Journal of Neuroscience Methods 251 (2015) 83–91

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Basic neuroscience

# Stress-free automatic sleep deprivation using air puffs

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## HIGHLIGHTS

- Gentle handling sleep deprivation is time- and personnel-intensive.
- Manual sleep scoring adds unwanted variability to studies.
- We developed an automated air-puff sleep deprivation procedure for rats.
- AP is no more stressful than gentle handling sleep deprivation.
- AP is a viable, automated alternative to current sleep deprivation techniques.

## ARTICLE INFO

*Article history:*
Received 16 December 2014
Received in revised form 21 April 2015
Accepted 14 May 2015
Available online 23 May 2015

*Keywords:*
REM sleep
EEG
EMG
Waking
Automated
Sleep deprivation
Air puffs
Power spectral density
Non-REM sleep
MATLAB

## ABSTRACT

*Background:* Sleep deprivation via gentle handling is time-consuming and personnel-intensive.
*New method:* We present here an automated sleep deprivation system via air puffs. Implanted EMG and EEG electrodes were used to assess sleep/waking states in six male Sprague-Dawley rats. Blood samples were collected from an implanted intravenous catheter every 4 h during the 12-h light cycle on baseline, 8 h of sleep deprivation via air puffs, and 8 h of sleep deprivation by gentle handling days.
*Results:* The automated system was capable of scoring sleep and waking states as accurately as our offline version (~90% for sleep) and with sufficient speed to trigger a feedback response within an acceptable amount of time (1.76 s). Manual state scoring confirmed normal sleep on the baseline day and sleep deprivation on the two manipulation days (68% decrease in non-REM, 63% decrease in REM, and 74% increase in waking). No significant differences in levels of ACTH and corticosterone (stress hormones indicative of HPA axis activity) were found at any time point between baseline sleep and sleep deprivation via air puffs.
*Comparison with existing method:* There were no significant differences in ACTH or corticosterone concentrations between sleep deprivation by air puffs and gentle handling over the 8-h period.
*Conclusions:* Our system accurately detects sleep and delivers air puffs to acutely deprive rats of sleep with sufficient temporal resolution during the critical 4–5 h post learning sleep-dependent memory consolidation period. The system is stress-free and a viable alternative to existing sleep deprivation techniques.



## 1. Introduction

Sleep plays a crucial role in the memory consolidation of learning tasks (Ferrara et al., 2008; Poe et al., 2010; Smith, 1995). In fact, it has been shown that sleep must occur within a critical 4 to 5 h window after the completion of the task for hippocampal memory consolidation to take place (Bjorness et al., 2005; Hagewood et al., 2010; Prince et al., 2014). Given this precarious sleep-dependent period for hippocampal plasticity and memory, acute use of sleep deprivation techniques can enable the elucidation of memory consolidation mechanisms during specific sleep stages.

A variety of techniques have been developed and validated to deprive laboratory animals of total or partial sleep using electromyography (EMG) and cortical electro-encephalography (EEG)

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http://dx.doi.org/10.1016/j.jneumeth.2015.05.010

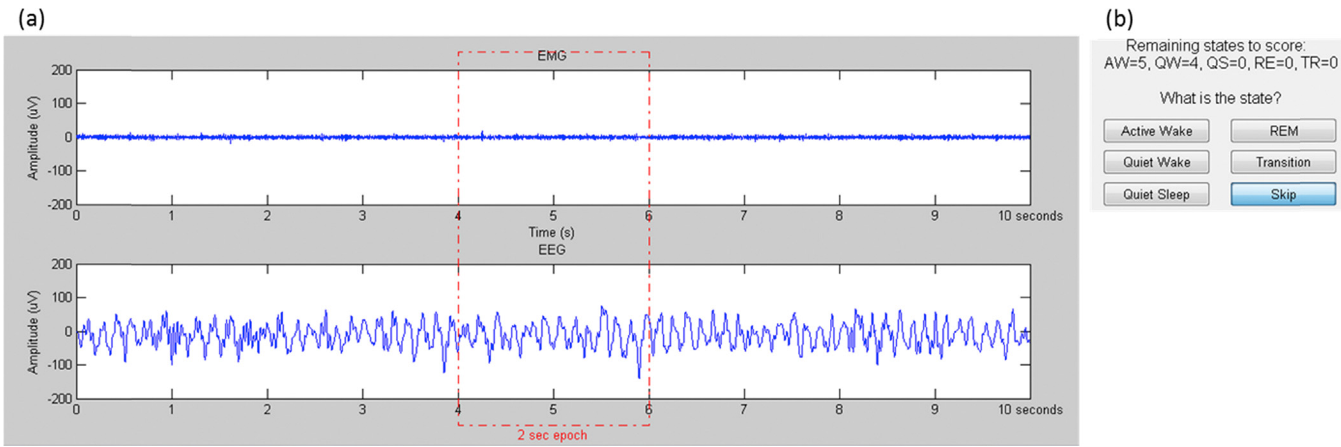


**Fig. 1.** The manual scoring window is used to determine threshold values for real-time autoscoring. (a) The user scores a 2-s epoch (dashed outline) by (b) selecting one of six pre-defined sleep states until each state is scored 5 times. The minimum number of scored epochs can be modified based on the interests of individual laboratories. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

