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Dose-Dependent Dual Effect of Morphine on Electrophysiologic Correlates of Positive Reinforcement (Reward Contingent Positive Variation: RCPV) in the Cat

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MARCZYNSKI, T. J. AND J. T. HACKETT. *Dose-dependent dual effect of morphine on electrophysiologic correlates of positive reinforcement (Reward Contingent Positive Variation: RCPV) in the cat.* PHARMAC. BIOCHEM. BEHAV. 5(2) 95–105, 1976. – In cats trained to press a lever for milk reward, the postreinforcement EEG synchronization (PRS) and the associated epicortical steady potential shift, known as "Reward Contingent Positive Variation (RCPV) restricted to the occipital cortex, were studied prior to and after administration of morphine sulfate. Contrary to what has ordinarily been described as a typical feline response to morphine, such as restlessness, aggressiveness, rage, and exaggerated startle reaction to environmental stimuli, associated with an increased tonus of the brainstem-hypothalamic arousal system and desynchronized EEG patterns, doses of 0.1–0.4 mg/kg, IM, caused a strong monophasic enhancement of the PRS-RCPV phenomenon. Doses of 0.6–1.0 mg/kg, IM, had clearly a biphasic action: the initial enhancement of the PRS-RCPV responses was followed by their strong suppression. Chlorpromazine (0.1–1.6 mg/kg, i.e.) promptly restored the EEG responses. During the peak effect of the enhancing doses of morphine, the reward-related EEG phenomena also occurred prior to or after the nonrewarded bar press when the animals licked the empty cup. This dissociation of the PRS-RCPV from consumption was much more conspicuous in animals whose control frequency of the PRS oscillations was higher, and after morphine showed more significant slowing. Despite the strong facilitation of the PRS-RCPV in the presence of light, morphine, in contrast to LSD-25, was not able to restore the reward-induced phenomena in the dark.

Reward Contingent Positive Variation (RCPV) Post-reinforcement EEG synchronization (PRS) Morphine-induced enhancement

IN INTACT cats, doses of morphine smaller than 1 mg/kg given parenterally have been reported to have no measurable effects on overt behavior, electroencephalographic activity (EEG), and evoked potentials to central or peripheral nociceptive stimulation recorded in various brain regions [5, 10, 21, 22, 27]. On the other hand, early reports [7,29] claim that the sensitivity of intact cats to morphine, as judged by overt gross behavior, approaches that of man, and doses as small as 0.1–0.2 mg/kg, SC, have sedative effects, whereas larger doses of 2–5 mg/kg cause behavioral excitement. Such a dual action of morphine was also demonstrated in acute decorticated cat preparation in which doses of 2–5 mg/kg, IV, depressed "sham rage", in contrast to the excitatory effects of larger doses [31].

In intact cats a dual action of morphine on gross behavior was interpreted as an initial "selective depression of adaptive behavior" followed by a phase of restlessness and exaggerated startle reactions to environmental stimuli [31]. To the best of our knowledge, there are no more recent reports on the biphasic action of morphine in the cat, in which an attempt was made to quantify these effects.

In previous studies we have shown that in the cat trained to press a bar for milk reward the so-called postreinforcement EEG synchronization (PRS) and the associated epicortical steady potential shift, termed Reward Contingent Positive Variation (RCPV), are very sensitive measures of central actions of drugs [12]. The maximum positivity of a typical RCPV lasting 3–5 sec is recorded over the primary and secondary visual projections (posterior marginal gyri of both hemispheres) and ranges on the average from 100–200 μV when recorded with reference to the subjacent white matter or a distant "neutral" cortex [18,19].

The present study, in agreement with our preliminary report [14], shows that the enhancing effect of morphine on postreinforcement EEG phenomena can readily be quantified after doses as low as 0.1 and 0.2 mg/kg, and this

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effect is blocked and even reversed by higher doses than 0.4 mg/kg, IM. Hence, the cat may be a more valuable animal than other species in which the intrinsic actions of morphine can be studied, because the activation of two different dose-dependent mechanisms appears to be separated in a short period of time.

