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Continuous Quantitative Monitoring of Spontaneous Opiate Withdrawal: Locomotor Activity and Sleep Disorders

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STINUS, L., C. ROBERT, P. KARASINSKI AND A. LIMOGE. *Continuous quantitative monitoring of spontaneous opiate withdrawal: Locomotor activity and sleep disorders.* PHARMACOL BIOCHEM BEHAV **59**(1) 83–89, 1998.—The time course of drug abstinence is not readily amenable to examination using intermittent observations, because abstinence is known to interfere with circadian rhythms of general activity. Accordingly, we propose a model for continuous assessment of spontaneous withdrawal without any intervention by the investigator. This model is based on the automatic recording of locomotor activity. Experiments were performed in rectangular activity cages equiped with two infrared photoelectric cells. In a parallel experiment, to confirm the locomotor activity effects, continuous monitoring of EEG activities was achieved from two cortical and one reference electrodes. Morphine dependence was induced by intraperitoneal injections of increasing doses of morphine twice daily for 10 days (from 5 up to 90 mg/kg). Behavioral and EEG activities were recorded for 8 to 10 days following the last injection of morphine. Although control rats displayed a typical locomotor activity pattern characterized by nocturnal hyperactivity that was markedly reduced during the light phase, opiate abstinent rats developed a constant motor activity during the first 3 or 4 postinjection days and that was associated with a drastic reduction of overall rapid eye movment sleep (REM) and non-REM sleep and with an increase of waking (W). Although morphine-abstinent rats slowly resumed a normal circadian cycle after the fourth day in terms of horizontal activity, REMS, NREMS and W, long-term effects were revealed by the permanent motor instability recorded during both the light and the dark phases when the total amount of photocell counts was considered, and by the perturbation of the circadian rhythm of the ratio of REM sleep to total sleep time. Automatic continuous recording of total motor behavior appears to be a useful index with which to follow, over an extended period of time, the acute and long-term consequences of opiate abstinence. Therefore, long-term withdrawal-induced changes in activity could be a suitable model for the validation of antiabstinence therapies. © 1998 Elsevier Science Inc.

Opiate Dependence Withdrawal Behavior Spontaneous EEG Rat

OPIATE antagonist-precipitated withdrawal in opiate-treated animals may be a reliable animal model for the exploration of the neurobiological basis and treatment of opiate dependence. However, because the duration of the withdrawal state depends upon several factors, including the pharmacological profile of the opiate antagonist, it does not easily allow one to evaluate the time course of the effects of opiate withdrawal. In contrast, spontaneous withdrawal in animals has a definite course of onset and duration, which parallels the abstinence syndrome in human addicts and can be studied over extended periods of time, not only to evaluate the intensity and the duration of the acute abstinence phase, but also to measure long-term effects of opiate abstinence.

Quantitative assessment of signs of withdrawal in opiate dependent rats, as measured either by naloxone-precipitated withdrawal or by abstinence from the drug, reveals a constellation of somatic and behavioral signs with different onset, duration and intensity (9,10) that have different neurobiological substrates (3,4,6,11,17,22,25). Any assessment procedure requires the imposition of some degree of arbitrariness, not

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only in selecting the signs that are to be included but also in ranking the relative importance of each sign (9). One alternative was to develop a withdrawal index method that derives from an ethological analysis of the somatic signs and behavioral changes that the rat displays during opiate withdrawal (8). However, this type of evaluation of an opiate withdrawal syndrome is based upon the intermittent monitoring of the rat's responses, which do not easily permit the continuous evaluation of opiate withdrawal over long periods of time. The purpose of this study was to evaluate a simple behavioral model for automatic, continuous, long-term monitoring of spontaneous opiate withdrawal without intervention by the investigator. Because one of the major complaints of opiate addicts during the first 3 to 4 days following opiate abstinence is sleep impairment, circadian changes in locomotor activity were recorded in activity cages that allowed characterization of horizontal locomotor activity as well as total motor activity generated by morphine dependent rats during 10 days following cessation of the pharmacological treatment. In a parallel experiment, to confirm the behavioral changes, continuous monitoring of sleep patterns was performed during abstinence from morphine.

