

New Insight for the Study of Mild Cognitive Impairment: The Novel Object Recognition Task and the Single Day Morris Water Maze in Total Sleep Deprived Rats

M. Pellitteri*, V. Colavito, E. Moscardo, F. Schio, B. Salvetti, M. Bentivoglio, P.F. Fabene, G. Bertini

Department of Neurological, Neuropsychological, Morphological and Motor Sciences, University of Verona, Italy.

**michele.pellitteri@univr.it*

Introduction

Mild cognitive impairment (MCI) is a brain disorder in which cognitive abilities are mildly impaired. Subjects with MCI are of major clinical importance because they have an increased risk of developing Alzheimer-type dementia. Appropriate animal models are necessary in order to understand the pathogenic mechanisms of MCI and develop drugs for its treatment. Although MCI is associated with a variety of symptoms, memory deficits are a dominant feature of the syndrome and is essential to select appropriately difficult behavioural tasks in order to point out the deficits can be associated with neuropathological alterations. We are all aware of the detrimental effects of sleep loss. Preventing or disrupting sleep after the acquisition of new information have been repeatedly shown to impair the recollection of memories. Few studies have investigated, however, the role of sleep prior to the learning experience in affecting subjects' ability to acquire new information. We examined the effect of 6h of total sleep deprivation (TSD) by gentle handling immediately before the learning process, in two different mnemonic tests: the novel object recognition task (NOR) and the single-day Morris-water-maze task (1d MWM).

Materials and Methods

We assessed recognition memory in Sprague-Dawley rats (4 months old) after 6hrs of total sleep deprivation by gentle-handling performed at 2 different periods of the resting phase, respectively during the first 6 hours (0-6h) and the last 6 hours (7-12h) after lights on. Both time points were tested in each of the subjects. Tests were carried out 3 weeks apart. Immediately after sleep deprivation, during the acquisition phase, animals were allowed to familiarize with 2 identical objects for 10 minutes in PhenoTyper cages (Noldus Information Technology, Wageningen, The Netherlands). To test recognition memory, after a 1h delay, animals were presented with 2 objects (recognition phase): one was identical to the previously presented objects (Familiar object), the other one was never seen before (Novel object). During the delay time animals were placed in their own home cages. Automated tracking was performed using the EthoVision[®] XT software (Noldus Information Technology, Wageningen, The Netherlands).

The distance moved by each animal in the cage (cm) and the duration (s) of when the nose of the animals was tracked within a 2 cm area around the objects were recorded. Recognition memory was inferred by comparing the time spent exploring the novel object vs. the familiar one, during the recognition session. Object type and position of the novel object were randomized.

Furthermore, we focused our studies evaluating the effects of TSD in a spatial learning task. A modified experimental classic protocol of the Morris water maze was adopted. A circular pool with a diameter of 130 cm and a height of 90 cm, filled with 20±1 °C water to a depth of 60 cm, was placed in a completely dark room. The maze was divided into four equal quadrants and release points were designed at quadrant as NW, SW and SE. A hidden circular platform (11 cm in diameter), was located in the centre of the NE quadrant, submerged 1.5 cm beneath the surface of the water. In order to increase the colour contrast between the water and the animal, a nontoxic paint was used to get the water darker. Fixed, extra maze visual cues were present at various locations around the maze. The task requires rats to swim to the hidden platform guided by distal cues. Immediately after 6h hours of total sleep deprivation (0-6h), rats were subjected to three blocks of 3 trials each, with an inter-trial time of 1h. Three different starting positions were equally distributed around the perimeter of maze and randomized within each block. After reaching the platform, rats were allowed to remain there for 30 s until the

start of the next trial. The animals were given a maximum of 120 s to find the platform; if they failed to find the platform in this time, they were placed by the experimenter on the platform and allowed to stay there for 30 s. After completion of each block of trials, the animals returned to their home cage. 1h after the end of the 3 blocks, an extra 120 sec trial (probe trial) was performed. Platform was removed and time spent in each quadrant was recorded. A camera was mounted above the center of the maze and automated tracking was performed using EthoVision® XT software. For all the trials, time (s) spent to find the platform (latency) and total travelled distance were measured. Moreover the time spent in each quadrants was recorded during the probe trial.

Results and Discussion

Regarding the NOR task, we found that locomotor activity was not affected by SD protocols. On the other hand, sleep deprivation impaired object recognition when it occurred during the first 6 hours of the light phase. When TSD was postponed to the last 6 hours of the light cycle, it had no effect on cognitive performance. Control rats behaved similarly both when the test was administered in the light and in the dark phase.

In the spatial learning task (1d MWM), learning occurred in both animal groups; animals during the probe trial spend majority of the time swimming within the quadrant where the platform was previously placed. The mostly interesting point is that total sleep deprived animals didn't learn the position of the hidden platform as fast as the controls animals.

These findings show that preventing the initiation of sleep at the end of a normal period of activity (i.e., when homeostatic sleep pressure is high) impairs subsequent learning. 1) TSD has been proved to be a valid challenge in order to induce acutely MCI in rodents; 2) we demonstrated that both NOR and single-day MWM can detect TSD-induced MCI; 3) Pros and cons of the two different techniques are weighted. Therefore these behavioural tests allow to study the role of drugs during the different stage of the memory shaping.

Ethical statement

The experiments received authorisation from the Italian Ministry of Health, and are conducted following the principles of the NIH Guide for the Use and Care of Laboratory Animals, and the European Community Council (86/609/EEC) directive. Rats are housed and used according to current European Community rules. Experiments on rats are approved by the research committees from the University of Verona and Italian National Institute of Health

References

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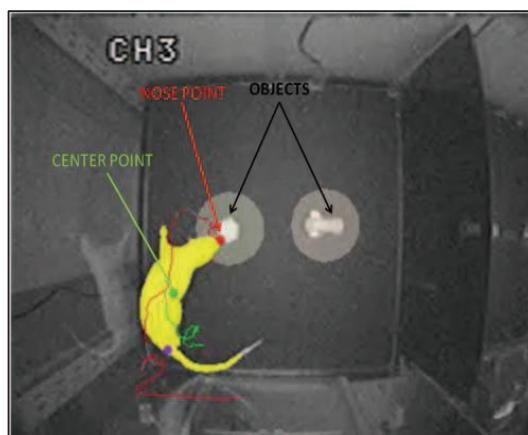


Figure 1. Automatic tracking of a rat during the recognition phase in the NOR test. The time spent by the animal with the nose within the virtual circular area drawn around the objects was counted as exploration time.