MATERIALS AND METHOD

The results are based on a total of 66 successful experimental sessions, each lasting 40–90 min, carried out in 6 adult cats (3 males and 3 females), trained to press a lever for an 0.8–1.0 ml of milk reward presented on a variable interval schedule such that pressing a lever produced the reward aperiodically, on the average once every 10 sec (VI-10 sec). During the initial stage of training, the experimenter shaped the behavior of the cats by producing the reward each time the animal touched the lever. Standard unsweetened milk was used. After 3–5 weeks of training during which the animals were allowed to perform each day for 1 hr, 5 days per week, 90% of the control and experimental sessions aimed at quantifying the PRS-RCPV were conducted using a fixed interval schedule in which the lever produced the reward once very 4 sec. The latter schedule was selected because it was observed that it markedly reduced the variability of the PRS-RCPV responses in terms of amplitude and duration, thus facilitating the statistical analysis of the data. All animals were kept on a 23-hr water- and food-deprivation schedule, 5 days a week. Since the occurrence of the PRS-RCPV responses depends upon the light input [3,18], the experiments were conducted in a well-illuminated chamber in which the light intensity was set at 26 candles/sq meter. The Lehigh Valley 4 cubic feet, sound-attenuating chamber, provided with a one-way window, was equipped with a transparent Plexiglas delivery cup attached to the wall 12 cm above the floor level, two levers protruded from the wall, each requiring approximately 20 g of force. A mirror on the floor below the delivery cup allowed the experimenter to see whether the cat consumed the milk or licked the empty cup. In a typical lever pressing performance, the electronically controlled delivery of milk was executed in a fraction of a second. The actual consumption of a 0.8 ml of milk lasted 2–3 sec and that of a 1.0 ml of milk 3–4 sec, as judged by the experimenter observing the transparent delivery cup. Licking outlasted the consumption by 1–2 sec, depending on the animal.

Under pentobarbital anesthesia, 4–10 solid-type nonpolarizable (0.6–0.8 mm in dia) Ag-AgCl electrodes, described by Bond and Ho [3], were implanted epidurally over the parieto-occipital cortex. One Ag-AgCl electrode was implanted in subjacent white matter of the posterior marginal gyrus 5 mm below the surface (AP 1.0; L 3.0) as a reference for transcortical recording of the steady potential shifts and electrocortical patterns from an epidural electrode placed 2–3 mm frontally or rostrally from the point of entry of the deep electrode. In many instances, very good and artefact-free recording of the PRS-RCPV was obtained over the posterior marginal gyrus with reference to another epidural electrode placed over the anterior ectosylvian gyrus of the same hemisphere (Fig. 2) which in previous studies of the topographical distribution of the RCPV responses was shown to be relatively "neutral", i.e. contributing only a small fraction (approx. 1/10) to the positive SP over the posterior marginal gyrus by developing a 15–25 μV surface negativity as measured in transcortical recording [19]. In addition to the epidural and the white matter electrodes, two stainless steel electrodes were implanted: one at the bottom of the frontal sinus, and the other in the occipital ridge as a reference to monitor the potentials associated with licking or lapping. In approximately 50% of instances, a more faithful monitoring of licking and lapping was obtained by converting the milk delivery cup into a drinkometer [20]. This was accomplished by feeding a continuous 40 c/sec train of impulses (3–5 V; 1 msec duration) from a Grass stimulator and an isolation unit into the main milk container on the top of the chamber, and recording this signal from the frontal sinus electrode with reference to the "common ground", each time the cat's tongue touched the cup (see Fig. 2). After the completion of experiments, the histological examination revealed that all recording electrodes were placed over an intact dura and normal looking cortex.

Quantification of RCPV Responses

The RCPV responses were recorded with a Grass Model 7P1 low-level DC amplifier coupled with a Model 7DA driver amplifier and written out on paper. The RCPV responses were integrated by feeding the output (smoothed out by filtering to half-amplitude response at 3 c/sec) from a Model 7DA driver amplifier into a Grass Model 7P10 cumulative DC integrator. The output of the latter was displayed on a separate channel in the form of constant amplitude sawtooth deflections whose rise time, and thus the frequency, were proportional to the magnitude of the positive steady-potential shift triggered by reward. The integrating system was calibrated to produce two pen deflections (30 mm each) in response to a 100 μV positive shift lasting 1 sec. This value was arbitrarily accepted as one unit of RCPV.

In a typical experiment session, after the DC electrodes have been balanced during the initial nonrewarded bar presses associated with maximal surface negativity and desynchronized EEG patterns, the baseline of the output from a Grass Model 7DA driver amplifier fed into the 7P10 DC integrator (filtered to half-amplitude response at 3 c/sec) was adjusted to maintain a zero output of the latter. If there was a shift in the baseline of more than 20 μV during the session, an additional adjustment was made. The RCPV responses were measured by counting the number of deflections of the integrator during the time period between two rewarded bar presses, as shown in Fig. 2 (a boxed response in the upper right corner).

