# Electromyographic Power Spectral Changes Associated with the Sleep-Awake Cycle and With Diazepam Treatment in the Rat<sup>1</sup>

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YOUNG, G. A. AND N. KHAZAN. Electromyographic power spectral changes associated with the sleep-awake cycle and with diazepam treatment in the rat. PHARMACOL BIOCHEM BEHAV 19(4) 715-718, 1983.—Power spectral analysis was used to study temporalis muscle EMG activities during the sleep-awake cycle in the rat. EMG spectra derived from EMG during the states of slow-wave sleep. REM sleep and wakefulness demonstrated qualitative and quantitative differences. Diazepam treatment produced reductions in EMG spectral power during wakefulness. Thus, our experimental model allows qualitative and quantitative delineation of EMG activity associated with behavioral changes or drug treatments.

Diazepam Sleep-wake cycle EMG activity

WE previously demonstrated that the cortical electroencephalographic (EEG) power spectra associated with the behavioral states of slow-wave sleep (SWS), rapid eye movement (REM) sleep and wakefulness in the freely-moving rat were qualitatively and quantitatively different [15]. SWS was associated with a predominance of spectral power in the lower frequency range (zero to 5 Hz) and a gradual dimunition of spectral power in the 5-20 Hz range; total spectral power was the largest among the three behavioral states. REM sleep was associated with a predominant peak of spectral power in the 6-9 Hz range. The awake state was associated with substantially less total spectral power than either SWS or REM sleep. We have further utilized the technique of power spectral analysis in the rat to delineate the CNS effects of opioids [3, 6, 8, 13, 16], ethanol [12,14] and  $\Delta^9$ -THC [1].

We have entertained the possibility that the spectral analysis of electromyographic (EMG) activities in the rat, in a manner similar to that already established for EEG activities, might be equally useful in studying any EMG changes associated with normal behavioral conditions or resulting from drug administration. Thus, we are now reporting qualitative and quantitative differences in EMG power spectra during the behavioral states of SWS, REM sleep and wakefulness in the rat. We are also providing data on diazepaminduced changes in EMG spectral power.

#### PROCEDURES

In the first experiment six adult female Sprague-Dawley rats, 250–300 g, were studied. For bipolar EMG recordings,

pairs of stainless steel wires were inserted under ketamine anesthesia (100–150 mg/kg, IP) into the left and right temporalis muscles. For bipolar EEG recordings, stainless steel screws (size 0-80  $\times$  <sup>1</sup>/<sub>8</sub> inch) were implanted over the frontal (2 mm anterior and 2 mm lateral to bregma) and ipsilateral parietal (3 mm posterior and 2 mm lateral to bregma) cortices. An additional screw was placed 6 mm posterior and 2 mm lateral to bregma and served as the indifferent electrode. All electrodes were soldered to a miniature Continental connector which was attached to the skull with dental acrylate and Eastman 910FS adhesive [4].

Each rat was maintained in an individual cage that was equipped with a swivel cable connector for EEG and EMG recordings. Direct EEG recordings on the Grass Model 7D polygraph were filtered to pass frequencies between 1 and 35 Hz, while EMG recordings were filtered to pass frequencies between 10 and 75 Hz. Behavioral states of SWS, REM sleep and wakefulness during the sleep-awake cycle were identified by the corresponding changes in EEG and EMG activities. Processing of the EMG samples was accomplished as follows. The EMG output (J6) from the polygraph to the analog input of the Nicolet MED-80 computer was filtered to pass frequencies between 10 and 750 Hz with a Krohn-Hite Model 3550R filter. The 5.46 sec samples of EMG were digitized on-line at a sampling rate of 1500/sec. Power spectral densities were estimated at 0.1 Hz intervals from zero to 750 Hz, and geometric smoothing over three neighboring frequencies was employed.

In the second experiment three rats that were prepared with EMG and EEG electrodes as above were also implanted with permanent indwelling intravenous cannulae into the

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FIG. 1. Direct cortical EEG and integrated temporalis muscle EMG recordings are shown for an individual rat during the behavioral states of wakefulness, slow-wave sleep and REM sleep.

right external jugular vein [10,11]. These rats received 1 mg/kg intravenous injections of injectable diazepam (Roche Laboratories). The effects of diazepam treatment upon EMG spectral power were compared with EMG spectral power associated with normal behavioral activities.