signals, including gentle handling (Franken et al., 1991; Tobler and Borbely, 1990), multiple platforms over water (Mendelson et al., 1974; Youngblood et al., 1997), and forced locomotion (Borberly and Neuhaus, 1979; Rechtschaffen et al., 1999). Manual sleep deprivation techniques can introduce unexpected confounds. For example, male experimenters have been shown to induce more stress (Sorge et al., 2014) and what procedures constitute gentle manipulation vary between experimenters. Manual scoring in sleep studies is also time-consuming, resulting in research delays and increased personnel costs. Furthermore, off-line scoring does not allow for real-time feedback control based on the sleep or waking state of the animal.

We have developed an on-line total sleep deprivation system based on our previously published off-line software, Auto-Scorer (Gross et al., 2009), for real-time automated detection of sleep states in animals outfitted with EMG and EEG electrodes and delivery of puffs of air to the animal's home cage to prevent further sleep in order to gain further understanding in sleep-dependent memory consolidation.

## 2. Materials and methods

The design and validation of both the software and hardware components of the system are described below, as well as a description of the animal experiments carried out to measure the real-time performance of the system. We also present the procedure used to characterize the stress response of rats to sleep deprivation via puffs of air.

### 2.1. Software design and testing

The software component of the air puff sleep deprivation system was adapted from our previously published, open source, logic-based, automated sleep scoring software, Auto-Scorer (Gross et al., 2009). The real-time software contains a manual scoring mode and an automated scoring mode that were developed in MATLAB, version 2012b (The MathWorks, Inc., Natick, MA). The sleep deprivation method relies on online automatic sleep scoring with thresholds initially set in manual scoring mode.

#### 2.1.1. Manual scoring mode to determine thresholds

The manual scoring mode allows the user to determine the threshold values of neck EMG and EEG (delta, theta and sigma) power for each animal that are used in the logic of the automated scoring algorithm (refer to Gross et al., 2009 for further details). The user scores a small number of epochs of each user-defined sleep or waking state (Fig. 1a and b). With the animal connected to the recording system, the most recent 10 s of the EEG and EMG signals are displayed in the graphical user interface (GUI) and the user marks which state the animal is in. Discussed in Gross et al. (2009) and by others Benington et al. (1994), Robert et al. (1999), Witting et al. (1996), an epoch size of 10 s is commonly used in manual scoring of rat sleep and wake states due to the trade-off between accuracy and manual labor. The user scores the state based on the middle 2-s window contained in a red rectangle. Once five epochs of each state are scored (countdown displayed in Fig. 1b), the software displays two 3-dimensional plots (Fig. 2a and b). The first plot includes power spectral densities (PSD) of the EEG signal in the delta, theta, and sigma frequency bands for each epoch. The PSD of the EMG, the delta/theta PSD ratio, and sigma × theta PSD of each epoch comprise the second plot. These graphs are inspected by the user to determine threshold settings for the logic-based automated scoring mode. Refer to Gross et al. (2009) for the protocol to select threshold values. Once the thresholds have been set, the GUI prompts the user to save the values (Fig. 2c) and then returns to the main menu.

#### 2.1.2. Auto-scoring mode

The automated scoring mode of the software requires the user to enter the PSD threshold values for each animal determined from the Manual Scoring mode, and the length of time (in seconds) that the program should run. Given that the goal is to provide real-time feedback control based on the sleep or waking state of the animal, an epoch size of 2 s was selected to decrease the likelihood of allowing micro-sleeps to occur. A shorter delay for feedback to deliver puffs of air would awaken the animal from sleep faster. Automated scoring in 2-s epochs has been shown to improve agreement with manual scoring (Ruigt et al., 1989), so this shortening of the epoch size in our algorithm was expected to have a beneficial effect on the logic algorithm's automated scoring performance.

The algorithm's auto-scoring performance of 2-s epochs was initially evaluated offline using a data set of six sleeping rats. The states scored by the algorithm were compared to manually scored states from three experienced technicians, and the agreement of each state between users and the algorithm was calculated.

### 2.2. Hardware design

The air puff sleep deprivation system (Fig. 3) includes Neuralynx data acquisition hardware (digital amplifier and Cheetah EDT