中 國 醫 藥 大 學

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碩士學位論文

全期睡眠剝奪對傷害性刺激及脊髓 c-fos 表現之影響

Effects of Total Sleep Deprivation on Nociceptive Response and Spinal c-fos Expression in Rats

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中 文 摘 要

背景

目前已知睡眠剝奪會影響許多正常的生理變化，如：免疫反應、傷口癒合、體溫及體重變化，其中包括了疼痛感知。睡眠主要包含了兩大部分：快速動眼期（rapid eye movement）及非快速動眼期（non-rapid eye movement），而前人的實驗多著重在快速動眼期睡眠剝奪對生理的影響。

研究目的

本實驗乃採取前瞻性之單盲隨機設計，以三十六隻250-350公克大白鼠分別來研究全期睡眠剝奪影響傷害感受的機轉。福馬林測試（formalin test）可量化測量長期疼痛刺激的行為反應，故較為近似真實臨床下的疼痛傷害。本實驗之目的在比較睡眠剝奪及允許自由睡眠之大白鼠在皮下注射福馬林後行為反應及脊髓背角c-fos活動表現上的差異性。

研究方法

本計劃把三十六隻大白鼠隨機分成 C1、C2、C3、D1、D2 及 D3 六組。先讓老鼠適應環境三天，再分別讓 D1、D2 及 D3 組大白鼠睡眠剝奪 1、2 及 3 天後給予福馬林測試。C1、C2、C3 組大白鼠，則在適應期後另加 1、2 及 3 天自由睡眠才進行測試。在測試後一小時內記錄大白鼠對福馬林刺激之疼痛行為反應。並在犧牲大白鼠後，記錄脊髓背角 c-fos 之活動表現。在完成資料蒐集後，分析比較各相關資料。

實驗結果

C1、C2、C3、D1、D2 及 D3 六組之大白鼠，對施打福馬林後，在疼痛行為反應上並無明顯的差異。然而脊髓背角 c-fos 之活動表現（number of c-fos-like immunoreactive neurons）却在睡眠剝奪 2 及 3 天後較對照組有明顯增加現象。此

增加現象遍佈於脊髓背角的 Superficial (I-II), Nucleus Proprius (III-IV) 及 Neck (V) lamina 內。

結論

全期睡眠剝奪對化學性疼痛刺激臨床上幾無影響，但經 48、72 小時全期睡眠剝奪後，大白鼠之脊髓背角 c-fos 之活動表現有明顯增加現象。此一結果說明相較於快速動眼期睡眠剝奪，全期睡眠剝奪對疼痛行為之影響恐較為複雜。

英文摘要

Background

Sleep deprivation has been reported to be related with many physiological changes, including compromise immune function, poor wound healing, decrease in body temperature and loss of body weight. Sleep consists of two major parts, rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. Previous studies focus mostly on the interaction of REM sleep deprivation and pain sensitivity; studies on total sleep deprivation were rare.

Aim of the Study

The aim of this study is to assess the effect of total sleep deviation on the reaction of rats subject to noxious stimuli and to determine the possible mechanism that how total sleep deprivation influences nociception.

Materials and Methods

Thirty six rats were randomly allocated into six groups—group C1, C2, C3, D1, D2 and D3. Formalin test was applied to Group D1, D2 and D3 rats on the morning of the second, third and forth day after confinement. Group C1, C2, and C3 rats had the test immediately after their adaptation period. Immediately after formalin injection, behavior reaction was recorded for an hour. The rats were sacrificed and c-fos immunoreactivity of the spinal cord was examined. Data were compared and analyzed to see whether behavior reaction and c-fos immunoreactivity of the spinal cord in rats after subcutaneous formalin injection were different between groups.

Results

For the chemical noxious stimuli, groups of total sleep deprivation of 1, 2

and 3 days and their corresponding control groups do not respond differently to formalin injection. However, c-fos response (numbers of c-fos-like immunoreactive neurons) to formalin injection was significantly greater in 2 and 3 days of total sleep deprivation compared with liberal sleep environment. This increase was observed in the Superficial (I-II), Nucleus Proprius (III-IV) and Neck (V) lamina of the dorsal horn.

Conclusion

Total sleep deprivation of 48 and 72 hours induces a significant increase in c-fos immuno-reactive neurons in the dorsal horns of rats. However, the pain related behaviors induced by the formalin test do not show a clinical difference between the sleep deprived groups and adequate sleep groups.

關鍵詞

睡眠剝奪；大白鼠；傷害感受；福馬林測試；c-fos 細胞致癌基因。

Sleep deprivation; Rats; Nociception; Formalin test; c-fos.

