Pharmacology Biochemistry & Behavior, Vol. 21, pp. 923–928, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Reassessing Morphine Effects in Cats: II. Protracted Effects on Sleep-Wakefulness and the EEG

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Received 21 February 1984

DE ANDRES, I., J. R. VILLABLANCA AND J. W. BURGESS. *Reassessing morphine effects in cats: II. Protracted effects on sleep-wakefulness and the EEG.* PHARMACOL BIOCHEM BEHAV 21(6) 923–928, 1984.—Adult cats were implanted with standard electrodes to record EEG, EOG, and EMG. After 15 days, morphine sulphate or saline placebo was given IP at 0.5, 1.0, 2.0, 3.0 mg/kg, at least 15 days apart. Cats were continuously recorded for 72 hr postinjection. Wakefulness, drowsiness, NREM and REM sleep percentages were scored from polygraphic features and statistically analysed. There was a dose-dependent suppression of NREM and REM sleep for at least 6 hours postmorphine, with a progressive sleep recovery thereafter. During the insomnia period there was an EEG/behavioral dissociation where bursts of high-voltage waves were seen over a background of desynchrony; meanwhile the animal was first aroused although quiet and later showed stereotypic behavior. There was a prolonged NREM sleep rebound which started later at the higher doses. A significant, relatively brief REM sleep rebound was seen only at the lowest dose. The latency for NREM and REM sleep onset was also dose-dependent. Possible brain sites of morphine actions and similarities with effects in other species are discussed.

Morphine Sleep-wakefulness EEG Cats Behavior

THIS study investigates the effects of single doses of morphine on the sleep-wakefulness cycle of the cat throughout a 3-day period. In particular, polygraphic recording events are contrasted with the behavioral manifestations that typify a range of drug doses [2, 29, 30].

In the bulk of the literature reports on this topic, observations are restricted to the same day of drug administration, and there is little or no attention paid to concurrent behavioral data. In general, studies of the short-term effects of opiate administration in humans [9, 10, 16, 21], dog [19, 22, 32], cat [5, 15, 18, 24], rabbit [13,18], and rat [14] find suppression of both non-rapid-eye-movement (NREM) sleep and rapid-eye-movement (REM) sleep. Furthermore, some types of EEG/behavioral dissociation have been described during the insomnia period, since the observed electrical patterns differ from the fully desynchronized EEG that is present during normal wakefulness (see Discussion). However, this phenomenon was not reported for the cat. Thus, we here report on the initial insomnia period and the EEG/behavioral dissociation following morphine administration, and the complex time-course of sleep-state recovery ensuing during the subsequent days. This full report follows an earlier abstract [4] and contributes to the description of the neurological and behavioral effects of low doses of morphine presented in the first paper of this series.

METHOD

Subjects

Under general anesthesia (ketalar 7 mg/kg; pentobarbital 22 mg/kg, IP), 7 adult male cats were implanted with neocortical, lateral geniculate, orbital, and neck muscle standard electrodes to record EEG, EOG, and EMG, respectively. Using the coordinates of the Reinoso-Suárez atlas [23], the neocortical electrodes were stereotaxically placed over the sensorimotor and primary visual cortices: i.e., 2 mm rostral to Bregma and 10 mm from the midline; 21 mm caudal to Bregma and 2 mm from the midline. An additional reference electrode was placed near the midline over the frontal sinus. These electrodes were terminated in an Amphenol strip connector and secured in a pedestal of dental cement.

