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Protocol for Rat Sleep EEG

Subjects

Male Sprague Dawley rats weighing 250-300 grams at the time of surgery are used. Food and water are available *ad libitum* throughout the experiment. Rats are group housed prior to surgery, and following surgery they are housed individually in polypropylene shoebox cages (47 x 26 x 20 cm). A 12h:12h light:dark cycle and constant room temperature are maintained.

Surgery

Sleep-wake states are identified electrophysiologically using chronically implanted EEG (N=4) and EMG (N=2) electrodes. The electrodes are implanted using stereotaxic coordinates. Anesthesia and analgesia are maintained using the following drug cocktails:

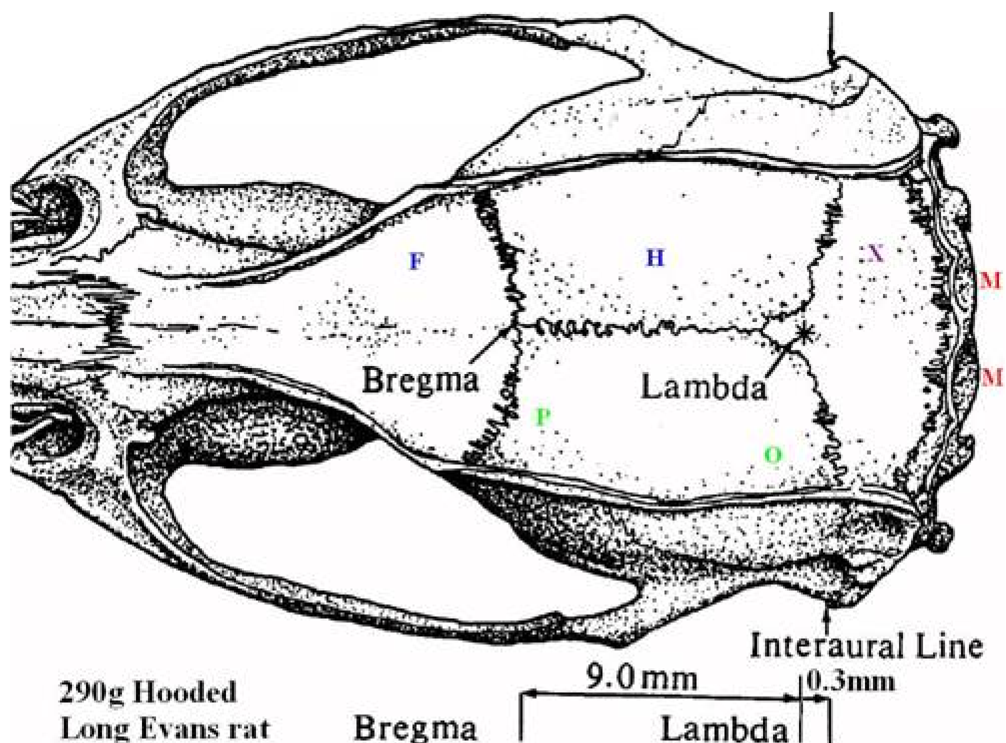
AGENT	DRUG (concentration)	DOSE	ROUTE
Induction	Ketamine (100mg/ml)	90mg/kg	IP
Induction	Xylazine (20mg/ml)	9mg/kg	IP
Induction	Acepromazine (10mg/ml)	2mg/kg	IP
Maintenance	Isoflurane/O2	0.5-2%	Inhalant
Analgesia	Metacam (5mg/ml)	1mg/kg	SQ
Hydration	Lactated Ringer's solution	10mg/kg	SQ
Reversal	Antisedan (5mg/ml)	1mg/kg	SQ

The EEG electrodes used are stainless-steel jeweller's screws threaded into holes drilled through locations on the skull as described (Mistlberger et al, 1983, 1987; Pollock & Mistlberger, 2003). Slow wave and spindle neocortical EEG activity are obtained by differential recordings from two electrodes positioned in the lateral parietal (P) and lateral occipital (O) cortices. Hippocampal theta rhythms are

obtained by differential recordings from two electrodes positioned in the dorsal hippocampus (H) and the frontal cortex (F) as a reference. EMG is recorded from two subcutaneous wire electrodes implanted between the occipital bone and the neck muscle (M). An extra screw (X) is placed on the skull above the cerebellum to provide additional anchor support. The following stereotaxic coordinates relative to landmarks on the skull, based upon the rat brain stereotaxic atlas by Paxinos & Watson (1997), are used to determine electrode placements:

LOCATION	A-P (mm)	M-L (mm)	D-V (mm)
Parietal cortex	-1.0 from Bregma	+2.5	-0.5
Occipital cortex	+1.0 from Lambda	+3.5	-1.5
Hippocampus	-4.3 from Bregma	-2.0	-2.3
Frontal cortex	+3.0 from Bregma	-2.0	-1.0

The pins from all 6 electrodes are connected to a protective plastic headcap. The entire electrode assembly is insulated and bonded to the skull with dental acrylic.