METHOD

Animals

A total of 42 Sprague–Dawley rats (IFFA-CREDO, Lyon, France) weighing 220–240 g were used (behavioral study, Experiment 1, $n = 30$; EEG study, Experiment 2, $n = 12$). The animals were housed individually in a thermoregulated room (22°C) with a 12 D:12 L cycle (lights on from 0800–2000). Food and water were available ad lib. These conditions were maintained constant during all the experiments. Experiments were performed in accordance with the declaration of Helsinki, the European Communities Council Directives (86/609/EEC, 24 november 1986), and the French Directives concerning the use of laboratory animals (décret No. 87-848, 19 octobre 1987).

Pharmacological Treatment

Morphine dependence was induced in 22 rats by intraperitoneal (IP) injections of morphine sulfate (Sanofi-Francopia, France) twice a day for 10 days according to the following design: 5 mg/kg the first day, 10 mg/kg the second, and subsequent increasas of 10 mg/kg/day to reach a dose of 90 mg/kg per injection on the 10th day. Injections were delivered at 0800 and 2000. The last injection (90 mg/kg) occured on the morning of the 11th day. Morphine was dissolved in isotonic saline (NaCl, 0.9% in water). For the first 4 days the injection volume was 1 ml/kg; for the following days it was increased to 2 ml/kg. Control rats ($n = 20$) received the same injections except that morphine was omitted. Morphine withdrawal was induced by the abstinence of the drug following its chronic treatment.

Apparatus for Activity Recording

Thirty rats were used in Experiment 1 (morphine-treated rats $n = 14$, vehicle-treated rats $n = 16$). Locomotor activity was measured in activity cages ($35 \times 25 \times 25$ cm), whose floor was formed of wire mesh (mesh opening 11 mm) similar to that used for home cages, and whose side walls were made of 10 mm Plexiglas. Two beams of infrared light across the long axis of the cage were 14 cm a part and 3 cm above the floor. They were connected to photoelectric cells so that each passage of the animal interrupted a beam. Each photocell count was recorded by a computer. A computer program analyzed the total number of activity counts (total beam interruptions on both cells) that represented the total motor activity of the animal, as well as the number of crossovers (sequential beam interruptions), which indicated the amount of horizontal locomotor activity generated by the rat.

Three days before the end of morphine treatment, rats were placed in the activity cages and locomotor activity was then recorded continiously for 11 days. The experimental room (permanently ventilated, 10 volumes per hour) contained 30 activity cages. Temperature, dark–light cycle and food and water availability were identical to the animal colony housing conditions. Water was available from a water bottle, and food sticks were provided in a food basket, both attached to front door of the cage, which was made of wire mesh similar to that used for the floor. A metal tray containing a layer of sawdust (3 cm thick) was located under each cage floor (8 cm below) to collect urine and fecal boli; It was not changed during the course of the experiment.

Electrocorticogram Recording and Hypnogram Construction

The real-time sleep–wake scoring system used was previously described (12).

Electrode implantation. Cortical EEG was recorded from two stainless steel screws implanted under general anesthesia over the right parietal cortex (PC) and occipital cortex (OC) and a reference electrode (REF) implanted over the left frontal cortex (relative to bregma, posterior, and lateral coordinates, respectively, PC: −5, 1.5; OC: −11, 0; REF: +4, 2). Electrodes were soldered to a microconnector cemented to the skull with a special glue (Superbond) and dental acrylic resin (16). Each animal was placed in a cylindrical Plexiglas enclosure and rats were connected to the amplifier through a screened cable via a ten channel rotating joint. ECoG recording started 8 days after surgery.

Signal processing. The sleep-wake analysis that was performed discriminated three main vigilance states (rapid eye movement sleep—REMS, nonrapid eye movement sleep—NREMS, and waking—W). The automatic system of electrocorticogram analysis used was previously validated; interscoere reliability with two independent scorers for 24-h tracings of six rats was 83% for REMS, 96% for W, and 97% for NREMS (12). Briefly, the signal was amplified, filtered (bandwidth 3.18–25 Hz) and then fed to a computer via an Intracell S200 data acquisition board. After Analog/Digital conversion (sampling at 512 Hz), the signal was split into 8-s epochs. REMS, NREMS, and W were identifed by analyzing three statistical variables (standard deviation, skewness, and kurtosis) and two harmonic variables (number of zero crossings and number of relative maxima and minima). In the present experiment, an expert observer selected for each rat and for every day recording and each state of vigilance (REMS, NREMS, and W), 100 characteristic epochs to produce a reference model. Using a quadratic distance, the nearest reference model permits one to associate the state of vigilance to the epoch under consideration.

