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Effects of Chronic Morphine Administration and Naloxone on EEG, EEG Power Spectra, and Associated Behavior in Two Inbred Rat Strains

LIESER MAYO-MICHELSON AND GERALD A. YOUNG[1]

Department of Pharmacology & Toxicology, University of Maryland School of Pharmacy, 20 N. Pine Street, Room 344, Baltimore, MD 21201

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MAYO-MICHELSON, L. AND G. A. YOUNG. *Effects of chronic morphine administration and naloxone on EEG, EEG power spectra, and associated behavior in two inbred rat strains.* PHARMACOL BIOCHEM BEHAV 42(4) 815-821, 1992. – Utilizing behavioral and electroencephalographic (EEG) assessments, two inbred rat strains, Lewis (LEW) and Fischer 344 (F344), were exposed to morphine (IV) over a period of 7 days to discern differences in tolerance development. Following morphine injection, the LEW group demonstrated a greater mean total amount, as well as a greater rate of reduction, of stuporous behavior across the 7 days tested. Differences in patterns of latency to onset of slow-wave sleep between the two strains were also exposed. EEG analysis of spectral parameters utilizing an analysis of variance with repeated measures revealed that peak frequency, mean frequency, and edge frequency differed as a function of inbred rat strain. All spectral parameters differed as a function of duration of morphine injection; linear trends were indicated for both strains. Naloxone was administered (IV) following the 7 days of morphine to delineate dependence differences. LEW animals reflected a greater amount of behavioral responses, for example, wet-dog shakes, diarrhea, body stretch, and sluggish behavior. However, F344 rats demonstrated a greater alteration in two spectral parameters assessed: peak frequency and total power. Genetic variability appears to play a major role in both morphine tolerance and dependence as indicated by differences in EEG and behavioral responses.

Morphine EEG power spectra Lewis inbred rat strain F344 inbred rat strain Behavior Tolerance Dependence

FOR over 10 years, our lab has employed electroencephalography (EEG), power spectral parameters derived from EEG, and behavioral assessment to evaluate the effects of opiates and other CNS-active agents (7). For instance, in nontolerant rats morphine (10.0 mg/kg, IV), primarily a μ-agonist, and ethylketocyclazocine (EKC), primarily a κ-agonist, when administered to freely moving female Sprague-Dawley rats resulted in the production of a behavioral stuporous phase associated with high-voltage cortical EEG slow-wave bursts. Chronic administration of both drugs resulted in a significant reduction of EEG and associated behavioral profiles (8).

We have reported EEG and behavioral differences in response between two inbred rat strains, Lewis (LEW) and Fischer 344 (F344), upon acute exposure to IV administration of morphine at doses of 3, 10, and 30 mg/kg. Duration of morphine-induced EEG slow-wave bursts and associated degree of behavioral stupor was greater in the LEW group. Analysis of latency to onset of slow-wave sleep revealed a dose-related effect for both strains. Global spectral parameters were extracted and compared. Except for peak frequency and edge frequency, all differed as a function of inbred rat strain. Regarding morphine dose, all spectral parameters differed except peak frequency. Multivariate factor analysis of morphine-induced EEG spectral parameters revealed a unique factor for each strain that was bipolar in nature and was associated with alterations between burst and interburst periods that occur in EEG after opiate administration (3).

LEW and F344 rat strains have had separate lineages for at least 75 years (4). To our knowledge, there are few pharmacogenetic studies that have focused upon EEG and opiate administration. Since differences were noted both behaviorally and electroencephalographically upon acute morphine admin-

[1] To whom requests for reprints should be addressed.

istration, it seemed reasonable to examine the effects of chronic administration of morphine, focusing primarily upon EEG and behavioral signs of tolerance development and dependence.

METHOD

Animals

Five LEW and five F344 rats (ages 3-6 months, 250-300 g) were implanted with bipolar epidural EEG stainless steel electrodes over the ipsilateral frontal (2 mm anterior and 2 mm lateral to bregma) and parietal (3 mm posterior and 2 mm lateral to bregma) cortices through small burr holes. Surgical procedures have been previously described (1,2). Electrodes were soldered to an Amphenol (Oak Brook, IL) connector and attached to the skull with dental acrylate. Seven days of recovery were permitted. Just prior to experimentation, rats were prepared with chronic silicone rubber cannulae inserted into the external jugular vein under halothane anesthesia (5,6).