Drug test procedure & sleep recording

Following one week recovery from surgery, an additional week is spent handling the rats (for 15 min/day) and allowing them to habituate to sleep recording conditions. During this habituation period, sample sleep recordings are taken to ensure proper functioning of the electrodes and to determine polygraphic signal amplitude cutoffs for each individual rat (see below). Recording chambers consist of plexiglass chambers (37 x 34 x 53 cm) within electrically-shielded and sound-attenuating enclosures (Model E3125AA-3 Animal Chest, Grason-Stadler Co., West Concord, MA). Each recording chamber is equipped with commutators (Plastics One Co.) to allow free movement of the animals while being connected to EEG recording cables, overhead passive infrared motion detectors (modified versions of Model 49-426, RadioShack Co.) to detect locomotor activity, and observational windows through which video recordings can be taken. Fresh air is continuously pumped into the recording chambers, which are housed in a temperature- and light-controlled isolation room with a white noise generator. During experimental recordings, no person is present in the isolation room to prevent possible disturbances of sleep.

Following this adaptation period, the rats are housed in the recording chambers for periods of two consecutive days: one day for a baseline recording followed by a second day for a drug/vehicle test recording session. Prior to each baseline recording day, the animals are placed in the recording chambers at the beginning of the dark period (their usual wake phase of the circadian cycle). On each recording day, the rats are removed from their recording chambers for a brief period (30 min.) immediately after light onset (the beginning of their usual sleep phase) in order to change their food, water, and bedding. On drug test days, each animal receives an injection immediately before being returned to the recording chamber. As soon as the animal is reconnected to the EEG recording cable, the baseline or drug/vehicle test recording commences, and continues for 23.5 hours. This procedure permits assessment of the wake promoting effects of the drug when sleep propensity is highest; by recording continuously for a full day, extended waking effects and/or subsequent ‘rebound’ increases in sleep (if any) are detectable. At the end of each 2-day recording period, the rats are permitted 2 days of recovery before the next 2-day recording session, thus providing a four-day interval between injections.

Each rat is tested with this procedure for each condition in the study design (e.g., 5 times to assess vehicle control, 3 drug doses, and a positive control, the latter being caffeine, modafinil or amphetamine). The order of treatments is balanced across rats.

Experimental Schedule

One week recovery following surgery						
One week habituation to sleep recordings						
Baseline day1	Test day1	Recovery day	Recovery day	Baseline day2	Test day2	Recovery day
Recovery day	Baseline day3	Test day3	Recovery day	Recovery day	Baseline day4	Test day4
Recovery day	Recovery day	Baseline day5	Test day5			

Polygraphic signals are amplified and bandpass filtered (0.3-30 and 30-300 Hz for EEG and EMG, respectively) by a polysomnograph (Grass Model 9, Grass Instruments Co.). Along with signals from the motion detectors, these polygraphic signals are digitized (sampling rate of 250 Hz) and stored on a computer using data collection software (SleepSign, Kissei Comptec Co.) for subsequent off-line analysis.

Data analysis

Waveform recognition analysis of EEG signals is performed using the following parameters:

WAVEFORMS	EEG DERIVATION	AMP MIN	AMP MAX	FRQ MIN	FRQ MAX	HALF-WAVE COUNTS
Slow waves	Parietal-Occipital	+67%		1 Hz	3 Hz	1
Theta rhythms	Hippocampal-Frontal	-50%	+50%	5 Hz	9 Hz	10
Spindles	Parietal-Occipital	+67%		10 Hz	15 Hz	4

Slow wave and spindle amplitude cutoffs are set relative to typical desychronized EEG levels seen in active wakefulness and REM sleep. Theta rhythm amplitude cutoffs are set relative to the typical theta rhythm amplitude exhibited in REM sleep.

Behavioral states are scored in 10-second epochs according to standard criteria (Pollock & Mistlberger, 2003). Each epoch is

classified as to the predominant state. The following seven sleep/wake states are recognized:

BEHAVIORAL STATE		MOTION	EMG	SLOW WAVES	SPINDLES	THETA RHYTHMS
Waking	Locomotor	+				
	Active	-	++			
	Quiet	-	+	-	-	
NREM sleep	Slow wave	-	±	+		
	Transitional	-	±		+	
	Low-amplitude	-	-	-	-	-
REM sleep		-	-	-	-	+

The minimum EMG integral cutoff for active wakefulness is set to within 25% of maximal EMG integral levels, which can be determined when the rat is first placed in the recording chamber and is actively locomoting. To accommodate phasic twitches which can occur within REM sleep, the maximum EMG integral cutoff for low-amplitude sleep and REM sleep is set to within 300% of minimal EMG integral levels, which can be observed during epochs of REM sleep with complete muscle atonia.

Sleep-wake states (averaged into hourly time intervals throughout the recording day) are graphed and evaluated for drug and time effects using repeated measures ANOVA and Bonferroni post-hoc tests (GraphPad Prism,Version 4.00, GraphPad Software, Inc.). Behavioral state episode durations and frequencies, and fast Fourier transform analysis of delta power (1-4 Hz) in all SWS epochs, are similarly analyzed.

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