Fifteen days after surgery, each cat was acclimated for 3 or more days to the presence of the recording cable and placed in the sound-attenuated, ventilated recording chamber where it could be observed through a 1-way mirror. After habituation, 4 cats received a single IP injection of

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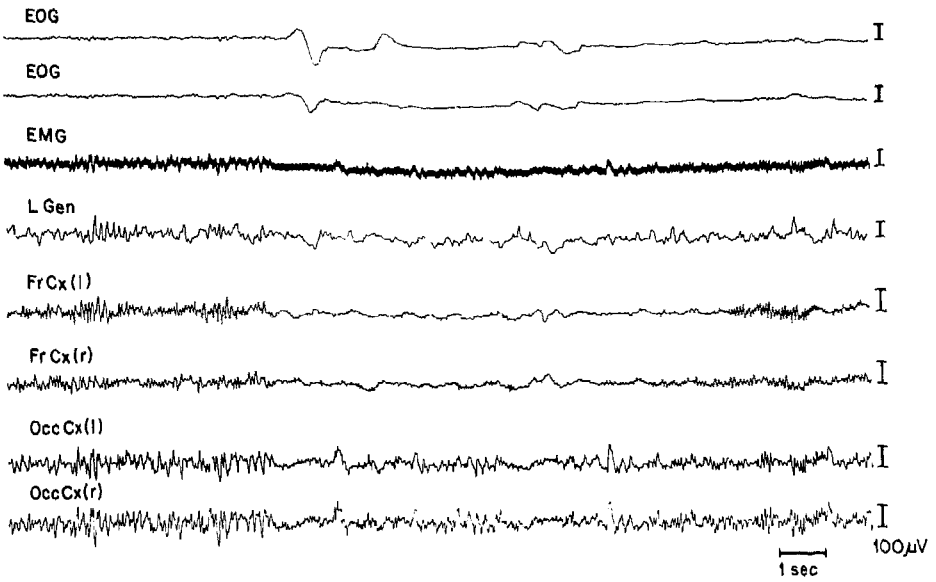

FIG. 1. Polygraphic recording taken from a cat 90 min after injection of morphine sulphate at 3.0 mg/kg dose. Note bursts of higher voltage in the electrocorticogram while the cat is behaviorally awake (see EMG, EOG). KEY: EOG=electrooculogram; EMG=electromyogram; LGen=lateral geniculate body; Fr Cx=frontal cortex; Occ Cx=occipital cortex; l=left; r=right. All recordings are bipolar with the reference electrode.

morphine sulfate at each of the doses of 0.5, 1.0 and 2.0 mg/kg, at least 15 days apart in a balanced administration design. The remaining 3 cats received a single injection at 3.0 mg/kg. All cats also received a single placebo injection of saline vehicle given prior to and/or following all morphine treatment. Two cats received an additional treatment with naloxone (1 mg/kg, IP) given 2 hr postmorphine.

Recording

Polygraphic records began at 10:00–11:00 (1 hr before injection), and continued throughout the next 72 hr. All records were made in the sound-attenuated chamber under an automatic 12/12 hr light/dark photoperiodic regime (lights on: 7:00). Animals were fed and cleaned briefly around 9:00 each morning and were left undisturbed throughout the remainder of the day.

In order to correlate behavioral stages with polygraphic events occurring during morphine administration, observations were made continuously for 6–7 hr after morphine administration on the first day, and for a few min every hr during the remaining recording time (except for about 8 hr during the 2nd and 3rd nights).

To compute the time spent by each cat in different behavioral states, recordings were scored according to the observed behaviors and the established polygraphic features of wakefulness, drowsiness, NREM sleep, and REM sleep [25].

Statistical Analyses

The percentage time spent each hour in each behavioral state was calculated for each cat. Pearson's product moment correlation coefficients were used to compare morphine dose level with the onset latency of NREM sleep and REM sleep. Two-factor, repeated measure analyses of variance for unweighted means (dose × time × subjects) [33] were used to compare the averages of every 6 hourly samples. Multiple pair contrasts were made using Tukey's pairwise comparison test [6]. All comparisons were between values for each of the doses and the placebo group values.

The observed EEG patterens closely followed the three characteristic successive stages of response to low doses of morphine in the cat (see [2, 30, 31]), and will be described accordingly. The polygraphic recording technique, including the presence of the connecting cable, did not visibly alter these previously described stages.