During experiments, rats were housed continuously in individual chambers 28.5 × 28.5 × 36.0 cm. Each cage was equipped with a swivel connector having concentric mercury pools for EEG recordings (1,2). Rats were allowed at least 24 h of acclimation in these conditions before experimentation. Lighting conditions were composed of a timer-regulated illumination period from 6 a.m.-10 p.m. Subjects were connected by flexible cables (permitting free movement) and mercury commutators to a Grass (Quincy, MA) model 7D polygraph.

EEG Recording and Analysis

For each rat, direct EEG activity, filtered to pass frequencies below 0.5 kHz, was recorded on a Grass polygraph and, simultaneously, on a Hewlett-Packard (San Diego, CA) FM tape recorder. Analog EEG (stored on FM tape) was filtered at a low pass of 50 Hz with a Kronhite (Avon, MA) filter and digitized at a rate of 103/sec by a Nicolet (Madison, WI) Pathfinder II computer using Pathlab II software (developed by Rick Gussio, Ph.D.). Digitized EEG was retrieved as 112 consecutive 10-s epochs (representing approximately 20 min of real time) and transformed by fast Fourier analysis. From this transformation, power spectral parameters were derived. Six spectral quantities (peak frequency, complexity, mobility, mean frequency, edge frequency, and absolute power) were extracted from these spectra in 10 specific frequency bands: 1.0-2.5, 2.5-5.0, 5.0-7.5, 7.5-10.0, 10.0-13.0, 13.0-20.0, 20.0-30.0, 30.0-40.0, 40.0-50.0, and 1.0-50.0 Hz. Mean values of global EEG spectral parameters (1-50 Hz) were analyzed as a function of inbred rat strain and morphine dose

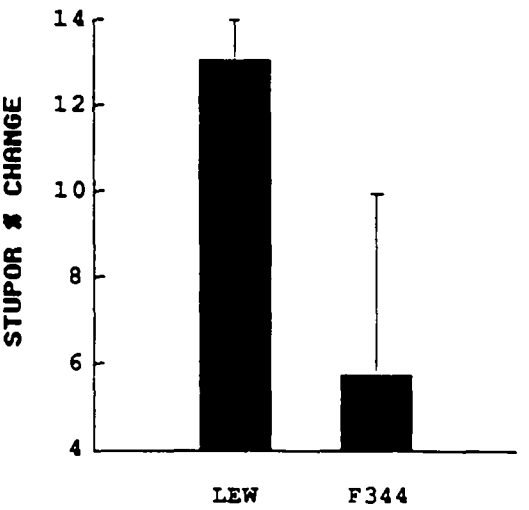

FIG. 2. Rate of reduction of stupor between days 1 and 7 of morphine administration is represented as percent change for LEW and F344 inbred rat strains. Data are presented as means ± SDs.

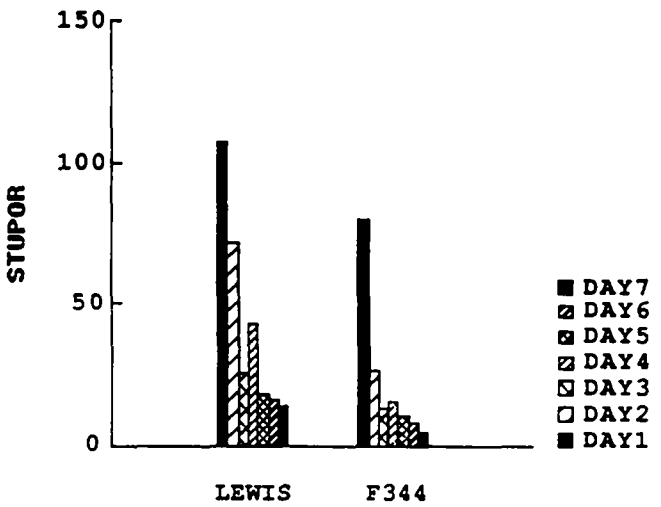

FIG. 1. Duration of stupor is shown as a function of chronic morphine administration in LEW and F344 inbred rat strains. Data are presented as means.