Experimental schedule. After a postoperative 8-day period, the first ECoG recording was performed over 24 h (reference day) following 4 days of habituation to the experimental conditions. As described before, morphine dependence was then induced over the following 10 days. ECoG recording started with the last injection (11th day) and ended after 10 days of morphine withdrawal. Eight morphine- and four solvent-treated rats were used. Results were expressed as the total duration (in seconds) of REMS, NREMS, and W recorded over the 3-h periods. The percentage of REMS out of total sleeping time expressed as %REMS/TST (REMS + NREMS), was

calculated in 3-h steps. Temperature, dark–light cycle, and food and water availability were identical to the animal colony housing conditions.

Statistical Analysis

Locomotor activity. Data were analysed by a three-way ANOVA with one between factor (pharmacological treatment) and two within factors (day postwithdrawal and time of day) followed by post hoc analysis for two × two comparisons (Dunnett's test). To clarify data presentation, results were plotted by 6 h. SEMs were omitted from the figures for clarity but extreme values are indicated in the figure legends.

States of vigilance. For each vigilance state, data were analyzed first by a three-way ANOVA with one between factor (pharmacological treatment) and two within factors (day and time of day). Due to the limitations of our CRUNCH4 statistical program, ANOVA was performed on only the first 6 days posttreatment. Further ANOVAs were performed by the comparison of each withdrawal day to the reference day ("within" analysis) and to data recorded the same day for the control group ("between" analysis). SEM were omitted from figures for clarity but mean values are indicated in legends.

RESULTS

Experiment 1: Rectangular Activity Cages

As indicated before, this device allowed us to analyze both horizontal locomotor activity (by measuring the crossover frequencies), and total motor activity (by summing the number of rear and front photocell counts). Figure 1 presents the evolution of crossovers for both groups of rats.

Control rats developed a biphasic pattern of locomotor activity with numerous crossovers at night and a reduced number during the day, with no difference in rhythmicity between days [ANOVA day 1 to 8: time of day effect, $F(3, 45) = 89$, $p < 0.001$; time of day × day interaction, $F(21, 315) = 1.13$, NS].

When compared to control group data, the evolution of crossovers in morphine-treated rats was disrupted during acute opiate abstinence [ANOVA through the first 6 days posttreatment, group × time of day × day interaction, $F(15, 420) = 4.0$, $p < 0.001$]. Further day by day ANOVA analysis (control vs. spontaneous withdrawal) indicated a statistical difference over the first 4 days, $F(3, 84) > 6$, for each day, $p < 0.005$, as well for the fifth day, $F(3, 84) = 3.65$, $p = 0.0158$. During the first 6 h following the last injection of morphine rats displayed an intense behavioral activation (Dunnett's test, $p < 0.005$). During the next 3 days, crossover frequency stabilized at a level that was intermediate between light and dark phase activity recorded from control animals. At this point, crossover frequency evolved with a circadian rhythm similar to the control group. Activity data were similar for both groups at days 5, 6, 7, and 8 [ANOVA: group effect, $F(1, 28) = 0.84$, NS; group × time of day × day interaction, $F(9, 252) = 1.80$, NS].

When the total number of photocell counts was considered as the dependent variable, striking differences were observed between the two groups of rats (Fig. 2). As seen with horizontal locomotor activity (crossovers), the evolution of global