序文及謝辭

首先感謝麻醉部吳世銓部長從我住院醫師時代以來的教學與訓練。十分有幸在取得專科醫師資格後，繼續在研究所接受吳部長的指導。對於資質駑鈍的我，吳主任總是有耐心的諄諄善誘、耐心教學孜孜不倦，令人如沐春風。另外要感謝動物實驗室黃九珍博士與技術員瓊昭小姐，在實驗過程及操作上的大力幫忙。最後要感謝一直在背後默默支持我的妻子，與在就學期間出生的長子，是你們讓我有繼續下去的勇氣與力量！！

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論文正文

第一章 前言

第一節 研究背景

目前已知睡眠剝奪會影響許多正常的生理變化，如：免疫反應、傷口癒合、體溫上升及體重減輕等種種變化，其中也包括了疼痛感知。長期睡眠剝奪睡眠甚至會造成死亡。睡眠主要包含了兩大部分：快速動眼期（rapid eye movement）及非快速動眼期（non-rapid eye movement），不同的睡眠階段，代表了不同的生心理機轉的修復。而前人的實驗多著重在快速動眼期睡眠剝奪對生理的影響，關於疼痛的研究也是如此。

Raymond等人發現在燒傷的病人身上可以使用其睡眠情形來預測其隔日疼痛程度。Lentz的團隊也報告在不影響總睡眠時間下，連續兩天剝奪受試者的慢波睡眠(slow-wave sleep deprivation) 會下降受試者的機械性疼痛閾值。Onen等人也利用大白鼠實驗證實，在連續三天的快速動眼期睡眠剝奪後，大白鼠對熱刺激及機械性刺激的耐受力會下降。然而在大老鼠身上對於全期睡眠剝奪與疼痛感知的文獻探討則十分缺乏。一直到近二十年，由於研究技術的進步讓我們有適當的動物模型可用來探討全期睡眠剝奪與疼痛感知的交互影響。

Formalin測試則提供一中度且連續性的疼痛刺激，目前已經被廣泛使用於疼痛及止痛藥物的相關研究中。

c-fos為一原細胞致癌基因，在受到外在刺激時引發其大量表現，形成Fos核蛋白，進而引發一連串的下游反應。Hunt團隊已發現在接受到包含熱、機械性或化學性惡性刺激（noxious stimulation）時，c-fos會在脊髓表現。因此其活動度可用來與傳統行為學疼痛測驗交互運用，為探討疼痛的方法中一理想之工具。

第二節 研究目的

本實驗乃採取前瞻性之單盲隨機設計，分別以三十六隻250-350公克大白鼠來研究全期睡眠剝奪影響傷害感受的機轉。而福馬林測試（formalin test）可量化測量長期疼痛刺激的行為反應，近似真實臨床下的疼痛傷害。本實驗之目的在比較睡眠剝奪及允許自由睡眠大白鼠在皮下注射福馬林後行為反應及脊髓背角c-fos活動表現上的差異性。

第二章 研究方法

第一節 研究材料與設計

本計劃把三十六隻大白鼠隨機分成 C1、C2、C3、D1、D2 及 D3 六組。先讓老鼠適應環境三天。再分別讓 D1、D2 及 D3 組大白鼠睡眠剝奪 1、2 及 3 天後給予福馬林測試。C1、C2、C3 組大白鼠則在適應期後另加 1、2 及 3 天自由睡眠才進行測試。在測試後一小時內記錄大白鼠對福馬林疼痛之行為反應。並在犧牲後記錄脊髓背角 c-fos 之活動表現。在完成資料蒐集後，分析比較各相關資料。

第二節 統計方法

資料將會以平均值±標準誤來呈現。行為學變化及脊髓的 c-fos 免疫反應則利用變異數分析佐以 Dunnett’s test，來做統計分析。以 $p<0.05$ 視為統計學上有意義。

第三章 研究結果

第一節 描述性統計分析

實驗結果發現 C1、C2、C3、D1、D2 及 D3 六組之大白鼠於施打福馬林之後，在疼痛相關行為表現出典型的雙相反應(biphasic pattern)。可是在睡眠剝奪組與非睡眠剝奪組之間，疼痛相關行為統計上並無明顯的差異。然而脊髓背角 c-fos 之活動表現（number of c-fos-like immunoreactive neurons）在睡眠剝奪 2 及 3 天之組別較對照組有明顯增加現象。此增加現象遍佈於脊髓背角的 Superficial (I-II), Nucleus Proprius (III-IV) 及 Neck (V) lamina 內。

第二節 推論性統計分析

實驗結果發現大白鼠在全期睡眠剝奪 48、72 小時後分別較自由睡眠組脊髓背角 c-fos 之活動明顯增加，表示在細胞層級全期睡眠剝奪會增加傷害刺激(nociceptive stimuli)之反應，然而在行為學上卻無相對應表現。

第四章 討論

第一節 結果討論

睡眠剝奪會造成嚴重的生理變化，甚至死亡。對身在加護病房或疼痛情況下的病人，其止痛藥的需求量可能會有不同。本實驗乃採取前瞻性之單盲隨機設計，以大白鼠來研究全期睡眠剝奪影響傷害感受的機轉，及其影響疼痛閾值之可能途徑。

實驗結果顯示在睡眠剝奪與非睡眠剝奪組間，行為統計上並無明顯的差異。然而脊髓背角 c-fos 之活動表現却在睡眠剝奪 2 及 3 天後較對照組有明顯增加之現象，暗示了全期睡眠剝奪對化學性疼痛閾質之影響，需到 48 小時以上，才有變化。

且近來研究顯示，脊髓中 microglia 的活性及其相關之 cytokines，有影響到疼痛行為的表現。其中一調節機轉乃是經由 p38 mitogen-activated protein kinase 之路徑，而其下游反應物即包含了 c-fos。故睡眠剝奪所導致之疼痛行為與脊髓背角 c-fos 之表現，可能與脊髓中 microglia 有關。