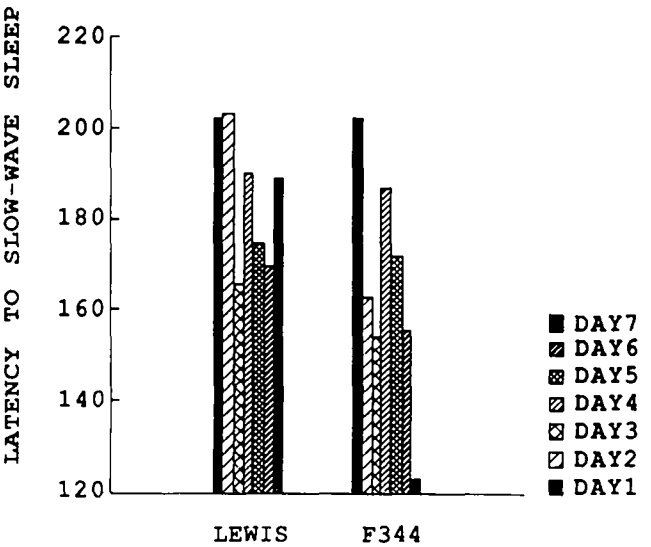

FIG. 3. Latency to onset of slow-wave sleep is shown as a function of duration of morphine administration in LEW and F344 inbred rat strains. Data are presented as means.