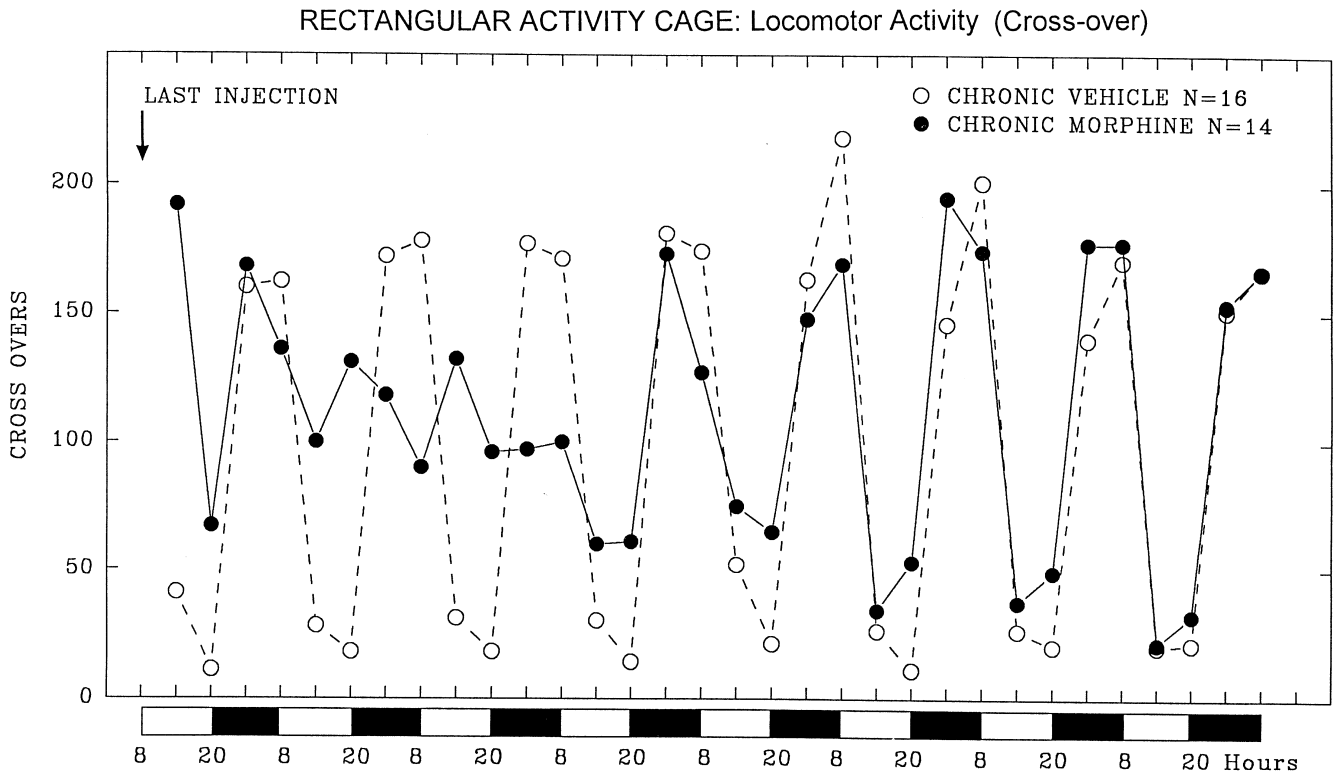

FIG. 1. Time course of horizontal locomotor activity (crossovers) recorded in rectangular activity cages over 8 days following opiate withdrawal. Morphine treated rats ($n = 14$) received increasing doses of morphine (up to 90 mg/kg IP) twice a day for 10 days (black circles and solid lines); vehicle treated rats ($n = 16$) received the same treatment except that the drug was omitted (white circles and dotted lines). Maximum and minimum standard deviation values (±SEM) were 8 and 12% of actitivty values, respectively.

