An analyis of the effect of paradoxical sleep deprivation on visuospatial component of working memory in wistar rats

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Abstract

Background: Working memory has two loops the phonological and visuospatial loops. Sleeps have a profound influence on these loops but the exact role played by REM and NREM sleep on working memory is controversial.

Aim: The present study focuses on the effect of paradoxical sleep deprivation on visuospatial component of working memory and also the gender wise difference in response to sleep deprivation.

Materials and Methods: Paradoxical sleep deprivation was done in wistar rats using flower pot technique. Before sleep deprivation the working memory was analyzed using radial maze. Sleep deprivation was done for 24, 48 and 72 hours and after each day working memory was analyzed.

Results: Statistical analysis was done using Paired T test and ANOVA. The maze running time decreased after 48 and 72 hrs of sleep deprivation while the number of wrong entries increased after sleep deprivation. On gender wise comparison, females were found to make more wrong entries with increased duration of sleep deprivation.

Discussion: Paradoxical sleep had increased influence on the visuospatial component of working memory and deprivation of this phase of sleep can result in wrong judgements and this effect was found to be more in females than males.

Keywords: Memory, Sleep deprivation, Flower pot technique, Maize, Gender differences.

Introduction

Memory is defined as the retention and storage of information. This process is highly dynamic and can be modified by various environmental factors. Working memory, which is a component of short term memory, maintains a limited amount of information for a brief period of time and this information is organized ,differentiated and used for future .Working memory involves two main components i.e. the phonological and visuospatial components. Phonological component deals with the auditory processing while the visuospatial is responsible for processing the visual information mainly the images and the location and placement of objects in space.[(1,2)] Research related to the organization of the memory process in humans is a burgeoning field and of the different factors influencing memory the role of sleep is always highlighted. Memories are believed to be processed and consolidated in sleep and there are considerable amount of experimental evidence proving this concept.[(3)]

The existence of two phases of sleep namely the non-rapid eye movement sleep (NREM) and the rapid eye movement sleep (REM) have been proved by researchers. The functions sub served by these two components of sleep is still controversial.[(4)] Lack of sleep is a common problem with severe consequences. Researchers have highlighted the role played by sleep in memory consolidation by noting the effects of sleep deprivation on the different aspects of memory. Studies related to sleep deprivation is mainly animal studies.[(5,6)] Experimental procedures resulting in total sleep deprivation and REM sleep deprivation have shown deleterious effects on memory and behavior. The organization of sleep-wake cycle in humans and rodents differ. Rodents spend 70 -80 % of the night in wakefulness and 70 to 80% of daytime sleeping but they do exhibit the NREM and REM pattern of sleep like humans. Experimental procedures of sleep deprivation, both in humans and animals have been widely employed to unveil the various aspects of sleep function, but the effect of duration of sleep deprivation on the visuospatial component of working memory is not studied in detail hence the present study was designed .

The present study focuses on the effect of paradoxical sleep deprivation on the visuospatial component of working memory and also the gender wise difference in response to sleep deprivation.

Materials and Methods

Six albino Wistar rats weighing 150- 200gms aged 8 – 10 weeks were used for the study. Animals were housed in metallic cages in the small animal research facility of Jubilee Mission Medical College and Research Center, Thrissur, Kerala, under standard conditions of temperature and light. They were maintained on a standard pellet diet and water ad libitum. Rats were kept exposed to the 12: 12 light dark cycle. This study protocol was approved by the Animal Ethics Committee.

Instruments Used: To analyze the visuospatial component of working memory the radial arm maze was used .it has a center platform with eight spokes radiating out from a central core. To test whether rats

remember which arms they have visited, a single food pellet is placed at the end of each arm and the rat is placed on the central platform. The rat visits each arm and eats the pellet. To successfully complete the maze, the rat must go down each arm only once. It must use short-term memory and spatial cues to remember which arms it has already visited. If a rat goes down an arm twice, this counts as an error. The rat's performance on the maze is considered a test of short-term memory.[7]

Sleep deprivation protocol: Flowerpot technique

Sleep deprivation was done in three male and three female albino Wistar rats. After initial assessment of memory, rats were introduced into separate sleep deprivation cages. Selective REM sleep deprivation can be achieved in rodents by means of "flower pot method". This technique allows the rats NREM sleep but it prevents the rats to enter into REM sleep. Flower pot technique sleep deprivation cages have (23 cm wide, 37 cm long and 30 cm deep) a central 14cm high and 6.5cm diameter platform. The bottom of the cage was filled with 22c water to within 2cm of the top of the platform. Cage closed by wire mesh lid with water which was easily accessible for the rats. The platform allows the animal to rest on it but as it enters into REM sleep the muscle tone decreases and the snout of the rat touches the water which wakes up the animal. Hence REM sleep was deprived.[8]

Study process

Working memory of all six rats was assessed by using the radial arm maze after giving them training in the maze. The rats were then housed in separate cages for sleep deprivation. They were made to run in the maze after 24, 48 and 72 hours of sleep deprivation subsequently. Time taken to complete all the four arms of the maze and the number of wrong entries made by each rat was tabulated.

Results

Time taken by the rats to cover all the eight arms of the maze was represented as mean and standard deviation .the number of wrong entries was represented as percentage .difference in maze running time and wrong entries between males and females were also represented as mean and standard deviation.

Statistical analysis was done using Paired T test and ANOVA. P value <0.05 was taken as significant.

Table 1: Effect on maze running time after time intervals of sleep deprivation

	Mean	N	Std. Deviation	P value
Initial. Time	6.4600	6	2.34250	.846
After 24 hours	6.1300	6	1.86207	
Initial. Time	6.4600	6	2.34250	.059
After.48hours	4.5267	6	1.02973	
Initial time	6.4600	6	2.34250	.102
After 72 hours	3.60667	6	1.951622	

None of the p values were found to be significant (pvalue>0.05).