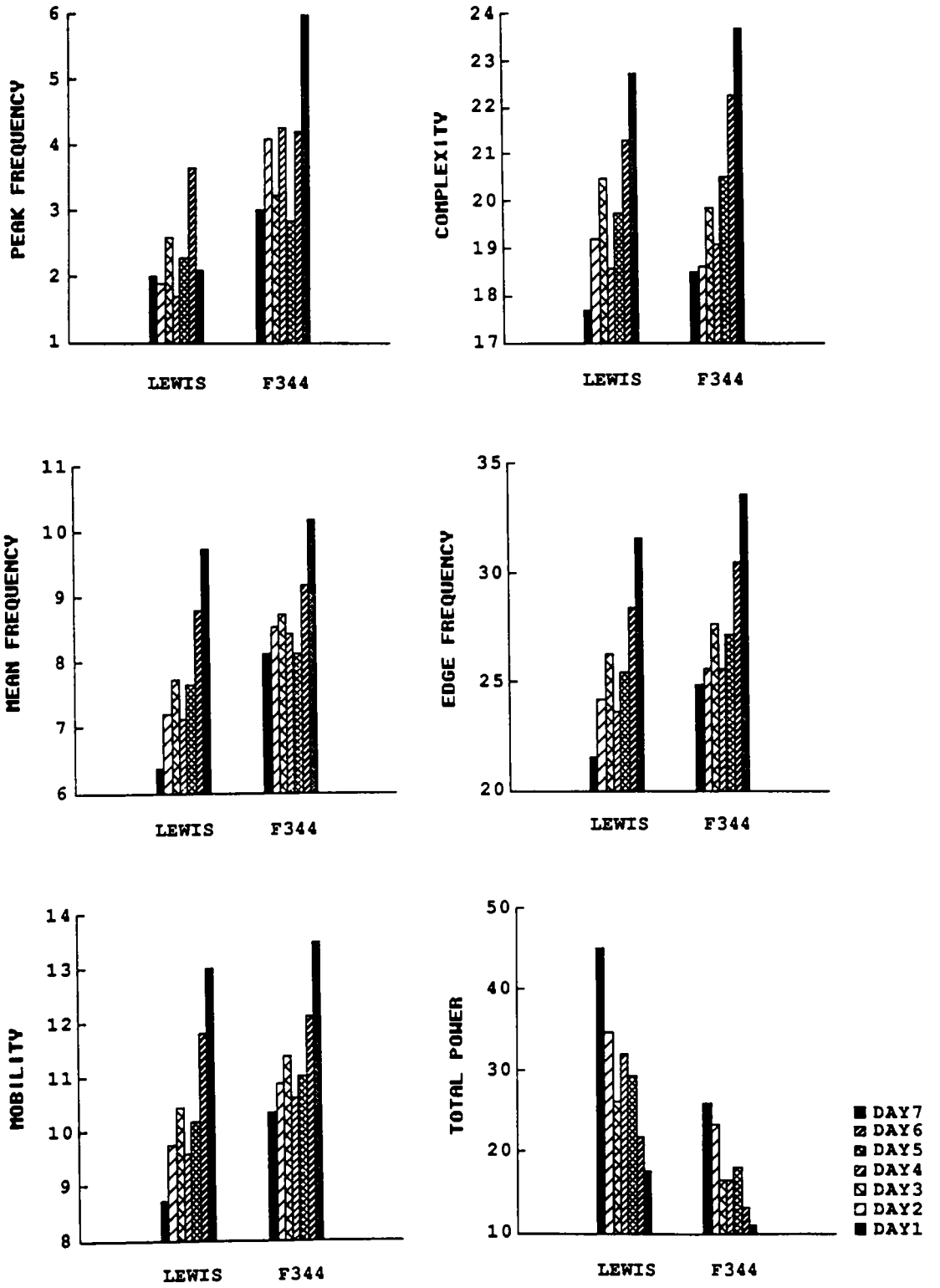

FIG. 4. EEG power spectral parameters are shown as a function of chronic morphine administration in LEW and F344 inbred rat strains. Data are presented as means.

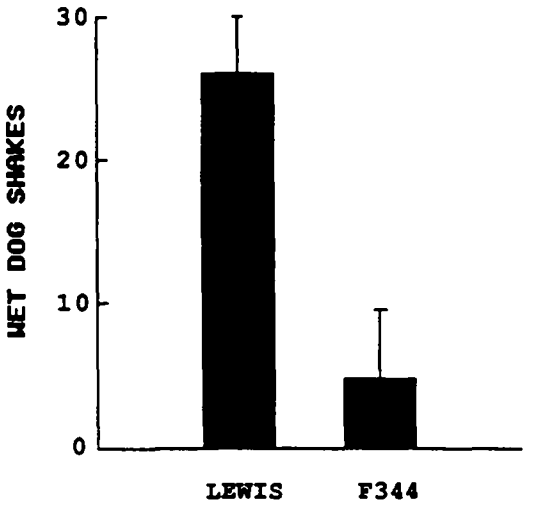

FIG. 5. Wet-dog shakes following naloxone treatment are shown for Lewis and F344 inbred rat strains. Data are presented as means ± SDs.

utilizing analyses of variance (ANOVAs) with repeated measures; when appropriate, posthoc trend analyses were performed.

Drugs

Morphine sulphate (Mallinckrodt, Inc., St. Louis, MO) was dissolved in isotonic saline and administered, IV, at doses of 10 and 20 mg/kg. Ketamine hydrochloride (Ketalar, Parke-Davis Div. of Warner-Lambert Co., Morris Plains, NJ), dosed at 100–150 mg/kg, and halothane (Halothane, U.S.P., Halocarbon Laboratories, Augusta, SC) were used to induce anesthesia. Naloxone (Sigma Chemical Co., St. Louis, MO) was dissolved in isotonic saline and administered, IV, at a dose of 1 mg/kg.

Procedure

EEG was recorded on a Grass polygraph for a minimum of 8 h prior to drug administration to screen for any abnormalities such as incorrect EEG electrode locations, bad solder connections, etc. and verify the presence of typical sleep-awake EEG activity.

Morphine was administered intravenously three times daily for 7 days with the exception of day 1, where two injections were given. Treatment times were set for 8:00 a.m., 2:00 p.m., and 8:00 p.m. On days 1, 2, and 3, rats received 10 mg/kg; on the remaining 4 days, the dose was increased to 20 mg/kg.

EEG was continuously recorded on a Grass polygraph for the 8 days of experimentation to ascertain amount of stupor (min) and latency to onset of slow-wave sleep (min). Measurement of degree of stupor following morphine administration has been utilized in our lab to assess one level of morphine's activity in rats. This behavioral tool has been defined as the simultaneous occurrence of high-voltage EEG slow-wave bursts and behavioral stupor that includes muscle rigidity, exophthalmos, suppressed respiration, Straub tail, and ptosis. Latency to onset of sleep is defined as the first occurrence of at least 5 min of high-voltage EEG associated with a sleep posture after morphine administration. This parameter has been utilized as an estimate of duration of morphine's effect. Data were analyzed as a function of inbred strain and morphine dose utilizing ANOVAs with repeated measures. Twenty minutes of EEG were also collected on the Hewlett-Packard FM tape recorder following each day's initial morphine dose. Global spectral parameters were extracted and analyzed as described above.

