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Effect of Sleep Deprivation on Wound Healing in Mice

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Summary : *In current literature there is little information about how sleep or sleep deprivation affects wound healing. With this study, we aimed to determine the effects of sleep and sleep deprivation on wound healing and wished our results to be considered in clinical practice. In this study, we used 4 week-old male mice as experimental animals and divided them into experimental and control groups. During the experiments, the animals were housed individually under the same standard conditions. We made full-thickness skin incisions on the dorsum of both groups of mice. The control group mice were allowed to have their normal sleep, while the mice in the experimental group were kept awake continuously for five days using a special apparatus designed in our laboratory. At the end of the 5th day, we sacrificed the mice by administering an overdose of thiopental sodium and tested the wound tensile strength with a computerized tensiometer and the skin thickness of wound edges by compass. We found that wound tensile strength and skin thickness of sleep-deprived group were less than that of controls. According to our data, sleep deprivation retarded wound healing. In addition we found a considerable weight loss in the sleep-deprived group in accord with the literature.*

Key words: *sleep deprivation, wound healing, mouse, tensile strength.*

Uyku Yoksunluğunun Farede Yara İyileşmesine Etkisi

Özet : *Uyku yada uyku yoksunluğunun yara iyileşmesini nasıl etkilediğine dair literatürde mevcut bilgi yetersizdir. Bu çalışma ile, uyku ve uyku yoksunluğunun yara iyileşmesine olan etkilerini ölçmeyi ve bu sonuçların klinikte dikkate alınmasını hedefledik. Bu çalışmada deney hayvanı olarak kullanılan 4 haftalık fareler deney ve kontrol grubu olmak üzere ikiye ayrıldı. Deney süresince, deney hayvanları aynı standart şartlar altında ayrı ayrı olarak muhafaza edildi. Her iki gruptaki farelerin sırtlarına tam-kesi sırt insizyonları yapıldı. Kontrol grubundaki deney hayvanlarının normal uykularını almalarına izin verilirken, deney grubundaki fareler laboratuvarımızda dizayn edilen özel cihaz yardımıyla 5 gün boyunca kesintisiz olarak uykudan yoksun bırakıldı. Beşinci günün sonunda aşırı doz sodyum pentotal ile hayvanlar feda edilerek, bilgisayarlı tensometre ile yara gerim dirençleri ve kompas ile de deri kalınlıkları ölçüldü. Uykudan yoksun bırakılan grupta yara gerim direncini kontrol grubuna göre daha düşük olarak tespit ettik. Bizim bulgularımıza göre uyku yoksunluğu yara iyileşmesini geciktirmektedir. Ek olarak, uykudan yoksun bırakılan grupta, literatürle uyumlu olarak, önemli bir kilo kaybı tespit ettik.*

Anahtar kelimeler: *uyku yoksunluğu, yara iyileşmesi, fare, yara gerimi.*

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INTRODUCTION

Why do we sleep? Why do we spend at least one third of lives doing something that provides most of us with only a few fleeting memories? It has been suggested that human sleep helps the re-programming of our complex computer-like brains, or that it permits some kind of emotional release in order to maintain our mental health. This is why many people are surprised to learn that virtually all mammals and birds sleep and that this sleep is much like ours; it is also characterized by high-amplitude, low frequency activity[1]. Even fish[2], reptiles[3], amphibians[4], and insects[5] go thorough periods of inactivity and unresponsiveness that are similar to mammalian sleep.