第二節 研究限制

在實驗中大白鼠脊髓背角 c-fos 之活動表現在睡眠剝奪 2 及 3 天後較對照組有明顯增加現象。然而施打福馬林後，在睡眠剝奪與非睡眠剝奪組間，行為統計上並無明顯的差異。對與此二種不同的疼痛反應，我們目前尚無適當解釋。

第五章 結論及建議

第一節 結論

全期睡眠剝奪對化學性疼痛刺激臨床上幾無影響，但經 48、72 小時全期睡眠剝奪後，大白鼠之脊髓背角 c-fos 之活動表現有明顯增加現象。此一結果說明相較於快速動眼期睡眠剝奪，全期睡眠剝奪對疼痛行為之影響恐較為複雜。

第二節 建議

未來可用更多疼痛檢驗方式來探討關於全期睡眠剝奪對疼痛之影響。而釐清 c-fos 基因之上下游調節表現機轉，對未來治療疼痛應有重大助益。

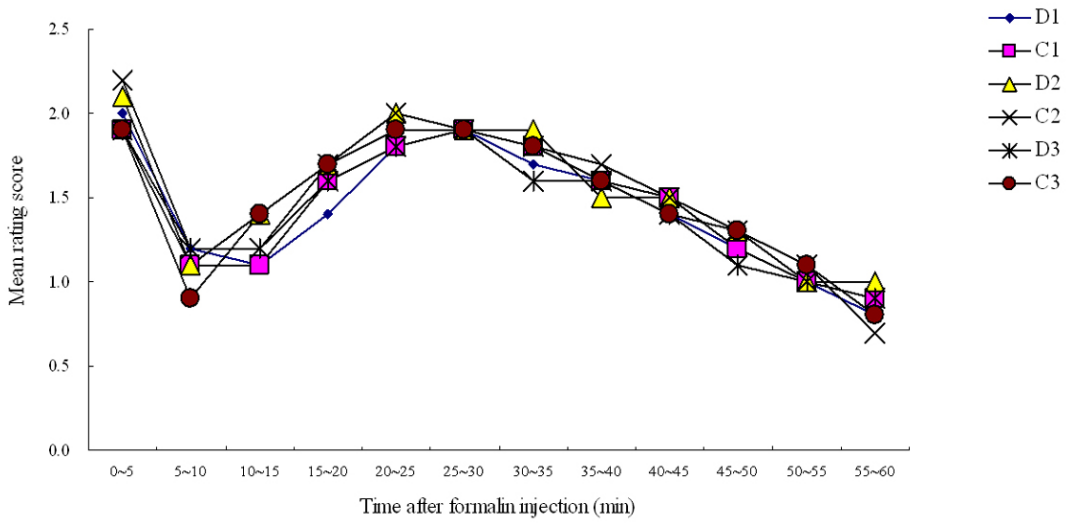

Figure 1. Time course of behavior response to hind paw formalin injection. Each data point represents the mean rating score of a particular time period. D1, D2 and D3 represented data from rats in sleep deprivation enviroment for 24h, 48h and 72 h respectively. C1, C2, and C3 represented data from rats in adequate sleep environments for 24h, 48h and 72 h, respectively.