On the eighth day of experimentation, rats received morphine (20 mg/kg) IV; 30 min later, naloxone (1 mg/kg) was injected, IV. Simultaneously, EEG was recorded on the Hewlett-Packard FM tape recorder for a period of at least 1 h. Spectral parameters were derived and analyzed pre- and postnaloxone administration as described above. EEG was also collected on a Grass polygraph for 24 h postnaloxone treatment. Wet-dog shakes, associated with brief high-voltage artifacts in the EEG, were counted for each rat strain for a period of 30 min following naloxone injection. Both strains were also observed for symptomatic signs of morphine withdrawal following naloxone, for example, diarrhea, body stretch.

RESULTS

Significant differences in mean total amounts of stupor (min) as a function of inbred rat strain, $F(1, 8) = 150.7$, $p < 0.05$, and duration of drug treatment, $F(6, 48) = 121.2$, $p < 0.05$, were revealed with an ANOVA with repeated measures. The mean total amount of stupor was greater in LEW than in F344 rats. Inbred rat strain × days of morphine administration interaction factor was significant, $F(6, 48) = 7$, $p < 0.05$. Trend analysis of the data indicated that over the 7 days of morphine administration mean amount of stupor declined in a linear direction for both LEW and F344 inbred rat strains (Fig. 1). The interaction factor was significant because rate of reduction was greater for the LEW group (Fig. 2).

Significant differences in mean latencies to onset of slow-wave sleep as a function of duration of morphine treatment, $F(6, 48) = 10.7$, $p < 0.05$, inbred rat strain, $F(1, 8) = 7.9$, $p < 0.05$, and were revealed with an ANOVA with repeated measures. Inbred rat strain × days of morphine administration interaction factor was significant, $F(6, 48) = 5.5$, $p < 0.05$. Analysis of the data indicated a quadratic trend ($p < 0.05$) in F344 rats (Fig. 3).

Analysis of global spectral parameters utilizing an ANOVA with repeated measures revealed that peak frequency, $F(1, 8) = 9.9$, $p < 0.05$, mean frequency, $F(1, 8) = 8.9$, $p < 0.05$, and edge frequency, $F(1, 8) = 9.9$, $p < 0.05$, differed as a function of inbred rat strain. Regarding days of morphine treatment, with the exception of peak frequency and edge frequency all spectral parameters differed: complexity, $F(6, 48) = 16.3$, $p < 0.05$, mobility, $F(6, 48) = 12.4$, $p < 0.05$, mean frequency, $F(6, 48) = 10.6$, $p < 0.05$, and absolute power, $F(6, 48) = 8.3$, $p < 0.05$ (Fig. 4). A linear trend was indicated in every case ($p < 0.05$). None of the interaction factors were significant.

Number of wet-dog shakes following naloxone treatment differed between rat strains, LEW animals exhibiting a greater amount of this behavior (Fig. 5). This strain also displayed a