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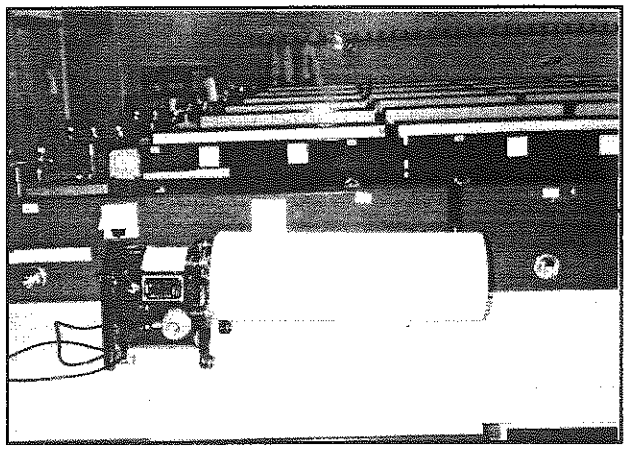
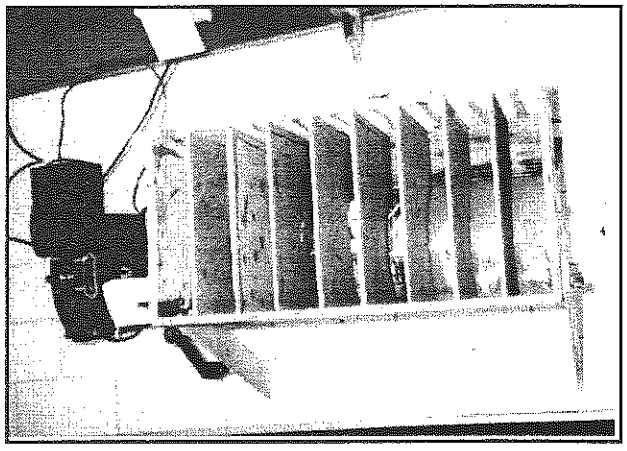
The fact that sleep is so common in the animal kingdom suggests that it serves a critical function, but there is no consensus on what this critical function is. There are two general theoretical approaches to the function of sleep (1): recuperation theories and circadian theories. The essence of the recuperation theories is that being awake disrupts the homeostasis of the body in some way and that sleep is required to restore it. In contrast, the circadian theories argue that sleep is not a response to internal imbalance. According to the circadian theories, a neural mechanism has evolved to encourage animals to sleep during those times of the day when they do not usually engage in activities necessary for their survival. The circadian theory views sleep as an instinct somewhat akin to the instinct to engage in sexual activity. In essence, recuperation theories view sleep as a nightly repairman who fixes damage produced by wakefulness, while the circadian theories regard sleep as a strict parent who demands inactivity because it keeps us out of trouble. According to the restorative theory, sleep restores the deficiencies in the body and mind caused by activity during wakefulness. If sleep has such a function, then it will probably play an important role in a process such as wound healing. There is no information on the extent that sleep or sleep deprivation affects wound healing. With this study, we aimed to determine the effects of sleep and sleep deprivation on wound healing.

METHODS

In our study, we used 4 week-old Swiss albino male mice. During the experiments, animals were housed individually in the same standard conditions and were maintained on a 12/12-hour dark/light cycle. Sixteen mice were randomly assigned to two equal groups (N=8). We made 2 cm length full-thickness skin incisions on the dorsum of all the mice. Before surgery, animals were anesthesized with Ketamin 75 mg/kg plus Rompun HCL 4 mg/kg. The skin wound was closed with silk stitches. All the mice in the control group, which were allowed to have their normal sleep, were kept in separate cages. The mice in both control and experimental groups were fed for 1 hour twice a day with standard mouse pellet and water. The mice in the experimental group were kept in the compartments of an electro-mechanical apparatus designed in our laboratory (Photograph 1 and 2). Each of the compartments in both experimental and control cages had similar volume of space. The sleep-deprivation-



Photograph 1: Cylinder connected to the electric motor.



Photograph 2: Sleep Deprivation Apparatus

apparatus is composed of a cylinder connected to an electric motor and has 9 compartments on the cylinder. The end of the compartments are covered by electric wires.. We applied 40 Volt 500 mA electric voltage to these wires. This voltage is sufficient to keep the mice awake but not to cause pain. If the mice do not move (if they sleep, of course they will not move), they slug to the electric wires, since the cylinder they stand on is turning continuously. In order not to touch the wires, each of the mice has to move in the opposite direction to the turning cylinder. An electronic device maintains the turning of the cylinder at 1 cycle per minute. Because of the low turning speed, it does not produce an exercise effect. Each mouse was fed outside the apparatus twice a day. In order to standardize the experimental conditions, we observed how many times the mice in the experiment group had contact with

electric wires, and we applied the same number and voltage of electric current to the mice in the control group. While the mice in the control group slept ad-lib, the mice in the experimental group were kept awake continuously by means of this special apparatus designed in our laboratory. Five days later, we sacrificed the mice with an overdose of thiopenthal sodium and measured the wound tensile strength with the Lloyd K model computerized tensometer. The computerized tensometer is composed of two units, an electro-hydraulic pump which applies a constant level of pressure to the ends of the device and a computer which detects the tensile strength on the ends of the machine. After we attached the skin samples to the ends of the computerized tensometer, constant pressure was applied and computer detected the pressure continuously. The value of the pressure at the moment the pressure decreased suddenly was considered the tensile strength value of that sample. Skin thickness of the wound edges was measured with a compass. Results were appraised with Student's t test.