#### RESULTS

Direct cortical EEG and direct or integrated temporalis muscle EMG recordings collected during the behavioral states of SWS, REM sleep and wakefulness in the rat are easily distinguishable from one another [2, 4, 5, 7]. For example, as illustrated in Fig. 1 for an individual rat, wakefulness is typically associated with low amplitude, fast frequency cortical EEG activity and high levels of integrated EMG activity. SWS is associated with high amplitude, slow frequency cortical EEG activity and low levels of integrated EMG activity. During REM sleep the cortical EEG consists of an intermediate peak-to-peak amplitude and is characterized by a further reduction of integrated EMG activity.

Representative power spectra derived from temporalis muscle activities during the EEG and behavioral states of quiet wakefulness, SWS and REM sleep are shown in Fig. 2 for an individual rat. During quiet wakefulness the overall spectral power was much greater than that during the SWS and REM sleep states. Spectral power peaked in the zero to 50 Hz range. During quiet wakefulness the EMG was also associated with an increase in spectral power over the 75 to 600 Hz range, peaking somewhere in the 100 to 200 Hz band. Even further increases in spectral power over the 75 to 750 Hz range were observed during wakefulness associated with behavioral activity. During SWS the EMG activity was associated with predominant spectral power in the zero to 50 Hz range along with relatively less spectral power distributed over the 75 to 200 Hz range. REM sleep was also associated with spectral power in the zero to 50 Hz range, but, in contrast to the SWS and wakefulness states, little spectral power was exhibited at other frequencies.

The degree of intersubject variability of the EMG power spectra associated with the three behavioral states of the rat is presented in Fig. 3. The relative power (% of total power) is shown as a function of frequency at 1 Hz intervals; standard deviations are shown as an indicator of variability among six rats. Since quiet wakefulness was associated with the largest amount of total power, the power associated with SWS and REM sleep was calculated as being relative to that of quiet wakefulness, with quiet wakefulness being 100%.

The effect of diazepam treatment upon EMG spectral power during quiet wakefulness is shown in Fig. 4 for an



FIG. 2. EMG power spectra derived from temporalis muscle activities in an individual rat are shown during the behavioral states of wakefulness, slow-wave sleep and REM sleep. Power is presented as a function of frequency (Hz).

individual rat. Following intravenous administration of 1 mg/kg of diazepam, a purported CNS skeletal muscle relaxant, temporalis muscle activity during quiet wakefulness was reduced over the 75 to 750 Hz range by over 50%. The 1 mg/kg dose of diazepam produced this reduction of muscle activity without disrupting sleep-awake activity.

#### DISCUSSION

The rat has been extensively used in studies related to experimental psychology and psychopharmacology. Relatively little information is available concerning EMG power spectra during normal sleep-awake behavior in freelymoving rats prepared with chronic EEG and EMG electrodes. This report describes the power spectra derived from the rat's temporalis muscle EMG activities during three behavioral states. All three behavioral states were associated with similar EMG spectral power in the zero to 50 Hz range; however, differences in EMG power spectra during the three behavioral states were most evident in the higher frequency bands. In the SWS state the EMG power spectra contained spectral power in the 75 to 200 Hz range. During REM sleep, EMG power spectra were characterized by the dimunition of the spectral power in the 75 to 200 Hz range that was associated with the SWS state. This dimunition of EMG spectral power in the 75 to 200 Hz range was correlated with the onset of muscle relaxation or atonia that is behaviorally observable as a rat enters REM sleep. Quiet wakefulness was associated with large increases in spectral power over the 75 to 600 Hz range.





FIG. 3. Power spectra derived from temporalis muscle EMG are shown during the wakefulness, slow-wave sleep and REM sleep states. For each of six rats a power spectrum was derived from a 5.46-sec EMG sample during each of the three behavioral states. These spectra from each of the six rats were then averaged relative to the total power associated with wakefulness, wakefulness being 100%. Standard deviations are indicated.