Maze running time was found to decrease but not to a statistically significant level after 24, 48, and 72 hours of sleep deprivation

Table 2: Wrong entries after time intervals of sleep deprivation

	Mean	N	Std. deviation	P value
Wrong entries before sleep deprivation	3.0000	6	.00000	.175
Wrong entries after sleep deprivation of 24 hrs	3.6667	6	1.03280	
Wrong entries before sleep deprivation	3.0000	6	.00000	.272
Wrong entries after sleep deprivation of 48 hrs	4.1667	6	2.31661	
Wrong entries before sleep deprivation	3.0000	6	.00000	.027
Wrong entries after sleep deprivation of 72 hrs	5.1667	6	1.72240	

P value 0.027 was found to be significant while the other two were not.

Wrong entries increased after different durations of sleep deprivation. After 72 hours of deprivation it increased to a statistically significant level.

Table 3: Effect on maze running time after time intervals of sleep deprivation in male & female rats

		Mean	N	Std. Deviation	P value
Male	**Initial Time**	7.0000	3	1.14595	
	After.24hrs	6.2567	3	1.87767	.675
	After.48hrs	4.5033	3	.81193	.006*
	After.72hrs	1.91000	3	.399500	.011*
Female	**Initial Time**	5.9200	3	3.39563	
	After.24hrs	6.0033	3	2.25711	.982
	After.48hrs	4.5500	3	1.41067	.498
	After.72hrs	5.30333	3	.852311	.807

On comparing the maze running time of males and females .it was found that the maze running time decreased to a statistically significant level in males after 48 and 72 hours of deprivation while females did not show any statistically significant changes.

Table 4: Wrong entries after sleep deprivation

RAT .No.	Male				Female			
	Initial	24 hours	48hours	72hours	Initial	24 hours	48hours	72hours
1	3	3	3	5	3	5	8	6
2	3	5	2	5	3	3	2	7
3	3	3	5	2	3	3	5	6
Total	**9**	**11**	**10**	**12**	**9**	**11**	**15**	**19**
Wrong entries increased by (%)		**22.22**	**11.11**	**33.33**		**22.22**	**66.67**	**111.11**

The percentage of wrong entries was found to increase in both males and females but the changes noted in females were more in comparison to males.

Discussion

In the present study the effect of duration of sleep deprivation on the visuospatialcomponent of working memory was analyzed .The time taken to cover all the eight arms of the maze was initially taken. After24, 48 and 72 hours of paradoxical sleep deprivation the time taken to run through the eight arms was analyzed. It was found those24 hours of sleep deprivation did not alter the maze running time while 48 and 72 hours of sleep deprivation resulted in decrease in maze running time but not to a statistically significant level. This could be attributed to stress and hunger in the rats after sleep deprivation. Further it is already proved that stress results in stimulation of the sympathetic system resulting in restlessness and anxiety.[(9)]

On analyzing the multiple entries made into the same arms i.e the number of wrong entries done after each intervals of sleep deprivation .it was noted that the number of wrong entries increased with the duration of sleep deprivation and after 72 hours of sleep deprivation it was found that the number of wrong entries increased to a statistically significant level. This result highlights that REM sleep plays a very important role in working memory .the increase in the number of wrong entries further strengthen the hypothesis that sleep deprivation results in impaired judgment too. The interesting Fact to be noted is that 24 hours of sleep deprivation does not affect the judgment component and neither do they result in stress response in rats. There are experimental evidences supporting our observation.[**(10)**]

Stress assessment in rats following paradoxical sleep deprivation has shown increase in corticosterone levels which resulted in increase in recovery sleep. Sleep deprivation activates the hypothalamopituitary axis (HPA) resulting in increase in levels of corticosteroids which can modulate sleep.[**(11,12)**]

On comparing the response of male and female rats to sleep deprivation, it was noted that the male rats had statistically significant decreased maze running time following 48 and 72 hours of sleep deprivation while in the female rats though the time decreased it was not to a significant level .on comparing the wrong entries it was found that the number of wrong entries increased in both males and females with the increase in duration of sleep deprivation but the wrong entries were noted more in females. The decrease in maze running time observed by the male rats can be attributed to the increase in sympathetic activity in them but it was found that though they had increased stress response in comparison with the females, the male rats had performed better in the radial maze with less number of wrong entries. This proves that females are more susceptible to the deleterious effects of paradoxical sleep deprivation. This was in support to the observations of Hajali.V.[(13)] Females sleep patterns are affected by gonadal steroids while no such effect was noted in males.[(14)]

Hence the difference in response noted in the present study can be due to the basic difference in male and female sex steroids effect on sleep pattern. Though the restlessness and stress levels in females were less when compared to the male rats as shown by no statistically significant difference in maze running time after 24,48 and 72 hours of sleep deprivation ,the visuospatial component of working memory was found to be affected in females. The sex difference in sleep behavior needs to be investigated in detail.

Conclusion

Paradoxical sleep deprivation affects memory is a well-known fact but the extent to which sleep deprivation can be tolerated is not yet known. The present study tries to shed light on this aspect .the results of this study shows that 72 hours of paradoxical sleep deprivation has a significant effect on visuospatial component of working memory while 24 hours of deprivation has no effects. On analyzing the gender difference in response to sleep deprivation we have concluded that PSD has more deleterious effects in females as the basic sleep pattern of males and females are different based on the effect of sex steroids on sleep. Here again 72 hours of deprivation had more deleterious effect in comparison to 24 hours.

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