Fig. 5). However, the analysis of EEG signals during SWS revealed the only significant change seen within Beta band for both frontal and parietal EEG.

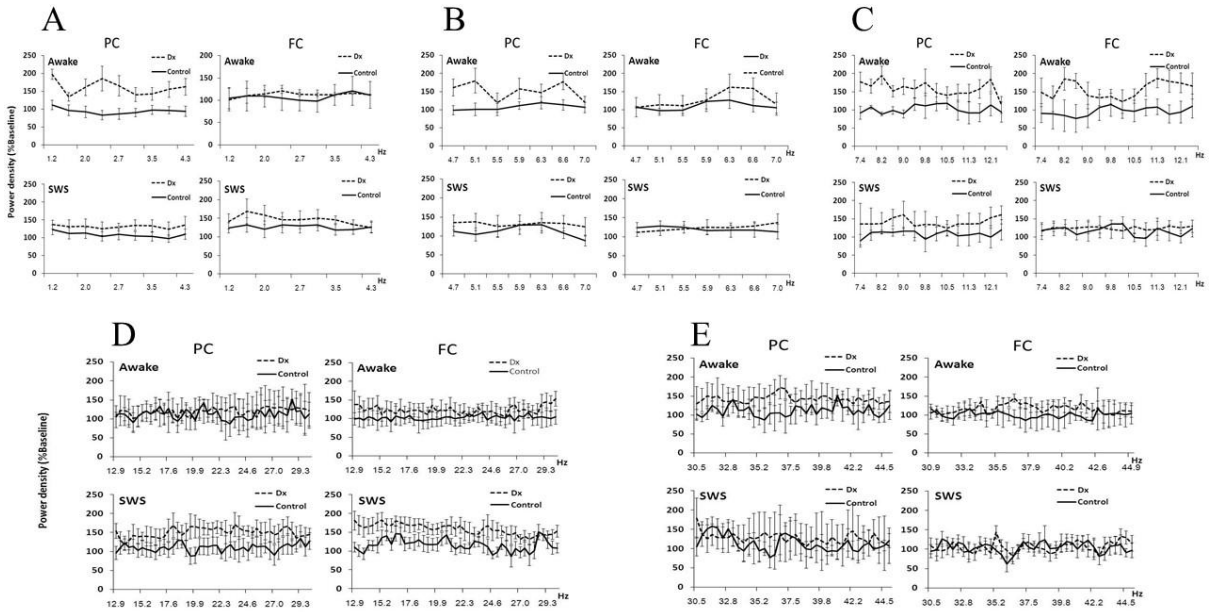

Fig. 4 Long term effects of dexamethasone on power spectra of Delta (A), Theta (B), Alpha (C), Beta (D) and Gamma (E) activities. EEG power activities were analyzed from parietal EEG (PC) and frontal EEG (FC) on day 21 of dexamethasone treatment. Data were taken from awake and slow wave sleep (SWS) 5-minute continuous periods.

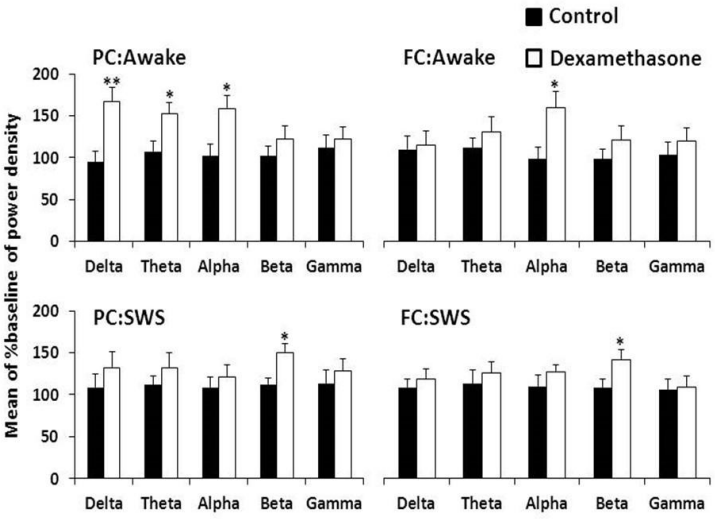

Fig. 5 Effects of long term dexamethasone treatment on mean power densities of each frequency waves during awake and SWS states. EEG signals from the frontal and the parietal cortices were analyzed. Data are expressed as mean ± S.E.M. of power density (% baseline). *p<0.05, **p<0.01 compared with control values.