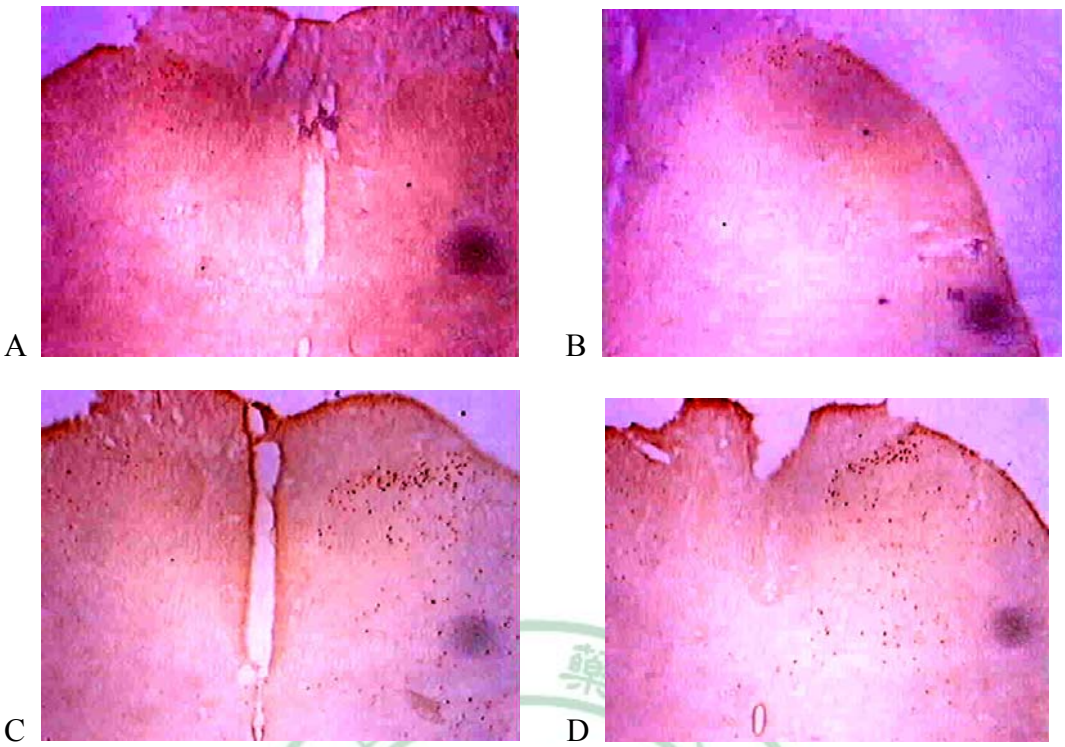

Figure 2. Appearance of spinal cord slice illustrating fos-like immunoreactivity at the L4-5 spinal segment ipsilateral to the hind paw injection of formalin. Four experimental situations are represented: control (A), total sleep deprivation for 24 hours (B), total sleep deprivation for 48 hours (C), and total sleep deprivation for 72 hours (D). The number of c-fos-like immunoreactive neurons increased significantly in the dorsal horns of rats that had total sleep deprivation for 48 h and 72 h.

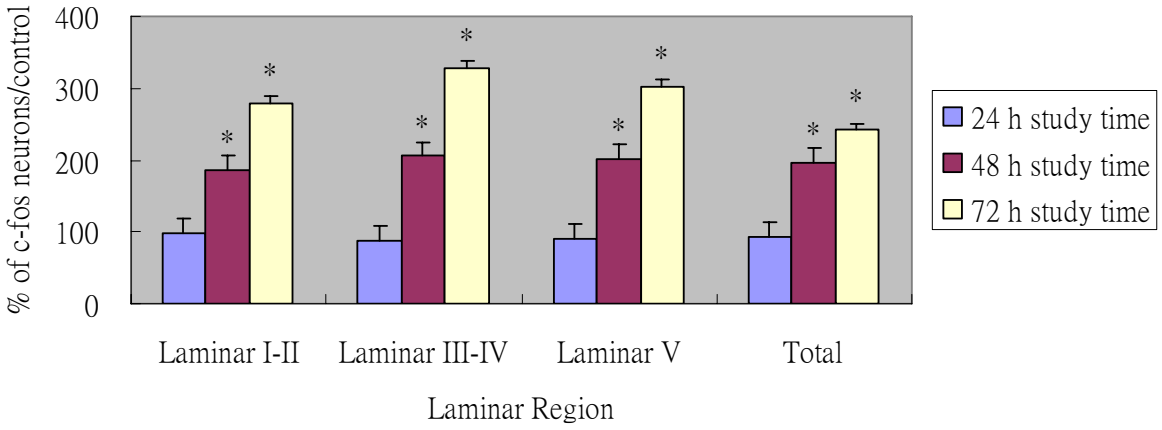

Figure 3. Effects of 24 h, 48 h, and 72 h of sleep deprivation on the expression of c-fos neurons in each laminar region (mean ± SEM percentage control). Ratio of c-fos neurons in sleep deprivation group to control group plotted at each lamina: Laminar I-II = Superficial Lamina, Laminar III-IV = Nucleus Proprius, Laminar V = Neck of the Dorsal Horn. * represented $p<0.01$ compared with control.

Time	Sleep Deprivation (number of positive c-fos neuron per section)				Adequate Sleep (number of positive c-fos neuron per section)			
	Lamina I-II	Lamina III-IV	Lamina V	Total	Lamina I-II	Lamina III-IV	Lamina V	Total
24 h	13.4±3.6	7.8±2.2	9.6±2.3	10.3±1.6	13.5±3.6	8.8±2.5	10.7±3.0	11.0±1.7
48 h	52.8±7.3*	33.0±4.8*	42.4±4.5*	43.0±3.6*	28.3±2.8	16.0±1.8	21.5±2.0	22.0±1.7
72 h	49.5±2.8*	29.5±2.2*	37.1±2.5*	38.7±2.4*	20.8±2.2	11.7±1.4	15.6±2.0	16.0±1.4

Table 1. Number of c-fos-like immunoreactive neurons was significantly increased in the dorsal horns of rats that had total sleep deprivation for 48 h and 72 h compared with adequate sleep environment. This increase was observed in the Superficial (I-II), Nucleus Proprius (III-IV) and Neck (V) lamina of the dorsal horn. * represents $p < 0.05$ compared with control

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附錄一 英文部分

Title: Effects of Total Sleep Deprivation on Nociceptive Response and Spinal c-fos Expression in Rats

Background

The relation between pain and sleep disturbance is a challenging issue. Pain and sleep disturbance affect each other. Understanding the interaction between the two may provide better strategies for pain management. Morin et al.[1] described that reported pain intensity is affected by frequency of sleep disturbances. Raymond et al.[2] found that the quality of night sleep in adult burn patients can significantly predict pain intensity on the following day. It has been reported that an increase of alpha waves during non-rapid eye movement (NREM) sleeps is associated with the decrease of the pain threshold in fibromyalgia patients.[3,4]