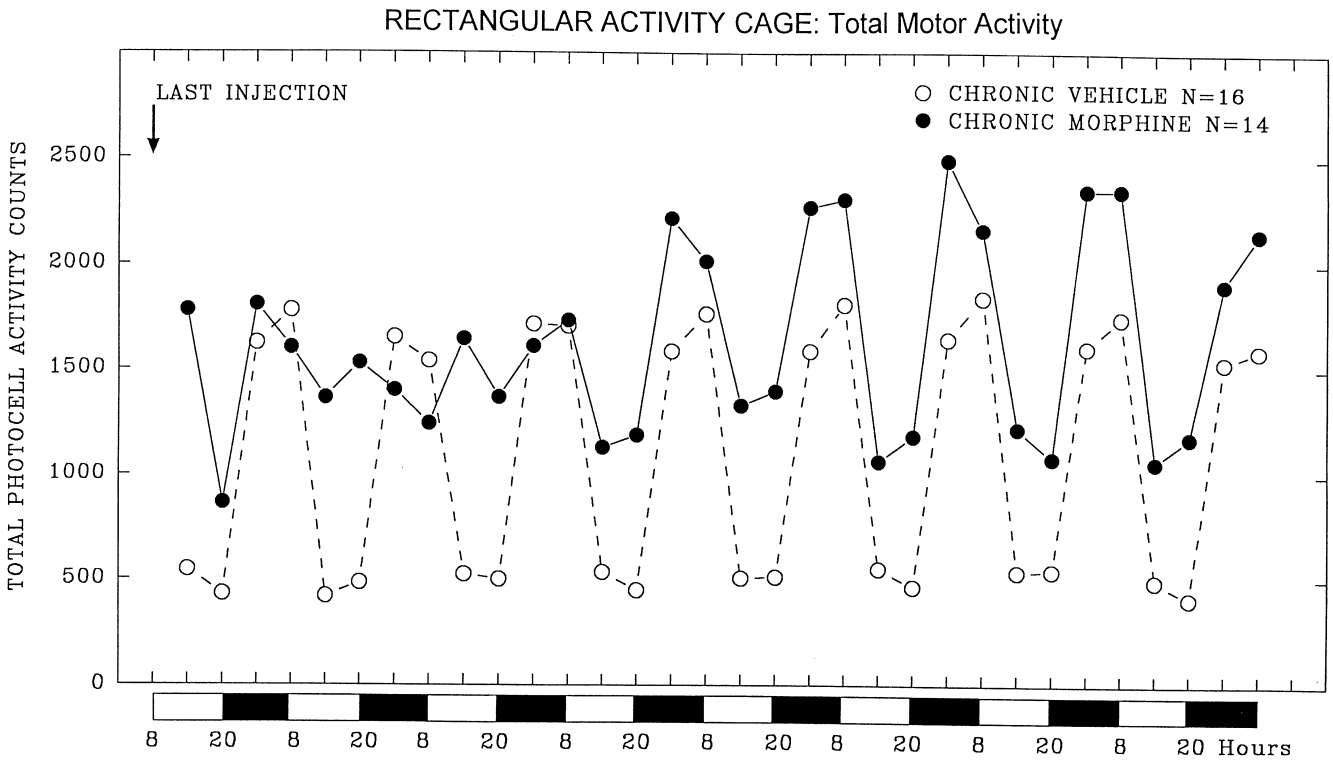

FIG. 2. Time course of total motor activity (total photocell activity counts) recorded in rectangular activity cages during the 8 days following opiate withdrawal. Chronic morphine treated rats: $n = 14$, black circles and solid lines. Chronic vehicle group: $n = 16$, white circles and dotted lines. Maximum and minimum standard deviation values (±SEM) were 8 and 12% of actitivty values. For pharmacological treatment, see legend Fig. 1.

motor activity was disrupted during acute abstinence in morphine dependent rats [ANOVA for the first 6 days: group effect, $F(1, 28) = 45$, $p < 0.001$; group × time of day interaction $F(3, 84) = 24$, $p < 0.001$, and group × time of day × day interaction $F(15, 420) = 5$, $p < 0.001$]. We also observed significant behavioral activation following the last morphine injection. However, during days 1, 2, 3, and part of the fourth following the beginning of abstinence global motor activity stabilized at a very high level, similar to the activity displayed by control rats during the dark phase [group × time of day interaction, $F(3, 84) = 9.4$ (first day), $F(3, 84) = 29.3$ (second day), and $F(3, 84) = 16.9$ (third day); $p < 0.001$ in each case]. Moreover, whereas the locomotor activity circadian rhythm returned to normal after the fourth day of abstinence, rats that underwent spontaneous opiate withdrawal were significantly more active than control rats, both during the dark phase and the light phase [group effect: day 5, $F(1, 28) = 19.6$, $p < 0.001$; day 6, $F(1, 28) = 10.4$, $p < 0.003$; day 7, $F(1, 28) = 22.2$, $p < 0.001$, and day 8, $F(1, 28) = 16.2$, $p < 0.001$].