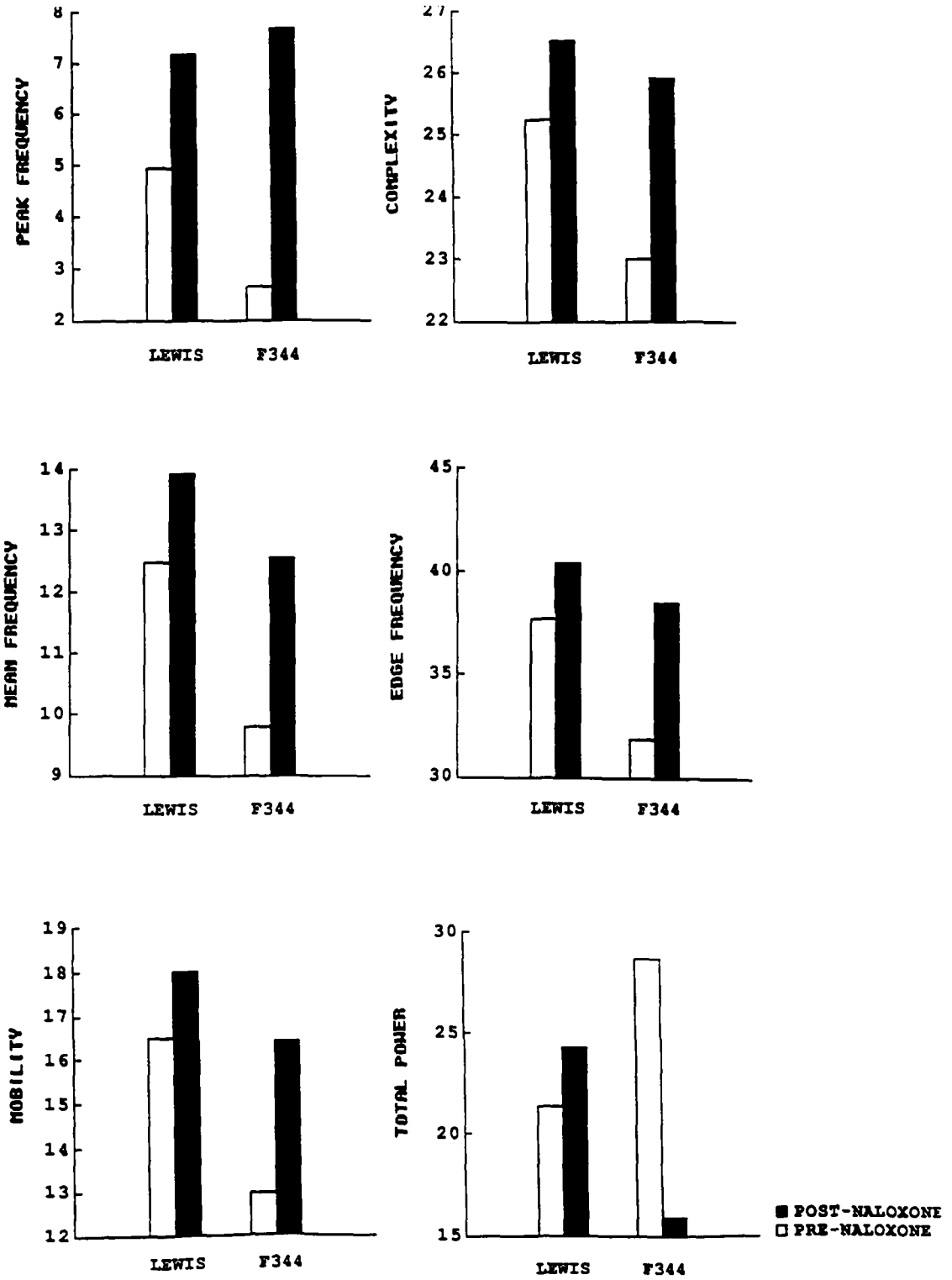

FIG. 6. EEG power spectral parameters are compared before and after naloxone treatment. Data are presented as means.