RESULTS

For approximately the first 10 min (while the animal exhibited mainly autonomic effects like salivation, licking, retching and vomiting), the EEG was fully desynchronized. After this "autonomic" stage [29,30] the cat calmed down and adopted a predominantly sitting or crouching posture with relatively fixed gaze, which contributed to relatively artifact—free records. From the onset of this "quiet stage" period [29,30], bursts of higher-voltage waves appeared against the background of EEG desynchrony. A representative recording (Fig. 1) shows that from the sensorimotor cortex these bursts have very regular waveforms with an amplitude of 50–100 μV, a frequency of 13–16 Hz, and a duration of about 2–6 sec. Desynchrony was less clear in the primary visual cortex, where these bursts of EEG activity were mixed with other, more irregular bursts of higher amplitude (100–150 μV) and slower frequency (9–10 Hz). This EEG activity was not abolished during the pinna movements or occasional eye movements occuring during this stage, although slight voltage decreases were observed.

Within 15 to 30 min, depending on the dose, a characteristic kind of head movement occurred in cats receiving these doses of morphine [29,30]. When fully developed by the end of the first morphine hour, these movements are best described in terms of visual behavior: they are complex

movements of the head which look as if the animal were visually tracking an object moving rapidly in his visual field and following an unpredictable trajectory. Although it also has other behavioral components, fully described in the first paper of this series, we have labeled this period the "head movement stage" of the morphine effect [29,30]. With the onset of the "head movement stage," the patterns of EEG activity already described continued but during bouts of head and body movements, long epochs of EEG desynchronization occurred in the sensorimotor cortex and clear decreases in burst amplitude were visible in the primary visual cortex (Fig. 1). Similarly, when the cat was involved in other behaviors such as rubbing or eating, EEG desynchrony was present.

The administration of naloxone 2 hr after morphine injection blocked the EEG and behavioral effects, and the cat entered into a state of drowsiness followed by NREM or even REM sleep with the concomitant polygraphic patterns characteristic of these states. Approximately 2 hr later, cats woke up and again exhibited fixed gaze and the previously described EEG pattern with high-voltage bursts.

The peculiar EEG activity seen during the initial hours of morphine administration was not an obstacle for identifying the behavioral components of the sleep/waking cycle. Initially, cats were strongly aroused and sitting, exhibiting fixed gaze and characteristic head movements during the morphine-typical EEG pattern. By the time sleep was first seen, the morphine-typical EEG rhythms were no longer present.

Sleep reappeared within 24 hr after morphine administration. The onset latency for NREM sleep was significantly correlated with drug dose (r=.83; $t(23)=7.137$, $p<0.00001$), as was the onset latency for REM sleep (r=.92; $t(23)=11.258$, $p<0.00001$). These values are shown in Table 1.

The percentage time cats spent in REM sleep was significantly dose-related throughout the entire experiment ($F(4,20)=5.077$, $p=0.005$). Moreover, the dose-dependency of all behavioral states changed significantly throughout the course of the 72 hr experiment, as shown by interaction effects between dose × time for waking, $F(44,220)=6.273$, $p=0.001$; drowsiness, $F(44,220)=1.626$, $p=0.01$; NREM sleep, $F(44,220)=3.335$, $p=0.001$; and REM sleep, $F(44,220)=1.502$, $p=0.03$.

The details of the S-W effects at the different doses are shown in the histograms of Fig. 2 where the percent time spent in each S-W stage during the 72 hr of the experiment is plotted. Changes in each 6-hr period were examined with multiple comparisons tests.

NREM sleep was significantly depressed at all dose levels for at least 13 hr compared to baseline placebo values ($p<0.01$; Fig. 2). At all but the highest dose, NREM sleep subsequently returned to placebo levels and a rebound (defined as a significant increase over baseline values) began as early as 18 (0.5 mg/kg) or 24 hr (1.0–2.0 mg/kg) postdrug. The time course of the highest dose was more protracted; at 3.0 mg/kg, NREM sleep was depressed for 24 hr ($p<0.01$), returned to placebo values, and showed significant rebound 30–42 hr after injection ($p<0.05$).