Experiment 2: Sleep–Waking Analysis

Figures 3 and 4 depict the time course of W, NREMS, and REMS episods as well as the percentage of REMS over total sleep, during the reference day (before any pharmacological treatment) and during the 10 days that followed the last injection of morphine (spontaneous withdrawal). Overall analysis for the 11 days of recording indicated a significant group × time of day × day interactions for the three states of vigilance, $F(70, 700) > 4.4$, $p < 0.001$ for W, NREMS, and REMS).

For control rats, the ANOVA indicated a perfect stability of each state whatever the day of recording or the time of the day all along the 11 days of testing. Moreover, further comparisons indicated that posttreatment data (days 1 to 10) were identical to the reference day recording for W, NREMS, and REMS.

For morphine-dependent rats, the overall analysis (reference day + 10 days of abstinence) indicated significant interactions between the time of day × day [W: $F(80, 490) = 20$, $p < 0.001$; NREMS: $F(80, 490) = 16$, $p < 0.001$, and REMS: $F(80, 490) = 12.6$, $p < 0.001$]. As a consequence of the last morphine injection, we observed first a 3-h period of behavioral activation that was associated with a decrease of NREMS and REMS followed by a period (6 h), during which W was decreased and NREMS and REMS increased. During the second day of abstinence, REMS episodes were reduced to less than 2%, NREMS was intermediate between the light and dark phase levels of control rats, and W increased to reach a peak at the beginning of the third day. During days 2, 3, and 4 of opiate abstinence, the sleep circadian rhythm was completely disrupted. The natural rhythm reappeared at the end of the fourth day and then resumed from that point on. Comparisons between each posttreatment day and the reference day (within-subjects comparison) showed statistical differences in the amount and evolution of the three vigilance states. For W and NREMS, statistical differences were detected from days 1 to 5 [time of day × day interaction: W, $F(7, 49) > 14.2$, $p < 0.001$ for days 1, 2, 3, and 4, $F(7, 49) = 2.5$, $p = 0.027$ for day 5; NREMS, $F(7, 49) > 18$, $p < 0.001$ for days 1, 2, 3, and 4, $F(7, 49) = 3.7$, $p = 0.026$ for day 5). For REMS,

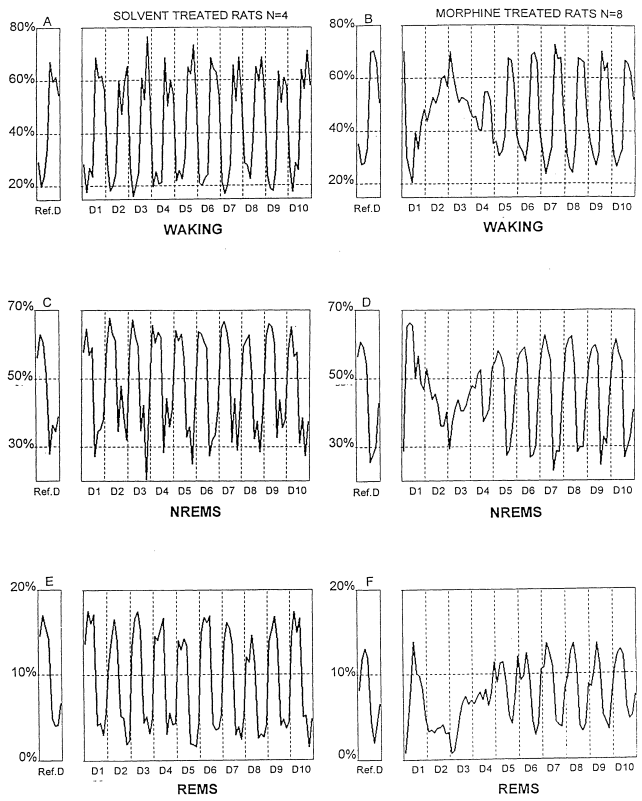

FIG. 3. Time course of the three main vigilance states waking W (A and B), nonrapid eye movement sleep NREMS (C and D), and rapid eye movement sleep REMS (E and F) before any pharmacological treatment (reference day: Ref.D) and OVER 10 days following opiate withdrawal (D1 to D10>). Each recording day began at 0800 h. The last injection of morphine or VEHICLE occured at 0800 h on day 1. Left panel: control group: A, C, and E ($n = 4$). Right panel morphine dependent group: B, D, and F ($n = 8$). Each figure first displays data from a reference day (Ref.D) recorded just before the pharmacological treatment, followed by the abstinence period (10 days, D1 to D10). Results were expressed as % of total time (3-h periods). To clarify data presentation the SEMs were omitted. At most, the SEMs it represented 3% of W, 4% of NREMS, and 2% of REMS. For information regarding the pharmacological treatment, see the legend for Fig. 1.