However, the findings of pain sensitivity after sleep deprivation in human studies were not in consistence. Lentz et al reported a decreased mechanical pain threshold after slow-wave sleep deprivation over two nights.[5] Onen et al published the result that mechanical pain tolerance thresholds were decreased after 40-hour total sleep deprivation in healthy adults.[6] In contrast, Arima et al reported that there were no effects after slow-wave sleep deprivation on pain thresholds in healthy patients with the regional pain of temporomandibular disorder.[7]

In rats, majority of experiment data are from studies of rapid eye movement (REM) sleep deprivation and their conclusions are much more consistent than those obtained from human. Under REM sleep deprivation in rats, nociceptive sensitivity to mechanical,[8] and electrical stimuli was increased.[9,10] REM sleep deprivation for 3 consecutive days in Wistar rats decreased thresholds to thermal and mechanical

stimuli but not to chemical stimuli (formalin test).[11] Thus all REM sleep deprivation studies in rats consistently suggested that REM sleep deprivation decreases nociceptive threshold. However, the data on whether total sleep deprivation or deprivation of other stages would affect nociceptive sensitivity are rare.

It is known that pathophysiologies of total sleep deprivation was difficult to apprehend and it was only in the past 2 decades with the successful development of animal models for total sleep deprivation that has permitted us to know the condition better.[12] Formalin test is a quantitative method for assessing pain and analgesia in rats and is increasingly used as a model of injury-produced pain.[13,14] It involves moderate, continuous pain generated by injured tissue and resemble pain as it is seen in actual disease states which could provide more information.

C-fos, an immediate early gene, is rapidly and transiently expressed in neurons in response to stimulation. Since the report by Hunt et al,[15] there have been many studies showing that various types of noxious stimulation, including thermal, mechanical and chemical stimuli, induce expression of c-fos in the brain and spinal cord.

Using spinal cord c-fos immunoreactivity combined with formalin test could quantitate responses to pain in related to conditions that may affect or treat pain and therefore is an appropriate model to study pain.[16-18]

Aim of the Study

The aim of this study is to assess the effect of total sleep deviation on the reaction of rats subjected to noxious stimulus and to determine the possible mechanism that how total sleep deprivation influences nociception.

Materials and Methods

Preparation of Animals

With the approval of the Animal Use and Research Committee of the China Medical University Hospital, 36 Sprague-Dawley rats weighing 250-350 g were used for the study. Initially, each rat was anesthetized with intra-peritoneal ketamine (70 mg/kg) and xylazine (6 mg/kg). Six stainless steel screws (Small Parts Inc., Miami Lakes, FL) were implanted through the skull to serve as dural electroencephalographic electrodes. At the conclusion of surgery the rat was placed into its original cage to restore for 7 days. After 7 days of restoration the rats were placed into the sleep deprivation apparatus for 3 days in order to adapt to the laboratory condition before the test.

Sleep deprivation apparatus

Total sleep deprivation was achieved by using the dish-over-water (DOW) method with the modified Rechtschaffen apparatus. The apparatus consisted of two plastic cages, each 60 cm (l) x 20 cm (w) x 60 cm (h), placed side by side, for the housing of two rats. A 40 cm diameter smooth circular plastic disc with its center in the alley between the cages and protruding 15.5 cm under each cage to provide a partial floor with approximately the same area as a home cage (492 cm^2) constitutes the ground the rats could stay. Beneath each side of the disc and extending beyond it to the walls of each cage was a tray of 2-3 cm deep water. The underside of the disc was attached to a computer control motor which can rotate the disc on demand. Cage temperature was regulated at 24-26 ºC; pan water was 3-4 degrees lower. Drinking water and food was available ad libitum from bottles and feeders hanging on the side of the side walls of the alley. The cage was maintained on a 12-hour light and dark cycle (lights on at 08:00 am).

Sleep deprivation procedure

At the start of sleep deprivation, the electroencephalographic electrodes were attached to a computerized MP 150 (BIOPAC System, Inc.). Upon detecting a sleep state, the computer started the motor beneath the disc to rotate the disc in a counterclockwise direction at a slow rotation rate of 3 rev/min. As the disc was rotating, the rats were disturbed and had to walk opposite to the direction of rotation in order not to fall into the water (rats are hydrophobic). A special board was used over the rotary disc on the side where the control rat resided so that the disc movement would not disturb the rat on it. The whole design is such that both rats were subjected to the same laboratory condition except that the rats on the covered disc will be able to sleep, whereas rats on the rotary disc would not.

Study design

The different experiments were performed to assess the sensitivity to chemical stimulus. To minimize changes caused by circadian rhythm,[19] all sensitivity to noxious stimuli measurements were performed in the morning between 7:00 a.m. and 12:00 noon. In each experiment, the investigator was blinded to the type of sleep manipulation the rat had experienced.

Assessment of nociception

For chemical noxious stimuli, thirty six rats were randomly allocated into 6 groups—group C1, C2, C3, D1, D2 and D3. After 3 days of adaptation period, rats from Group D1, D2 and D3 were subjected to 1, 2 and 3 days of sleep deprivation respectively. Group C1, C2, C3 rats acted as control and were given a condition that allowed adequate sleep for 1, 2 and 3 days respectively. Formalin test was applied to Group D1, D2 and D3 rats on the morning of the 2nd, 3rd, and 4th day after confinement. Group C1, C2, C3 rats had the test on the morning immediately after the corresponding sleep period.