Effects of dexamethasone on EEG spindles

The distribution of spindles was observed from raw EEG signals. Types of EEG spindle were characterized by power spectrum analysis of frontal EEG signals using FFT. Raw EEG signals were filtered for 6-10 and 10-14 Hz oscillations to extract EEG spindles and inspect their features. Two main types of spindles were characterized. Sleep spindles were recognized from frontal EEG with relatively low power when filtered for 10-14 Hz activity (Fig. 6). When filtered for 6-10 Hz activity, high-voltage spindles were detected. They exhibited high amplitude loops with peak frequency in the range. Therefore, numbers of both types of EEG spindle were counted from 2-hr period of EEG recordings after dexamethasone treatment for 21 days. The results showed that prolonged exposure to dexamethasone significantly increased number of high-voltage spindles during AW and SWS states (Fig. 7). No significant change was observed for normal sleep spindles.

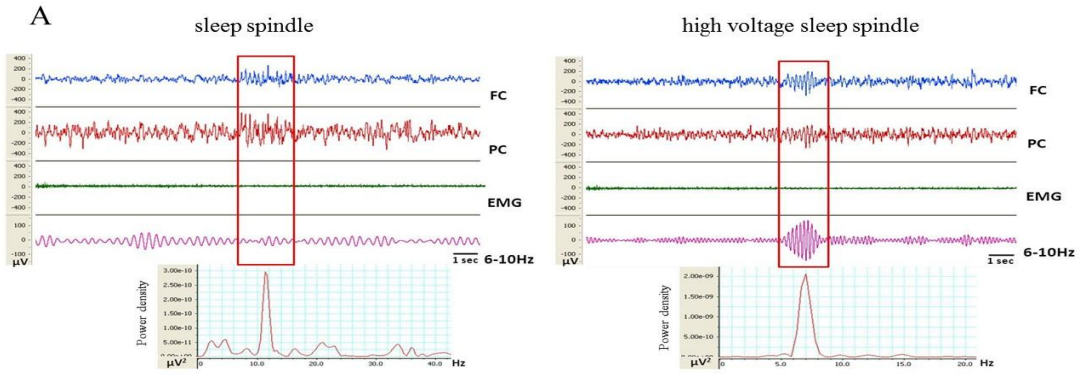

Fig. 6 Identification of regular and high-voltage sleep EEG spindles. EEG signals recorded on day 21 of the treatment were used. EEG spindles were divided into 2 types according to their features and EEG power densities: regular sleep spindle (10-14 Hz) and high-voltage sleep spindle (6-10 Hz).

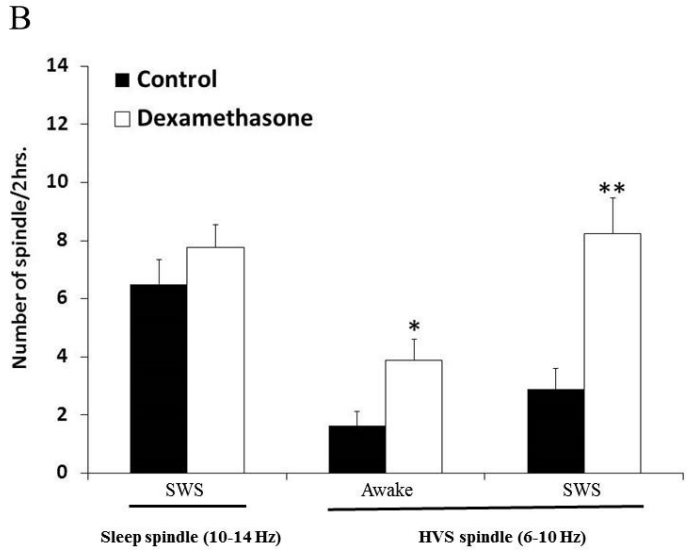

Fig. 7 Mean of numbers of spindles during slow wave sleep (SWS) and awake (AW) periods following a 21-day period of dexamethasone treatment. Data are expressed as mean ± S.E.M. of number of spindle (times/2hr.). *p<0.05, **p<0.01 compared with control group.