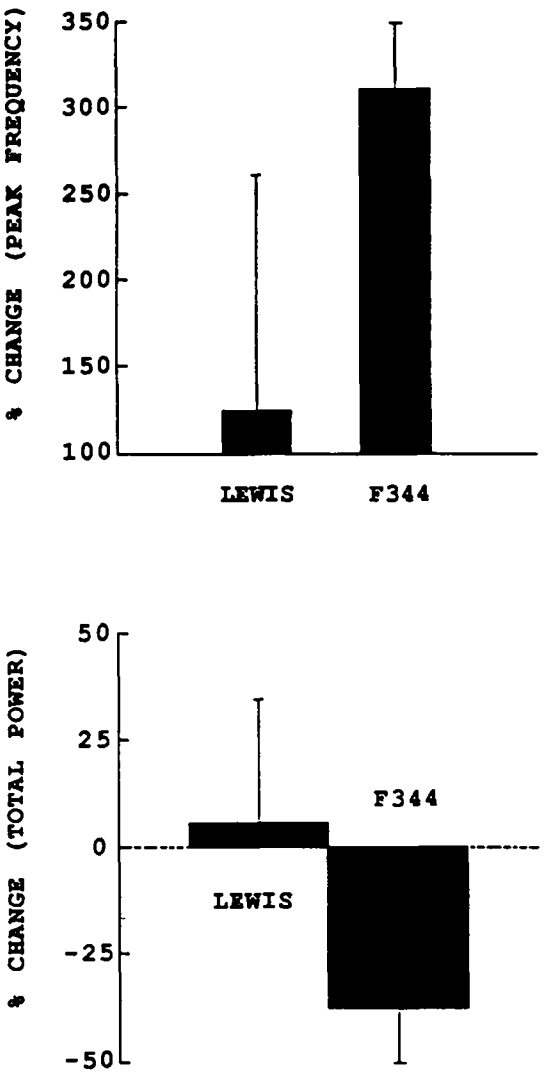

FIG. 7. EEG power spectral parameters are represented as percent change before and after naloxone treatment. Data are presented as means ± SDs.

greater degree of other behavioral effects associated with morphine dependence, for example, diarrhea and sluggish behavior.

No differences were noted with global spectral parameters as a function of inbred rat strain. With the exception of total power, all spectral parameters differed as a function of naloxone treatment: peak frequency, $F(1, 8) = 49.2$, $p < 0.05$, complexity, $F(1, 8) = 6.5$, $p < 0.05$, mobility, $F(1, 8) = 5.9$, $p < 0.05$, mean frequency, $F(1, 6) = 6.5$, $p < 0.05$, and edge frequency, $F(1, 8) = 6.7$, $p < 0.05$. Interaction factor between treatment and inbred group was significant for peak frequency, $F(1, 8) = 7.6$, $p < 0.05$, and total power, $F(1, 8) = 7.6$, $p < 0.05$ (Fig. 6). Furthermore, comparisons of spectral parameters before and after naloxone revealed a greater percent of change in peak frequency and total power for F344 rats (Fig. 7).

DISCUSSION

The purpose of this study was to evaluate and compare the effect of chronic morphine administration on EEG and behavior in two inbred rat strains, LEW and F344. Degree of associated behavioral stupor, latency to slow-wave sleep, and global spectral parameter changes were assessed over the course of 7 days of morphine treatment as a means of gaining insight into tolerance development between the two strains. To discern opiate dependence, behavioral and electroencephalographic effects were compared pre- and post-naloxone administration subsequent to the 7 days of morphine treatment.

Comparison of stuporous response following morphine treatment revealed a greater mean total amount of this behavior in the LEW group. Reduction of stupor occurred across the 7 days of testing in both strains; rate of reduction was greater for LEW rats.

Differences in latency to onset of slow-wave sleep also emerged during chronic morphine injections. Onset to sleep decreased for the F344 group, producing a quadratic profile. This effect could be interpreted as tolerance development.

Of the six spectral parameters quantified during the period of 7 days of morphine administration, three differed between strains, namely, peak frequency (frequency at which the greatest power density occurs), mean frequency (indicative of a central tendency), and edge frequency (reflecting the frequency below which 97% of the total power lies). All these descriptors were greater for F344 rats, indicating a predominance of faster frequencies.

For both LEW and F344 rats, chronic morphine treatment did not result in a change in peak frequency and edge frequency. Complexity, reflecting deviation of the analyzed EEG waveform from a theoretical sine-wave shape, increased. Mobility, a time domain descriptor calculated as a ratio of the second and zeroth spectral moment, increased. Mean frequency, a frequency descriptor based upon the ratio between the first and zeroth spectral moments, also increased over the week of morphine administration. Conversely, total power, defined as the summation of all absolute power spectral density values over the 1- to 50-Hz range, decreased.

Upon naloxone administration, LEW animals exhibited a greater number of wet-dog shakes and associated opiate withdrawal behavior (diarrhea, body stretch), implying greater physical dependence. EEG comparison pre- and postnaloxone injection revealed greater changes in both peak frequency and total power for the F344 group. In a previous report in which we compared opiate withdrawal in morphine- and EKC-tolerant rats, we found that there was a reduction in EEG spectral power in the morphine group but not in the EKC group. We interpreted these results as indicating that there was more physical dependence in morphine-tolerant rats than in EKC rats (8). In the present study, the wet-dog shakes data suggests LEW rats are more dependent. The reduction in total power indicates that F344 rats are more dependent. The reason for this apparent paradox may actually represent the contribution of genetic variability. That is, we have shown that the EEG and behavioral profiles of morphine withdrawal differ as a function of inbred rat strain.

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