All rats were placed in an observation chamber for 15 minutes before the formalin injection. The chamber was a 30 x30 x 30 cm Plexiglas box with a mirror below the floor at a 45 ºangle to allow an unobstructed view of the paws.

Formalin test began with a subcutaneous injection of 50µl of sterile 5% formalin into the rat's plantar surface of the left hind paw using a 1 ml syringe with a 26-G needle. The fluid was delivered as rapidly as possible while the rats were immobilized. Immediately after formalin injection, effects of formalin on overt behavior were assessed by observing spontaneous flinching and licking in rats for 60 min. Measurement of the behavior reaction was based on a scoring system described by Dubuisson and Dennis.[13, 21] The system divided behavior reaction into 4 categories: 0 = the injected paw was not favored (i.e. foot flat on floor with toes splayed); 1 = the injected paw has little or no weight on it with no toe splaying; 2 = the injected paw is elevated and the heel is not in contact with any surface; 3 = the injected paw is licked, bitten or shaken. The amount of time spent in each of the four behavior categories was recorded and calculated every minute by an anesthesiologist blinded to the treatment. At every 5 minutes interval an average score was calculated and recorded.

Results are expressed as mean ± standard error of the mean (SEM) occurring in a given time period.

Within one hour after behavioral testing, rats were deeply anesthetized with ketamine (40~80 mg/kg body weight, i.p.) perfused intracardially with heparinized saline (1-2 min) followed by freshly prepared 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4, Sigma). The complete spinal cord segments from L3-L6, where majority of sciatic afferent are, were removed. The segment was post-fixed in the same fixative for 12 h at 4 degree C and then transferred to a PBS containing sucrose (15-20%). The next day spinal cord segments were serially sectioned at 36-um thickness in a transverse plane with a freezing microtome. The sections were stained using a standard avidin-biotin-peroxidase complex (ABC) technique. Free-floating sections were incubated overnight in the primary rabbit antiserum directed against c-fos protein (Oncogene Res. Products) diluted 1:10,000 in PBS. After several washes in PBS the sections were incubated in biotinylated goat antirabbit IgG, diluted 1:200 in PBS and 3% normal goat serum for 2 h, rinsed in PBS and reacted with avidin-biotin reagents 1:100, for 1 h at room temperature. After three washes in Tri-buffered saline (TBS), sections were developed in diaminobenzidine tetrahydrochloride solution containing H2O2 in TBS. When a light background appeared, the reaction was stopped by four washes with PBS. Sections were then washed in distilled water, mounted on slides, air-dried, dehydrated through graded ethanol solutions followed by xylene and then coverslipped with Permount. The rats were sacrificed and c-fos immunoreactivity of the spinal cord was examined. Density of the labeled cells in the lumbar spinal laminae, conventionally divided into three groups (I-II, III-IV and V), was evaluated by a researcher unaware of the animal group assignment. Cell counts were made in all the processed section of each spinal

cord at a final x40 magnification. The number of Fos positive cells of each processed section of each spinal cord was divided by the number of counted tissue sections, in order to evaluate the average number of labeled cells for each rat.

Statistical analysis

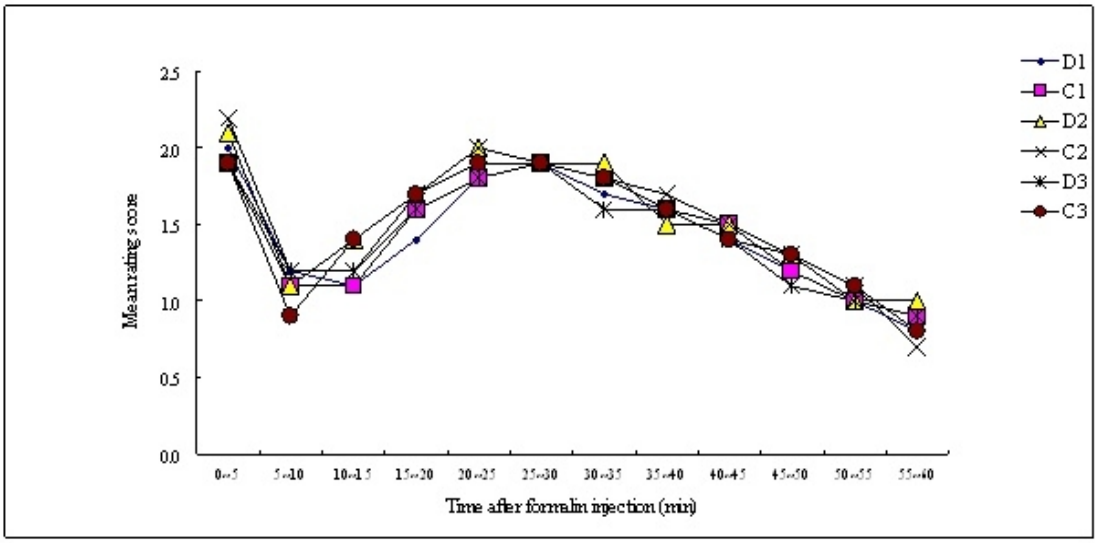
Data are presented as mean ± standard error of the mean (S.E.M.). The data for behavior reaction and c-fos immunoreactivity of the spinal cord of rats in the formalin test were analyzed and compared between groups (control rats and total sleep deprived rats) by ANOVA followed by Dunnett's test. $P<0.05$ was regarded as significant for all comparison.