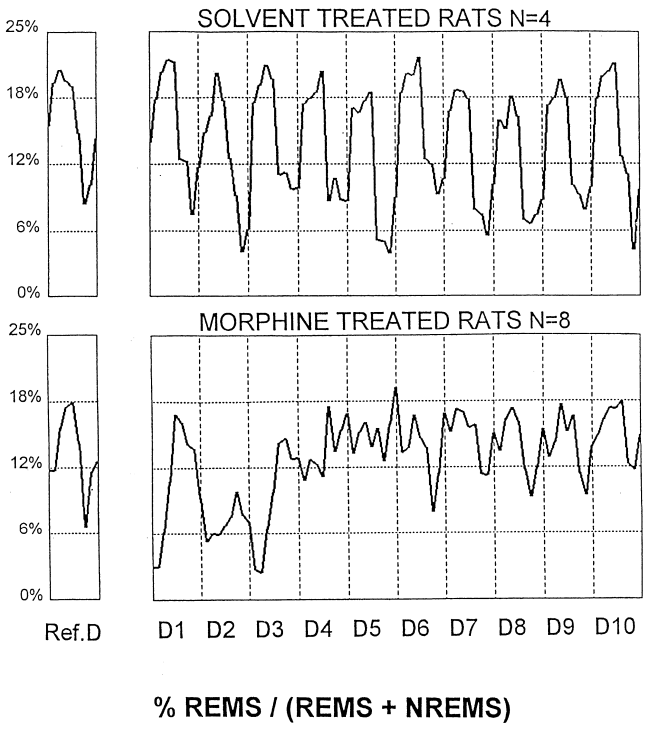

FIG. 4. Time course of REMS presented as percent of total sleep time (REMS + NREMS) (% REMS/TST) during a first reference day (Ref.D) recorded just before the pharmacological treatment, and during the first 10 days following abstinence (D1 to D10). The last injection of morphine or solvent occurred at 0800 h on day 1. To clarify data presentation, SEMs were omitted; at most, they represented 2.2%. For information regarding the pharmacological treatment, see the legend for Fig. 1.

statistical differences were noted from day 1 to day 6 (time of day × day interaction $F(7, 49) > 7.1$, $p < 0.001$ in each case). During the following posttreatment days (7 to 10) no difference in the amount or the evolution of W, NREMS, and REMS was recorded. The day by day comparison between morphine dependent rats and saline-treated rats (between-subjects comparison) revealed exactly the same differences and statistical results as those obtained with the within analysis method.

The overall comparison of the time course over the 11 days of recording (1 before and 10 after treatment) of the percentage of REMS over the total sleeping time (%REMS/TST) for solvent and morphine-treated rats indicated significant interactions between treatment × day $F(10, 100) = 5.5$, treatment × time of day $F(7, 70) = 42$ and treatment × day × time of day ($p < 0.001$ in each case) (Fig. 4). Considering the vehicle-treated rats, the data indicated the stability of %REMS/TST throughout the 11 days of recording. Moreover, this parameter was very stable both through the light and the dark phases (mean values over the 11 days: light 19.5 ± 0.4%, dark 9.4 ± 0.5%). In contrast, the circadian evolution of %REMS/TST was disrupted following morphine abstinence; during the first day it was inverted when compared to control rats. From that point on, for a day and a half, %REMS/TST was gradually reduced to a minimal value of 6%. After the fourth day of abstinence, %REMS/TST stabilized to a value between 13 to 17%, with significant small variations between the light and dark phases ($F(35, 245) = 2.54$, $p < 0.001$).