REM sleep was also depressed 0–12 hr after drug at all doses ($p<0.05$–0.01; Fig. 2) but a rebound was seen only with the lowest doses, and occurred only for the 30–36 hr epoch ($p<0.01$). At the higher doses, significant depression of REM sleep lasted longer (up to 18 hr for 1.0–2.0 mg/kg and up to 24 hr for 3.0 mg/kg) such that baseline levels were recovered later on but there was no rebound.

TABLE 1

ONSET LATENCIES OF NON-RAPID-EYE-MOVEMENT SLEEP (NREMs) AND RAPID-EYE-MOVEMENT SLEEP (REMs) AFTER ADMINISTRATION OF DIFFERENT DOSES OF MORPHINE SULPHATE

Dose	(mg/kg)	NREMs	(hr)	REMs (hr)
0.5	(N=4)	8.0	(±2.2)	10.0 (±2.4)
1.0	(N=4)	9.5	(±1.3)	13.5 (±2.5)
2.0	(N=4)	10.8	(±1.0)	15.0 (±4.0)
3.0	(N=4)	12.0	(±2.6)	25.3 (±1.1)
Placebo	(N=10)	0.1	(±0.3)	1.5 (±1.7)
			r=.83*	r=.92†

Latency values are means and standard deviations for the experiments with each dose, r=correlation coefficient; *$p<0.01$, †$p<0.001$.

Drowsiness showed a similar depression 0–6 hr postdrug at all doses ($p<0.01$) but a drowsiness rebound was found only after the highest dose between 12–24 hr ($p<0.05$–0.01); other changes are shown in Fig. 2.

Wakefulness increased at the expense of total sleep during the first 12 hr at all doses ($p<0.01$) and together with drowsiness during the first 6 hr, and only at the expense of total sleep 12–24 hr at the highest dose ($p<0.01$). A significant late decrease in wakefulness (concurrent with NREM sleep rebound) was found 30–42 hr postdrug only at the highest dose ($p<0.05$).

In summary, insomnia or hyposomnia for both NREM and REM sleep were seen after morphine at all doses and the latency, duration and magnitude of these effects directly depended on the dose. A 12 hr duration NREM sleep rebound was also seen at all doses and the latency to onset of this effect was likewise dose-dependent. REM sleep rebound was minimal and seen only with the smaller dose and after NREM sleep rebound. No changes from baseline were evident after 42 hr. In addition, we showed a partial EEG-behavioral dissociation lasting for the entire period of specific discrete behavioral effects and continuing for most of the insomnia period.

DISCUSSION

The high-voltage, slow-frequency bursts described from our polygraphic recordings have not previously been reported for the cat. Other authors [5, 15, 24] described no EEG abnormalities with smaller doses. Increased desynchrony after morphine (1–10 mg/kg, IP) was reported by Navarro and Elliot [18]; however, their preparation differed from ours in that a number of their animals were under gallamine (with artificial respiration) and in that their recording sessions were apparently fairly brief.

Additional data have been reported from experiments with other species, notably man [9,10] dog [22,31] and rat [1, 13, 14, 17, 26]. In rats, Khazan [12] described bursts of high-voltage, slow EEG activity similar to our results; these bursts increased in frequency of occurrence 1–1.5 hr post-morphine, and decreased 2–3 hr postinjection. The first period was coincident with a state characterized as behavioral depression, where the animal "is awake with the eyes open, but is immobile and stuporous," while during the second period the rat exhibited increased motor activity and