Having characterized the EMG power spectra associated with normal sleep-awake behavior in the rat, we have also provided data following diazepam treatment that demonstrate a diazepam-induced reduction in EMG spectral power during quiet wakefulness. Such a finding was expected since

FIG. 4. EMG power spectra derived from temporalis muscle activities in an individual rat are shown during control quiet wakefulness and during quiet wakefulness after diazepam (1 mg/kg, 1V) administration. Power is presented as a function of frequency (Hz).

diazepam acts upon reticular neuronal mechanisms that control muscle tone [9]. Thus, it appears that comparative studies of drug-induced changes in EMG spectral power will be able to provide useful qualitative and quantitative information.

### REFERENCES

- 1. Buonamici, M., G. A. Young and N. Khazan. Effects of acute  $\Delta^{9}$ -THC administration on EEG and EEG power spectra in the rat. *Neuropharmacology* **21**: 825–829, 1982.
- Colasanti, B. and N. Khazan. Agonistic properties of narcotic analgesics and antagonists on the electroencephalogram and behavior in the rat and their reversal by naloxone. *Neurophar*macology 12: 619–627, 1973.
- Kareti, S., J. E. Moreton and N. Khazan. Effects of buprenorphine, a new narcotic agonist-antagonist analgesic, on the EEG, power spectrum and behavior in the rat. *Neuropharmacology* 19: 195-201, 1980.
- 4. Khazan, N. The implication and significance of EEG and sleep-awake activity in the study of experimental drug dependence on morphine. In: *Methods in Narcotics Research (Modern Pharmacology-Toxicology*, vol 5), edited by S. Ehrenpreis and A. Neidle, New York: Marcel Dekker, 1975, pp. 173–215.
- Khazan, N., J. R. Weeks and L. A. Schroeder. Electroencephalographic, electromyographic and behavioral correlates during a cycle of self-maintained morphine addiction in the rat. J Pharmacol Exp Ther 155: 521–531, 1967.
- Lukas, S. E., J. E. Moreton and N. Khazan. Differential electroencephalographic and behavioral cross-tolerance to morphine and methadone in the l-alpha-acetylmethadol (LAAM)maintained rat. J Pharmacol Exp Ther 220: 561–567, 1982.

- Moreton, J. E., T. Rochrs and N. Khazan. Drug selfadministration and sleep-awake activity in rats dependent on morphine, methadone or 1-alpha-acetylmethadol. *Psychophar*macology (Berlin) 47: 237-241, 1976.
- 8. Steinfels, G. F., G. A. Young and N. Khazan. Opioid selfadministration and REM sleep EEG power spectra. *Neuropharmacology* 19: 69-74, 1980.
- Tseng, T.-C. and S. C. Wang. Locus of action of centrally acting muscle relaxants, diazepam and tybamate. J Pharmacol Exp Ther 178: 350-360, 1971.
- Weeks, J. R. Experimental morphine addiction: Method for automatic intravenous injections in unrestrained rats. *Science* 138: 143-144, 1962.
- Weeks, J. R. Long-term intravenous infusion. In: *Methods in Psychobiology*, edited by R. D. Myers, New York: Academic Press, 1972, pp. 155–168.

- Wolf, D. L., G. A. Young and N. Khazan. Comparison between ethanol-induced and slow-wave sleep synchronous EEG activity utilizing spectral analysis. *Neuropharmacology* 20: 687–692, 1981.
- 13. Young, G. A., L. Neistadt and N. Khazan. Differential neuropharmacological effects of mu, kappa and sigma opioid agonists on cortical EEG power spectra in the rat. *Res Commu Psychol Psychiatry Behav* 6: 365–377, 1981.
- Young, G. A., D. L. Wolf and N. Khazan. Relationships between blood ethanol levels and ethanol-induced changes in cortical EEG power spectra in the rat. *Neuropharmacology* 21: 721-723, 1982.
- Young, G. A., G. F. Steinfels, N. Khazan and E. M. Glaser. Cortical EEG power spectra associated with sleep-awake behavior in the rat. *Pharmacol Biochem Behav* 8: 89–91, 1978.
- Young, G. A., G. F. Steinfels, N. Khazan and E. M. Glaser. Morphine self-administration and EEG power spectra in the rat. *Pharmacol Biochem Behav.* 9: 525–527, 1978.