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Sleep-Wake Recording in the Turek Laboratory

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We use this protocol and it's working

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Abstract

Surgery and recording protocol for measuring sleep-wake behavior in mice

Surgery

1 The mouse is anesthetized by intraperitoneal injection with a ketamine/xylazine cocktail. Once the animal is anesthetized (as assessed by toe pinch), the fur on the top of the head is wetted with diluted alcohol and clipped.

2 The animal is positioned in the stereotaxis apparatus (David Kopf Instruments).

3 A single dose of meloxicam is given while the mouse is anesthetized. Sterile eye ointment is applied to the eyes to prevent corneal drying. Heat support during surgery is provided by small re-usable gel bead disks heated to 37 degrees Celsius.

4 The incision area is cleaned with 3 alternative washes of betadine scrub and alcohol, and sterile drapes are positioned over the animal's back.

5 An approximately 1 - 1.5 cm incision is made at the midline over the top of the skull. No skin is removed.

6 Mice are surgically implanted with a headmount (Part #8201, Pinnacle Technologies, Lawrence, KS) for sleep recording. The headmount consists of three electroencephalographic (EEG) electrodes and 2 electromyographic (EMG) electrodes connected to a printed circuit board,
which is affixed to the exposed skull via a small amount of cyanoacrylate (Vet-bond or similar) and four stainless steel screws (Part #8209: 0.10” screws for anterior positions; Part #8212: 0.12”
screws for posterior positions) that serve as the EEG leads.

7 Using the stereotaxic apparatus (David Kopf Instruments), the implant is positioned on the skull such that the front edge of the implant is placed 3.0-3.5 mm anterior to bregma. This configuration places all four screws over the cerebral cortex region in adult mice. Silver epoxy (Pinnacle
Technologies, Lawrence, KS) may be applied to insure a secure electrical connection to the screws is achieved.

8 A small pocket is made in the nuchal muscles using a pair of forceps for the placement of the EMG wires. Dental acrylic (Lang Dental Manufacturing Co., Wheeling, IL) is used to seal and protect the headmount, and the skin is closed around the headmount with a single suture using 4-0 or 5-0 chromic gut (United States Surgical, Norwalk, CT) at the front and back of the implant. Absorbable suture is used to minimize risk of damage of the fragile wires that could occur if sutures needed to be removed.

9 After surgery, a circulating water blanket is used for heat support as animals recover from anesthesia.

10 An additional dose of meloxicam is given for analgesia approximately 24 hours later.

Recording

11 Supplies and software utilized for sleep-wake recordings are from Pinnacle Technologies (Lawrence, KS). At least one week after surgery, mice are put into sleep recording cages (Product #8228). A preamp (Part #8406) plugs into the implant (Part #8201), and wires attached to the preamp connect to a commutator (Part #8408), which then plugs into an A/D converter (Part #8401).

12 Pinnacle software PAL 8400 is used to record. Recordings are obtained at a sampling rate of 250
Hz. A bandpass filter is applied between 0.5 Hz and 100 Hz on the EEG channels and between 1
Hz and 100 Hz on the EMG channel.

13 Recordings are obtained after at least 5 days of habituation to the sleep-wake chamber and continued over 48-72 hours.

14 Mice are maintained on a constant 12-hr light:12-hr dark light:dark (LD) cycle for the duration of the experimental protocol.