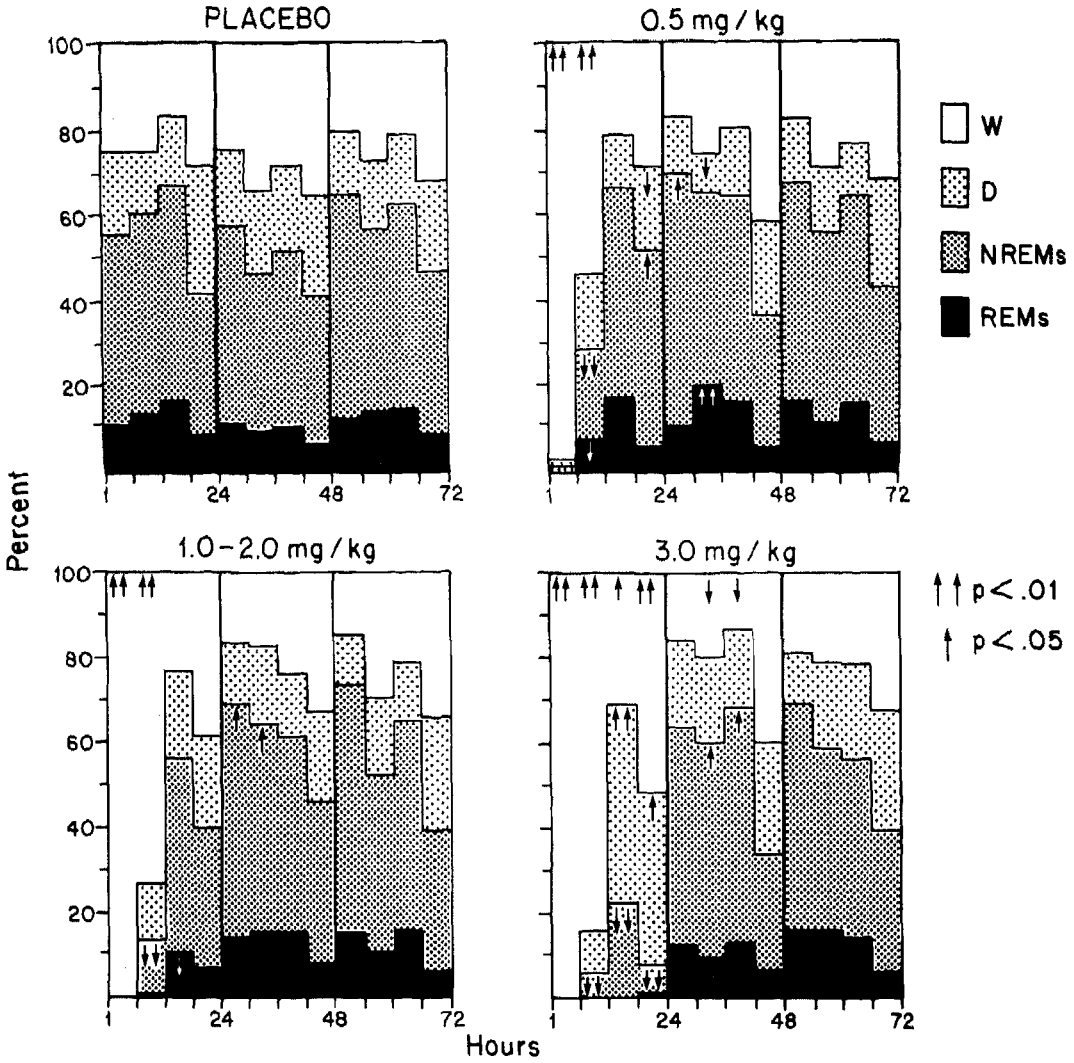

FIG. 2. Frequency histograms of mean percent values of the sleep-wakefulness states during 6-hr epochs recorded throughout 3 days following the administration of saline placebo or morphine sulphate in the doses indicated at the top of each graph. KEY: W=wakefulness; D=drowsiness; NREMs=non-rapid-eye-movement sleep; REMs=rapid-eye-movement sleep; Percent: cumulative percentage of the four behavioral states for each 6 hr epochs; the individual percentage for each of the states is indicated by the different shadings in each of the bars.

stereotypic behavior. These two behavioral states appear to correspond to the "quiet stage" and "head movement stage" that we have described in the cat. Similar observations in man [9,10] and dog [19,22] are also reminiscent of our data, suggesting that the morphine-induced EEG phenomena described here may transcend species barriers.