The paired Student's t test was used to evaluate the significance of the differences between the mean RCPV responses obtained during the control sessions and those during which the effect of morphine sulfate was tested. The average RCPV responses were based on 20 single RCPVs taken from each animal during various periods after administration of 0.5% morphine sulfate in doses of 0.07–1.0 mg/kg, IM. The average values were then compared to those obtained from the same animals during a comparable time interval after IM injection of saline 24 or 48 hr prior to the experimental session.

All 6 cats received doses of morphine sulfate ranging from 0.07 mg/kg to 1.2 mg/kg, IM whose effects are listed in Table 2 and Fig. 7. Doses of morphine were administered in a random order, each being separated from the next one by a time interval not shorter than 7 days. Approximately

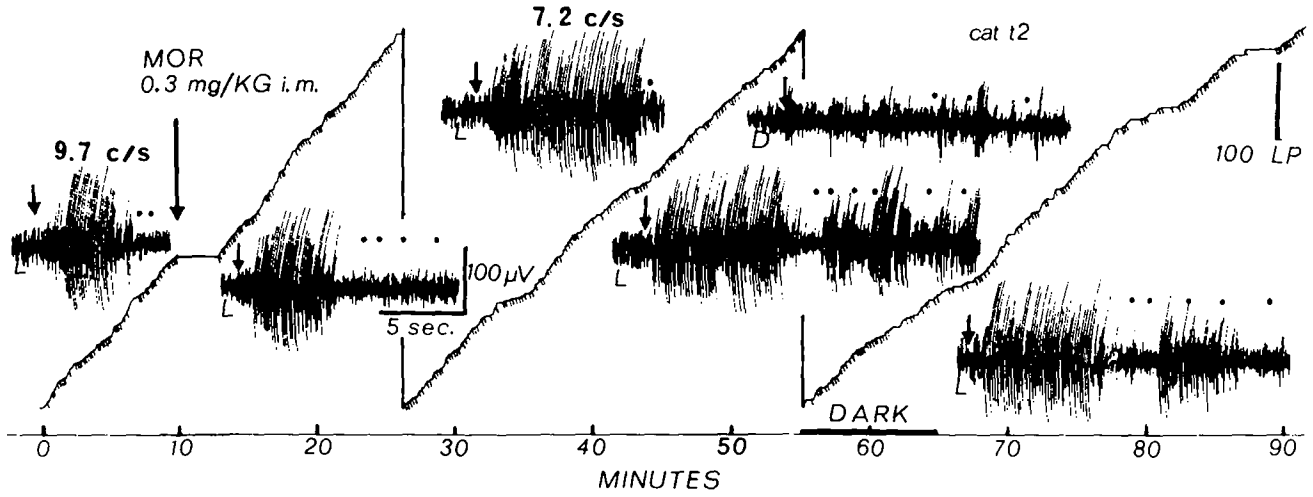

FIG. 1. Cumulatively recorded bar pressing performance on a VI-10 sec-Schedule postreinforcement EEG synchronization responses (PRS) prior and after 0.3 mg/kg, IM, of morphine sulfate in cat No. 5. Oblique short spikes on the slopes of the cumulative record mark the presentations of 0.8 cc of milk reward. Arrows above the EEG excerpts mark the time of rewarded bar press, whereas dots mark the nonrewarded bar presses. D and L below each EEG excerpt tell whether the response was recorded in the dark or light. Note that the amplitude and duration of PRS bursts increased after morphine administration. Initially, the nonrewarded bar presses were not associated with any signs of synchronization. However, during the peak facilitatory effect of morphine on PRS, bursts of PRS-like activity occurred prior and after the nonrewarded bar press. The PRS and the dissociated EEG responses were not observed in the dark (D). Note also that morphine caused a slowing in the PRS rhythm from 9.7 c/sec to 7.2 c/sec.

20% of the doses were repeated to find whether the results are replicable.