Correlations between types of spindle and EEG power spectra

Overall data were combined for the overview of these findings (Table 1). The spindle types and slow frequency waves appeared to be important factors for the discrimination between control and dexamethasone groups. Therefore, regression analyses between types of sleep spindle and EEG power of 3 slow frequency waves were performed. No significant correlation was seen for the normal sleep spindle. During awake period, number of high-voltage spindles was positively correlated with only Theta power. Greater relationship was seen when analyzed number of high-voltage spindles during SWS and EEG power of slow frequencies. Significant correlations were observed for all 3 frequencies analyzed. The distributions of slow frequency powers against numbers of high-voltage spindles completely distinguished values of control and dexamethasone groups (Fig. 8A-D).

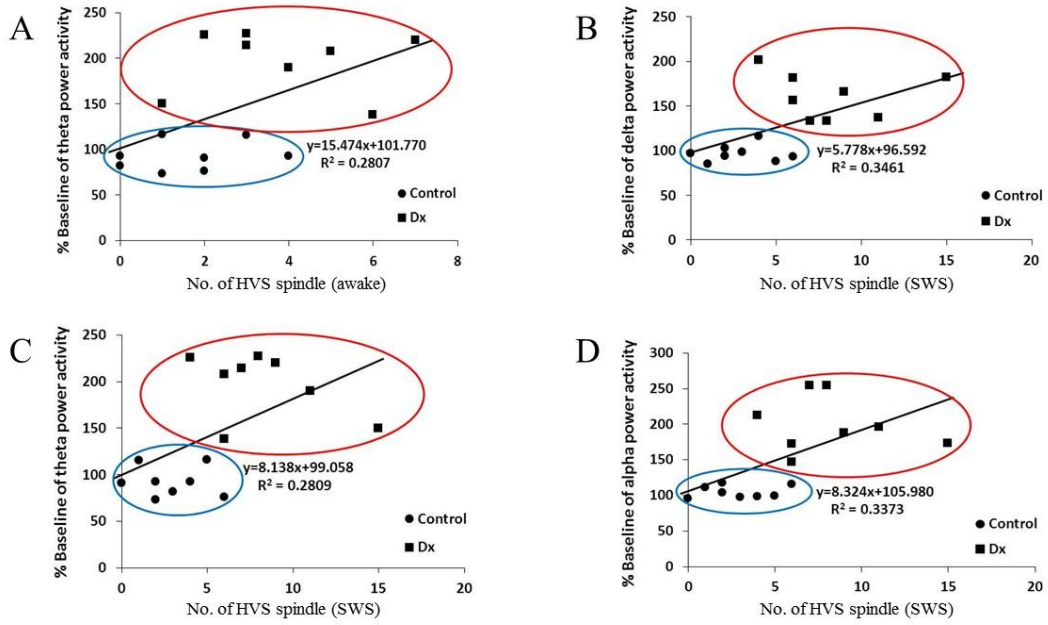

Fig. 8 Linear regression analyses of EEG powers and number of high-voltage sleep (HVS) spindles during different sleep-wake states: Theta vs HVS spindle during awake (A), Delta vs HVS spindle during SWS (B), Theta vs HVS spindle during SWS (C), and Alpha vs HVS spindle during SWS. Data were analyzed from EEG signals of control and dexamethasone treated rats. Correlations are expressed as R-square (R2) and linear equation.