Results

Nociceptive behaviors

Recording of pain related behaviors (licking, clutching, elevating, shaking or biting) was visually measured at 5-min intervals for 60 min post-injection in all rats. The data clearly demonstrated a classic formalin test biphasic response (Figure 1). There was no significant difference in the mean duration of phase 1 and phase 2 nociceptive behaviors between Group C1, C2, C3, D1, D2, and D3 rats.

Figure 1. Time course of behavior response to hind paw formalin injection. Each data point represents the mean rating score of a particular time period. D1, D2 and D3 represented data from rats in sleep deprivation enviroment for 24h, 48h and 72 h respectively. C1, C2, and C3 represented data from rats in adequate sleep environments for 24h, 48h and 72 h, respectively.



c-fos immunoreactivity

The intensity of c-fos immune reactivity significantly increased in the dorsal horns of rats that had total sleep deprivation for 48 h and 72 h compared to the rats in adequate sleep environment (Figure 2). This increase was observed in the Superficial (lamina I-II), Nucleus Proprius (lamina III-IV) and Neck (lamina V) of the dorsal horn at the L4-5 spinal segment ipsilateral to the hind paw injection of formalin. (Table 1 & Figure 3)

Figure 2. Appearance of spinal cord slice illustrating fos-like immunoreactivity at the L4-5 spinal segment ipsilateral to the hind paw injection of formalin. Four experimental situations are represented: control (A), total sleep deprivation for 24 hours (B), total sleep deprivation for 48 hours (C), and total sleep deprivation for 72 hours (D). The number of c-fos-like immunoreactive neurons increased significantly in the dorsal horns of rats that had total sleep deprivation for 48 h and 72 h.

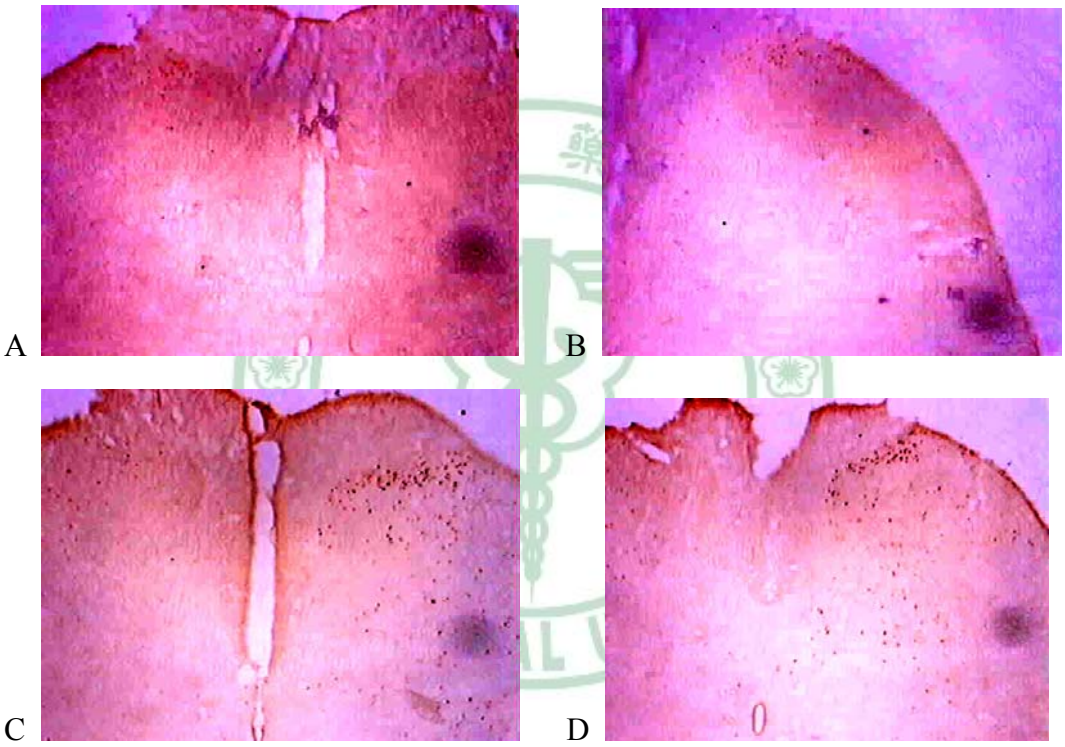


Figure 3. Effects of 24 h, 48 h, and 72 h of sleep deprivation on the expression of c-fos neurons in each laminar region (mean ± SEM percentage control). Ratio of c-fos neurons in sleep deprivation group to control group plotted at each lamina: Laminar I-II = Superficial Lamina, Laminar III-IV = Nucleus Proprius, Laminar V = Neck of the Dorsal Horn. * represented $p<0.01$ compared with control.