Dissociation of EEG Phenomena

In order to test the effect of morphine on the dissociation between the actual consummatory behavior and its EEG expression, the animals were allowed to perform on a variable interval schedule (VI-10 sec) in which several nonrewarded bar presses intervened between 2 rewarded ones. The degree of dissociation or uncoupling between the PRS-RCPV phenomenon and the actual consumption was determined by counting the number of the PRS responses triggered prior to a rewarded bar press and during or after a nonrewarded bar press when the animal licked the empty cup (Fig. 6, bottom). As mentioned earlier, the actual consumption of 1 ml of milk lasted not longer than 4 sec, the licking outlasting the latter by approximately 1 -2 sec. Thus, all lapping that occurred after 4 sec from the moment of reward presentation should be regarded as licking the empty cup. However, the PRS-RCPV response that continued uninterruptedly beyond the 4 sec time period, after it was triggered by reward presentation, was not counted as a dissociated response, even though it outlasted the actual consummatory period, as often observed after doses of morphine that potentiated the PRS-RCPV during performance on a FI-4 sec (Fig. 2, top right) and on a VI-10 schedule (Fig. 6, bottom). Only those PRS responses were counted that produced more than 0.5 units of RCPV in DC recording (see above for the definition of the RCPV unit). The ratio of the dissociated PRS-RCPV responses to the sum total of all responses was expressed as percentage. The mean values were based on 50 responses from each animal during the peak effect usually 20–40 min after administration of a particular dose of morphine sulfate.

Frequency of PRS Rhythm

Changes in the mean frequency were determined by counting the number of oscillations in 3 series of 10 PRS responses in each animal during the peak effect of a particular dose of morphine. The mean values were then compared to those obtained during a corresponding control period after saline injection in a session run 24 hr or 48 hr prior to the experimental one.

RESULTS

Modulation of PRS-RCPV Responses

Following a rewarded bar press (on a FI-4 sec or VI-10 sec-Schedule) during consumption of milk all 6 cats showed well-developed postreinforcement synchronization (PRS) consisting 7.5 -10.8 c/sec large amplitude (150 -200 μV) alpha-like activity over the posterior marginal gyrus of both hemispheres, with lower amplitude over the medial and marginal posterior suprasylvian gyri. The PRS was always associated with the positive epicortical steady-potential shift, i.e., the Reward Contingent Positive Variation (RCPV) with maximal amplitude over the primary and secondary visual projections (posterior marginal gyri of both hemispheres; for a description of a more detailed topographical distribution of the RCPV, see reference [27]). The effect of morphine on the PRS and RCPV is illustrated in Figs. 1 (VI-10 sec-Schedule) and 2 (FI-4 sec-Schedule), respectively. The smallest dose of morphine sulfate capable of significantly enhancing the RCPV was 0.1 mg/kg (Table 1). This effect was observed 15- 25 min after administration, lasted approximately 30 -40 min, and occurred clearly in 3 out of 6 animals, thus indicating that there are significant individual differences in sensitivity to low doses of morphine. Doses of 0.2 0.4 mg/kg markedly enhanced the RCPV responses in all 6 animals. A dose of