Factor A	Factor B	R square values	*p* values
Sleep spindle (SWS)	Delta	0.1140	0.2009
	Theta	0.2294	0.0605
	Alpha	0.1022	0.2274
High-voltage sleep spindle (Awake)	Delta	0.2322	0.0588
	Theta	0.2807*	0.0348
	Alpha	0.1334	0.1642
High-voltage sleep spindle (SWS)	Delta	0.3461*	0.0165
	Theta	0.2808*	0.0347
	Alpha	0.3373*	0.0183

Table 1 R-square data of correlation between types of spindle and slow wave oscillations. *$p<0.05$.

7.2.2 Discussion

The present findings demonstrated that the analyses of EEG spectral powers and sleep components provided differential parameters between control and dexamethasone treated rats. Longitudinal study of EEG also allowed for the detection of changes in slow wave as early as day 11 of dexamethasone treatment. These findings may be among the highlights of EEG study when repeated measures are possible and convenient. The suppression of REM sleep and the detection of high-voltage spindles induced by long term treatment of dexamethasone also clearly distinguished electrical brain signals between groups.

Dexamethasone is a synthetic steroid with high glucocorticoid activity. It is widely used according to its anti-inflammatory effects (Choksi et al., 2013;Leggas et al., 2009). However, its use has been linked with mechanisms that increase oxidative stress through long-lasting upregulation of reactive oxygen species (Kraaij et al., 2011;Schafer et al., 2005). Down-regulations of genes involved in the mitochondrial respiratory chain and those that encode antioxidant enzymes were induced by dexamethasone (Mutsaers and Tofighi, 2012). Therefore, oxidative stress induced by dexamethasone has been extensively used as a model for evaluation of protective effects of novel therapeutic substances (Gao et al., 2010;Hu et al., 2009).

The increases in slow wave activity has been found as typical findings in neurodegenerative disease (Coben et al., 1983;Kwak, 2006). Global cortical dysfunction was also associated with increased Theta activity in EEG spectra (Helkala et al., 1996). Significant increase in Delta power was observed in later stages of deterioration in Alzheimer's disease (Prichep et al., 1994). Neuronal loss is proposed to underlie the increased slow wave oscillation. In urethane-anesthetized rats, high dose of cholinergic agonist or cholinesterase inhibitor were demonstrated to decrease slow wave activity including low frequency power (Toth et al., 2012). Basically, acetylcholine is synthesized mainly in the nucleus basalis of Meynert and basal forebrain as the major sources of cholinergic afferents terminating in many brain regions including the areas involved in learning and memory processes and the neocortex that would impact neocortical EEG as well as sleep-wake stages (Selden et al., 1998;Semba, 2000). Moreover, increased Delta and Theta amplitudes in rats were induced by lesioning of the nucleus basalis and, therefore, reversed by anticholinesterase and pilocarpine (Riekkinen, Jr. et al., 1991). The elevated acetylcholine release is associated with low voltage fast oscillation during wakefulness and REM sleep, while its reduced release is found during SWS when delta waves dominate the EEG (Kanai and Szerb, 1965;Celesia and Jasper, 1966). Degeneration of cholinergic basal forebrain was commonly found in Alzheimer's diseases (Grothe et al., 2014). Moreover, monoamines also have a role in these actions when combined cholinergic-monoaminergic stimulation exhibited stronger effects in reversing learning impairments and EEG slowing than cholinergic enhancement alone in a rat model (Dringenberg, 2000). EEG analysis in Alzheimer's patients also indicated slowed mean frequency activity and reduced interplay among cortical regions (Jeong, 2004). Previously, the losses of neurons and glial cells in brain regions of the hippocampus were seen following long term dexamethasone treatment (Issuriya et al., 2014).

These consistent reports suggest possible links between the elevated slow wave EEG and degeneration of neuronal substrates that may also underlie neuropathology induced by long term dexamethasone treatment.