DISCUSSION

In the present study, we developed and tested a long-term reliable animal model for continuous monitoring of spontaneous opiate abstinence. This model did not require any intervention by the investigator and involved the recording of motor and locomotor activity of rats. It was seen that disturbances in the circadian cycle of activity paralleled sleep disorders during acute abstinence and postabstinence phases.

Control rats that received no morphine displayed a pattern of locomotor activity that was characterized by hyperactivity during the dark phase and hypoactivity during the light phase. In contrast, opiate abstinent rats showed a constant level of motor activity over the first 3 or 4 postinjection days. Along with thisdisruption in the day/night pattern of locomotor activity, there was a drastic reduction of overall REMS and NREMS and an increase of W. These early disturbances of locomotor activity could be considered as being representative

of acute opiate abstinence because they paralleled the time course of somatic and behavioral symptoms of acute withdrawal in morphine dependent animals (18,22).

Morphine abstinent rats slowly resumed a normal circadian cycle of activity after the fourth day postinjection. This was seen for horizontal activity, REMS, NREMS, and W. However, long-term effects were revealed by a permanent motor instability recorded in rectangular activity cages during both the light and the dark phases. This was reflected in total photocell counts, and by the changes in the circadian rhythm seen in the %REMS/TST ratio.

Measurement of horizontal locomotor and total motor activities indicated that changes in these behavioral parameters evolved differently over the course of abstinence. In accordance with previous results (1,5,23,24), we confirmed that during acute opiate abstinence horizontal locomotor activity was reduced during the dark phase, whereas it increased during the light phase. Interestingly, overall motor activity during opiate abstinence, as measured by total photocell interruptions, also was reflective of a prolonged motor instability. During the first 4 days following the last injection of morphine, overall motor activity during the dark and light periods equalled the dark phase motor activity of control rats.

During acute abstinence behavioral disturbances were associated with a dramatic decrease of REMS and NREMS and increased W. This was similar to observations of other investigators who used different techniques to produce morphine dependence (7,14,20). However, in our experimental conditions the short-lived REMS rebound reported elsewere was not detected (7,14).

Following the acute withdrawal phase, there were no apparent protracted effects of morphine dependence on measures of horizontal locomotor activity (Fig. 1) or REMS or NREMS or W (Fig. 3). However, total motor activity and %REMS/total sleep revealed long-term effects of opiate dependence. Thus, Fig. 2 shows that after the acute phase of abstinence (first 3 to 4 days after withdrawal), motor instability persisted during the subsequent testing days. The total motor activity of abstinent rats remained higher than normal (twofold) both during the dark and light phase even though the normal circadian rhythm was reestablished on the fourthy. From Fig. it is seen that on the fourth and fifth postinjection days %REMS/total sleep ratio was completely erratic. Subsequently, there was a slow reestablishment of a regular circadian rhythm; however, it never reached the amplitude of the control rats. These protracted effects of morphine dependence are in accord with several reports. For example, Martin et al. 18) reported that from day 4 to day 63 after withdrawal from morphine treatment, rats develop an increased respiratory rate, metabolic rate, and body temperature, as well as decreased fluid intake. In addition, postdependent animals show tolerance to the depressant effect of high doses of morphine 15 days, and 1 year, after morphine abstinence. This was seen as a reduction, compared to controls, in early EEG activity and behavioral stupor with subsequent increases in EEG activity and behavioral arousal (2,5,13,26). Moreover, whereas naloxone has no effect on locomotor activity of control rats, it has a depressant effect in postdependent rats long after morphine treatment has been stopped (5).

In conclusion, continuous recording of rats' activity in photocell cages during abstinence following chronic morphine treatment offers a reliable method for assessing the duration of acute and long-term consequences of morphine dependence. Measurement of the total photocell counts allows the detection of a permanent behavioral instability, which is present not only during the acute phase of the abstinence (4 days), but also at intervals up to 10 days after withdrawal and beyond. This animal model of short- and long-term effects of opiate abstinence is easy to implement. Furthermore, for the study and evaluation of the efficacy over time of putative antiabstinence therapies, the present method may be more appropriate than the intermittent observation of rats. It should be noted, however, that further studies are needed to characterize the exact nature of the behavioral instability recorded during the postdependent period and to systematically evaluate the ability of our experimental procedure to reveal long-term effects over a period of time greater than 10 days.

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