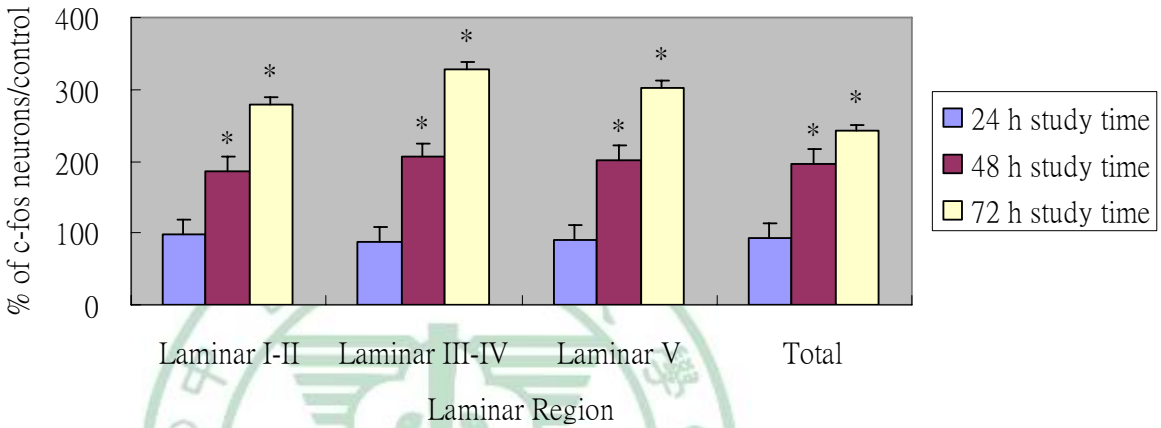


Table 1. Number of c-fos-like immunoreactive neurons was significantly increased in the dorsal horns of rats that had total sleep deprivation for 48 h and 72 h compared with adequate sleep environment. This increase was observed in the Superficial (I-II), Nucleus Proprius (III-IV) and Neck (V) lamina of the dorsal horn.* represents $p < 0.05$ compared with control.

Time	Sleep Deprivation (number of positive c-fos neuron per section)				Adequate Sleep (number of positive c-fos neuron per section)			
	Lamina I-II	Lamina III-IV	Lamina V	Total	Lamina I-II	Lamina III-IV	Lamina V	Total
24 h	13.4±3.6	7.8±2.2	9.6±2.3	10.3±1.6	13.5±3.6	8.8±2.5	10.7±3.0	11.0±1.7
48 h	52.8±7.3*	33.0±4.8*	42.4±4.5*	43.0±3.6*	28.3±2.8	16.0±1.8	21.5±2.0	22.0±1.7
72 h	49.5±2.8*	29.5±2.2*	37.1±2.5*	38.7±2.4*	20.8±2.2	11.7±1.4	15.6±2.0	16.0±1.4

Discussion

Total sleep deprivation of 48 and 72 hours induces a significant increase in c-fos immuno-reactive neurons in the dorsal horns of rats. However, the formalin test induced pain related behaviors do not show a clinical difference between the sleep deprived groups and adequate sleep groups.

Sleep consists of two major parts: REM sleep and NREM sleep, and every stage in sleep has different physiological functions. Sleep deprivation may induce enormous physiological changes such as disturbed liver functions, serum lipid levels and hyperphosphatemia,[23] alteration in endocrine and hormonal functions,[24,25] leukocytosis and increases in natural killer activity.[26] Sleep deprivation could even lead to death as a result of deterioration of body tissue and increase in the ratio of catabolism to anabolism.[27] Frequently, there are patients who need to receive anesthesia or in pain conditions suffering previous night/s sleeplessness. Patients from the ICU with no sedation during their previous ICU stay and patients who are too anxious or too painful to be able to sleep are among the many examples.[28] As sleep deprivation generates enormous physiological changes,[29] it will be valuable to know how it may influence anesthesia and the management of pain.

Previous studies focus mostly on the interaction of REM sleep deprivation and pain sensitivity; the studies on total sleep deprivation were rare. With a blind randomized controlled design we performed this experiment.

Our result presented a biphasic pattern of pain related behaviors that are usually found in formalin test.[14] The early phase seems to be caused predominantly by C-fibre activation due to the peripheral stimulus, while the late phase appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord.[31] The rating score does

not change significantly after sleep deprivation groups compared to the adequate sleep groups. However, the number of c-fos-like immuno-reactive neurons was significantly increased in the dorsal horns of rats that had total sleep deprivation. This augmentation was observed in the superficial (I-II), nucleus proprius (III-IV) and deep (V) lamina of the dorsal horn. That finding suggested total sleep deprivation affects nociceptive perception in a more complex manner than REM sleep deprivation. The proto-oncogene c-fos when activated makes the immunologically detectable nuclear protein Fos that are in the coresponding locations for nociceptive somatic sensory integration.[32] Thus, expression of Fos or c-fos indicates populations of neurons activated or excited by nociceptive input.[33] Recent studies showed that activation of p38 mitogen-activated protein kinase in spinal microglia is a critical link in inflammation-induced spinal pain processing.[34] Therefore, the increasing expression of c-fos immuno-reactive neurons in the dorsal horn after total sleep deprivation suggests that microglia might play a role in the upstream pathway of sleep deprivation induced hyperalgesia.

Our finding is important as the effects of total sleep deprivation on pain threshold in rats were poorly investigated before. However, we do not have enough information in the literature to explain why formalin test showed no significant difference but on the other hand increased the number of c-fos positive neurons. Further investigation to study the pathway which regulates c-fos expression in the future may be necessary.

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