In addition to EEG abnormalities, sleep disturbances have also been widely reported in cases of neurodegenerative diseases (Gagnon et al., 2002;Jackson and Snyder, 2008). Mostly, sleep disruption is detected before the onset of relevant neuropsychological impairments (Tranah et al., 2011;Ju et al., 2013). In Alzheimer's disease, sleep disturbances are positively correlated with amyloid deposition (Ju, McLeland, Toedebusch, Xiong, Fagan, Duntley, Morris, and Holtzman, 2013). In particular, REM sleep deprivation was hypothesized to be linked with impairment in

learning recent information (Christos, 1993). A decrease in REM sleep was among changes in parameters found in mild cognitive impairment subjects and to a greater extent in Alzheimer's patients (Maestri et al., 2015). In animal models of Alzheimer's disease, a reduction of REM and changes in EEG spectra were observed earlier than the occurrence of behavioral changes (Schneider et al., 2014). It was hypothesized that loss of REM in patients and mouse models is associated with a decrease in cholinergic tone. Therefore, donepezil, a cholinesterase inhibitor, was demonstrated to restore some normal sleeps in animals (Wisor et al., 2005) and Alzheimer's patients (Mizuno et al., 2004).

The present study analyzed features of spindle. 6-10 Hz and 10-14 Hz EEG oscillations were particularly investigated. Significant increases were observed for 6-10 Hz but not 10-14 Hz activity. Previously, relatively similar sleep spindles to 6-10 Hz EEG oscillation have been characterized in rats (Marini et al., 2008). Therefore, this type of electrical activity has been known as high-voltage spindle with the increase in relative power seen in rats with dopamine depletion (Ge et al., 2012) or systemic blockade of dopamine D2-like receptor (Yang et al., 2013). In a rat model of Parkinson's disease induced by 6-hydroxydopamine lesioning, a high frequency stimulation of the sub-thalamic nucleus, the brain area in cortical-basal ganglia loop, was found to restore the high-voltage spindles and motor deficits to normal states (Yang et al., 2015).
In terms of mechanisms, long term treatment of dexamethasone might be proposed to affect at least cholinergic and dopaminergic groups of neuron in the brain. Glucocorticoid receptors are present on dopaminergic cells (Harfstrand et al., 1986) and found to mediate some essential processes of cholinergic neurons in the brain (Guijarro et al., 2006). The prolonged dexamethasone administration is likely to give excessive stimulation to these neuronal cells through glucocorticoid receptors and ultimately cause cell death and neurodegenerative symptoms that might mimic those induced by oxidative stress.

Finally, the present study pointed out several correlations between EEG power spectra and high-voltage sleep spindles in a rat model. These findings highlighted some of the most relevant parameters for possible application in clinical levels. In this longitudinal monitoring of EEG signals, the analysis of slow frequency would allow for early detection of neurodegenerative signs. Though, the reduction of REM sleep was also obvious and consistent to those previous reports but, however, it became evidenced relatively late when most of symptoms already emerged. Anyway, it is of particularly interest that the excessive high-voltage sleep spindles especially during SWS was strongly correlated with increased Theta oscillation.

7.2.3 Conclusions

Taken together, the present data demonstrated that change in slow frequency oscillation appeared to be the first warning signal. Therefore, REM suppression and high-voltage sleep spindles may follow at later stages. Monitoring of slow frequency EEG powers especially that of Theta wave at the early stage would offer predictive value for early detection of neurodegenerative diseases. Moreover, these findings also suggest that this animal model of

neurodegeneration has high validity that may be useful as a model for testing novel neurodegenerative disease-modifying drugs or substances.

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7.3 ข้อคิดเห็นและข้อเสนอแนะส าหรับการวิจัยต่อไป

Further studies might be performed to address neural signaling of neurodegenerative pathology. Animals are used for intracranial electrode implantation in order to record local field potential directly from relevant brain regions as targets of the pathology. Experimental designs are made specific as models of Parkinson’s or Alzheimer’s diseases.

7.4 บทความวิจัยที่นำเสนอที่ประชุมวิชาการ (Proceeding) (ถ้ามี)

(ดังเอกสารแนบที่เป็น reprint)

หมายเหตุ: ข้อ 7.1 ขอให้ระบุฐานข้อมูลของวารสารที่ตีพิมพ์ตามเงื่อนไขที่มหาวิทยาลัยกำหนด (ISI หรือ SCOPUS หรือ อื่น ๆ)