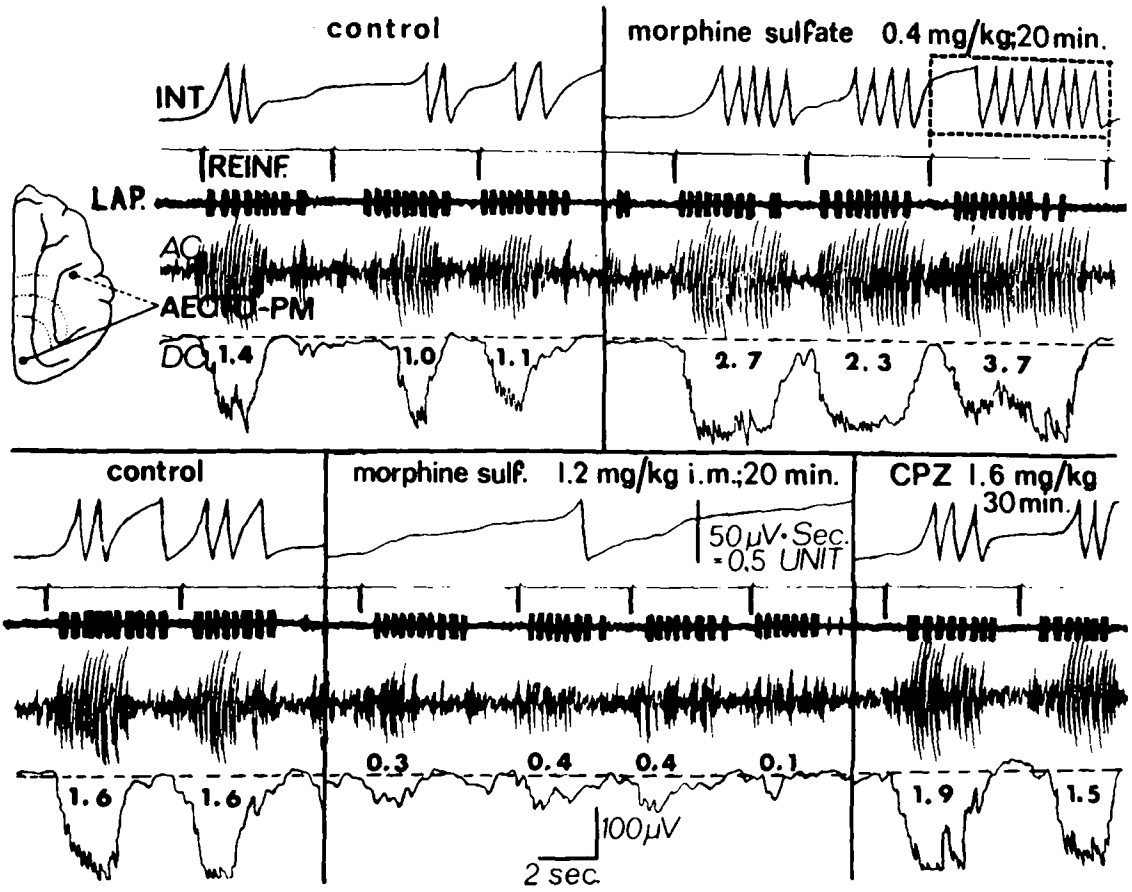

FIG. 2. Typical excerpts of EEG simultaneously recorded with an AC- and a DC-coupled amplifier from the visual cortex (posterior marginal gyrus) with reference to the anterior ectosylvian gyrus (cat No. 1). In the DC channel, the filter was set at 3 c/sec half-amplitude responsiveness. Two full deflections of the integrator (INT) are equal to one unit of RCPV. The numbers above each RCPV response were obtained by summing up the deflection of the integrator during the time period between two rewarded bar presses, as shown in the upper-right corner (boxed INT record). LAP-potentials caused by lapping and licking.

0.5 and 0.6 mg/kg still had a monophasic enhancing effect in 3 animals, but in the remaining 3 the effect was clearly biphasic, i.e., the initial enhancement was followed by a suppression which became most conspicuous 40–50 min after morphine administration. The biphasic action was particularly well developed in all 6 animals after a dose of 0.7 and 0.8 mg/kg as shown for the cat No. 2 in Fig. 3. With larger doses (1.0 and 1.2 mg/kg), the initial enhancement periods of the PRS-RCPV phenomenon, became shorter and less conspicuous, and, in most instances, were replaced by a monophasic suppression of the RCPV which occurred despite the fact that the animals, during the period between 15–25 min after injection, showed no significant changes or only slight slowing of their bar pressing performance (Fig. 2, bottom, middle). During the periods of RCPV suppression, approximately 60% of rewarded bar presses were followed by 16–18 c/sec oscillations of relatively low amplitude (60–80 μV) which were not associated with any significant steady-potential shift. Switching the filter of the DC amplifier from 3 c/sec to 15 or 33 c/sec half-amplitude responsiveness did not augment the abortive RCPV responses (not shown in Fig. 2).

The monophasic suppression of RCPV by larger doses of morphine and the associated restless behavior that interfered with bar pressing performance were readily antagonized by 1.0–1.6 mg/kg of chlorpromazine, as shown in Fig. 2 (bottom right). A typical time course of suppressant effect of 1.2 mg/kg of morphine sulfate on RCPV and bar pressing performance, and the effect of chlorpromazine are shown in Figs. 4 and 5.