RESULTS

Tensile Strength:

Results were shown in Table 1. Mean tensile strengths of the control and sleep deprived groups are 0,259 0,013 and 0,205 0,020 Newton\mm^2 respectively. This difference is statistically significant ($p<0,05$).

Skin Thickness:

Our results show that skin thickness in the sleep-deprived group is less than that of the control group (Table 1). In the sleep deprived group skin thickness was 0.34 ± 0,026 mm while this value was 0.49 ± 0,018 mm in the control group ($p<0.05$).

Table 1. Tensile Strength and Skin Thickness[a]

	n	Tensile Strength (Newton/mm^2)	n	Skin Thickness (mm)
Controls	8	0,259 ± 0,013 [b]	8	0,49 ± 0,018 [d]
Sleep Deprived	8	0,205 ± 0,020 [c]	8	0,34 ± 0,026 [e]

[a] Values are mean ± SEM

[b-c] Difference is significant ($p<0.05$)

[d-e] Difference is significant ($p<0.05$)

Body Weight Variances:

The mice in both groups were weighed before and after the experiment. At the end of the fifth day, while there was no significant difference in the control group; 23 % weight loss was found in the sleep-deprived group ($p<0,001$). Weights of mice in gram were shown in Table 2.

Table 2. Weights of mice before and after the experiment [a]

	n	Before experiment (gr)	n	After experiment (gr)
Controls	8	27,26 ± 1,24 [b]	8	29,08 ± 1,22 [c]
Sleep Deprived	8	28,85 ± 0,83 [d]	8	21,97 ± 0,85 [e]

[a] Values are mean ± SEM

[b-c] Difference is not significant ($p>0.05$)

[d-e] Difference is significant ($p<0.01$)

DISCUSSION

According to the Restorative Theory, sleep restores the deficiencies in body and mind, produced by wakefulness activity. If sleep has such an effect on body restoration, it might play an important role in such a process as wound healing which has both immunological and metabolic aspects. We made skin incisions on the dorsum of the mice. Five days later we found that wound healing was delayed in the experimental group forced to stay awake. We observed a 23 % weight loss in the sleep-deprived group at the end of the fifth day ($p<0,001$) in accord with the literature[6,7] while we could find no statistically significant difference in the control group. In order to standardize the experimental conditions, we observed how many times each of the mice in the experiment al group had contacted to electric wires, and we applied the same number and voltage of electric current to each of the mice in the control group. Thus we propose that the observed weight loss and wound healing retardation in the experiment group is caused by sleep deprivation per se and is not associated with stress induced by the experimental conditions. Several studies have examined various biochemical changes in humans during sleep loss. There is generally no significant change in cortisol (73 % of studies reported no change in humans), epinephrine and related compounds (80 % of human studies reported no differ-

ences) and catecholamine output (no difference in 83 % of human studies)[8]. These findings support our proposal.

It was shown that rats who were sleep-deprived for a long time developed a negative energy balance and showed some symptoms such as protein malnutrition secondary to increased energy consumption[9]. It was proposed that increased metabolism was the result of a thermoregulation mechanism, which works to prevent the lethal hypothermia[6]. We measured the tensile strength of wounds and the thickness of their edges. In the sleep deprived mice, both the tensile strength of wounds and the skin thickness were lower than those of controls. Decreased strength and thickness are possibly due to increased catabolic activity of sleep deprived metabolism because the body uses the fat tissue (also subcutaneous fat) for thermoregulation[10]. We believed that for this reason the subcutaneous fat tissue of mice in the sleep-deprived group would be thinner than that of the control group. In addition, hypothermia causes vasoconstriction of the skin vessel[10]; it is well known that vasoconstriction is a negative factor in wound healing since it decreases tissue perfusion[11,12]. It is possible that using energy and protein to produce heat, instead of for wound healing, will retard wound healing. In summary, we suggest that the reduction in tensile strength and skin thickness of mice in the sleep-deprived group is the result of a negative energy balance.

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