Little information is available to determine the CNS site of morphine action responsible for the EEG effects here discussed. These effects share some similarities with the EEG/behavioral dissociation described after atropine administration by ourselves and others [11,28] and may shed light on the underlying mechanisms involved. With both agents, drug-induced EEG synchrony is disrupted by strong behavioral activation in the treated animals [22, 24, 28] and thus, this peculiar EEG activity may reflect increased tendency to synchrony in neocortical circuitry. Indeed, the atropine effect on EEG persists in the permanently isolated forebrain [27], as well as in the isolated hemisphere of the cat [11,28], suggesting that the atropine effect is mediated by direct action of the drug on neocortical circuitry. In a preliminary experiment with morphine, we have seen drug-induced bursts in the surgically isolated forebrain of one cat. Thus, a similar research stategy may help to extend this analogy and clarify the action of morphine on EEG and behavioral phenomena.

The period of insomnia that we found immediately following morphine administration agrees with all reports in the literature describing the same phenomenon in all studied species, including cats (see Introduction). We now present additional evidence that the duration of sleep suppression is dose-dependent. For example, NREM sleep appeared first under all doses, and its latency was dose-dependent. Perhaps the only other report of dose-dependency comes from studies by Kay *et al.* [9,10] of the first night following mor-

phine in post-addicted men: their published data show a statistical dose-dependency of sleep suppression, at least for REM sleep.

In contrast to the early, prolonged rebound of NREM sleep, it is interesting that significant REM sleep rebound was seen only in a single epoch at the smallest dose. In shorter (8–24 hr) experiments, using only a single, smaller dose (0.3 mg/kg), Echols and Jewett [5] observed significant sleep rebound only for REM sleep in the cat. Thus, although we have no explanation for the phenomenon, their data fit our finding of a lower-dosage REM sleep rebound. In addition, compared to NREM sleep, the total amount of recovered REM sleep was modest, only slightly exceeding baseline levels for all doses and times following the initial decrease. Therefore, to the extent that NREM sleep recovers before and to a greater extent than REM sleep, it seems that sleep rebound following morphine is not markedly different from the rebound seen after other sleep-suppressing agents [7,16].

The morphine-induced suppression of both NREM and REM sleep appears to be an important generalized phenomenon present across species. This suggests that morphine posseses an overall, short-term excitatory action upon arousal in all animals including man, which is only later on compensated for by depression of arousal and consequent increase of sleep during rebound. The post-morphine NREM and REM sleep suppression could be masked immediately after injection by other events including apparent motor inhibition and drowsiness [8], as well as peculiar EEG patterns. We suggest that it is the magnitude and threshold of the latter events which contribute to the differences between species, thereby conveying an exaggerated sense of species differences in morphine effects. For example, strong motor inhibition in the rat can explain the "stuporous" state following injection that has strengthened the concept of "depressive" effects of morphine on this species.

We are now attempting to locate the brain sites underlying the sleep-suppressing effects of morphine. In experiments preliminarily reported [3] we showed that, with some qualifications, a similar sleep suppression is seen in cats permanently deprived of telencephalon (diencephalic). More recently (unpublished) we have observed that post-morphine insomnia was also present in 2 chronic decerebrate (mesencephalic) cats. Therefore, morphine action on REM sleep depends upon basic lower brainstem processes controlling this state of sleep. Further investigation of the differences between the diencephalic and mesencephalic animals may help clarify this issue as well as our understanding of morphine effects on NREM sleep.

ACKNOWLEDGEMENTS

This research was supported by USPHS Grants DA-02518 and HD-04612, and FISSS, Grant 51881, Spain. We wish to thank S. Belkin for her help with the illustrations.

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