Dissociation of the EEG Phenomena from Consumption

In an experimental paradigm in which milk reward was presented on a variable interval schedule (VI-10 sec), all 6 animals, if not treated with morphine, showed an almost perfect correlation between the actual consumption and the occurrence of the PRS-RCPV phenomena, since, out of the total 1750 considered responses, 98% of them were triggered only 0.5–1.0 sec after the rewarded bar press and a fraction of a second after the onset of consumption, as evidenced by the potentials caused by lapping (Fig. 6, top). This correlation was not disturbed even when as many as 8–12 nonrewarded bar presses intervened between 2 rewards, and the animal licked the empty cup. In this

TABLE 1

BIPHASIC, DOSE-DEPENDENT EFFECT OF MORPHINE SULFATE UPON DURATION AND AMPLITUDE OF THE REWARD CONTINGENT POSITIVE VARIATION (RCPV) EXPRESSED IN UNITS (ONE RCPV UNIT = 100 μV POSITIVE SHIFT OF ONE-SEC DURATION)*

Morphine sulfate (mg/kg, IM)	Average % change in RCPV as compared to control responses	Approximate time period of peak effect after administration (min)	Number of animals that showed significant change
0.07	-19 ± 1.6†(NS)	15-25	0/6
0.1	+23 ± 8.8 (S)	15-25	3/6
0.2	+118 ± 6.5 (S)	15-30	6/6
0.3	+190 ± 15.4 (S)	15-25	6/6
0.4	+210 ± 2.2 (S)	15-40	6/6
0.5	+199 ± 8.4 (S)	20-30	3/6
	−55 ± 19.8 (S)	40-50	6/6
0.6	+174 ± 12.5 (S)	20-25	3/6
	-59 ± 10.7 (S)	40-50	3/6
0.7	+85 ± 15.6 (S)	10-20	6/6
	−70 ± 18.0 (S)	40-55(R)	6/6
0.8	+75 ± 19.7 (S)	10-20	6/6
	−79 ± 12.2 (S)	40-60(R)	6/6
1.0	+28 ± 13.0(NS)	10-20	2/6
	−97 ± 11.0 (S)	40-90(R)	6/6
1.2	-99 ± 0.5 (S)	40-120 (R)	6/6

*Combined results based on 3–5 series of 10 RCPV responses recorded in each of 6 animals during the peak effect of morphine, and compared to the same number of control responses during a comparable time period following saline injection.

†Standard deviation.

(S) = Significant ($p<0.05$).

(NS) = Nonsignificant ($p<0.05$).

(R) = Restless behavior interfered with bar-pressing performance

situation, only sporadic small-amplitude, abortive PRS-like oscillations occurred (Fig. 6, top left), and were not associated with significant steady-potential shifts that exceeded 0.5 units i.e., the arbitrary minimum qualifying such a response as a dissociated RCPV phenomenon (now shown in Fig. 6). As shown in Fig. 6, top, the duration of the PRS bursts lasting 3–4 sec more exactly reflects the duration of the consumption of a 1.0 ml of milk than the lapping followed by licking of the empty cup. After administration of 0.3- 0.5 mg per kg of morphine sulfate, the well-timed PRS-RCPV responses, i.e. triggered during or after presentation of reward, markedly outlasted the actual 4 sec consummatory period, and continued uninterruptedly during the licking of the empty cup. In addition, a high percentage of fully developed dissociated responses were observed after a series of nonrewarded bar presses when the animal licked the empty cup (Fig. 6, bottom; "d" responses). Some dissociated responses occurred 0.5–1.5 sec prior to rewarded bar press, showed no decline during the subsequent rewarded bar press, and uninterruptedly continued through the period of consumption (Fig. 6, bottom right and left).

The relationship between the increasing doses of morphine and the per cent occurrence of dissociated RCPV responses recorded during a 10-min performance on VI-10 sec-Schedule at the peak effect of a particular dose of morphine is shown in Fig. 7 and Table 2. The animals were divided into Groups A and B which clearly showed different susceptibility to morphine. For instance, a dose of 0.3 mg/kg induced 58% dissociated RCPV in Group A and only 19.5% in Group B, the difference being significant at the 0.01 confidence limits. The greatest difference between the two groups was observed after a dose of 0.4 mg/kg, and occurred between the 15 and 40 min after morphine administration, i.e. during the time period of maximum potentiation of the RCPV after the same dose (Table 2). With still increasing doses of morphine and the emergence of suppressant action on RCPV, the occurrence of dissociated responses declined in both groups and the differences between groups became nonsignificant.

Sex differences did not seem to correlate with the groups, since in Group A there were 2 males and 1 female and the latter showed sensitivity to morphine that was comparable to that of the 2 males. Also in Group B the 2 females and 1 male appeared to respond in a comparable fashion. Although the rate of bar pressing performance was

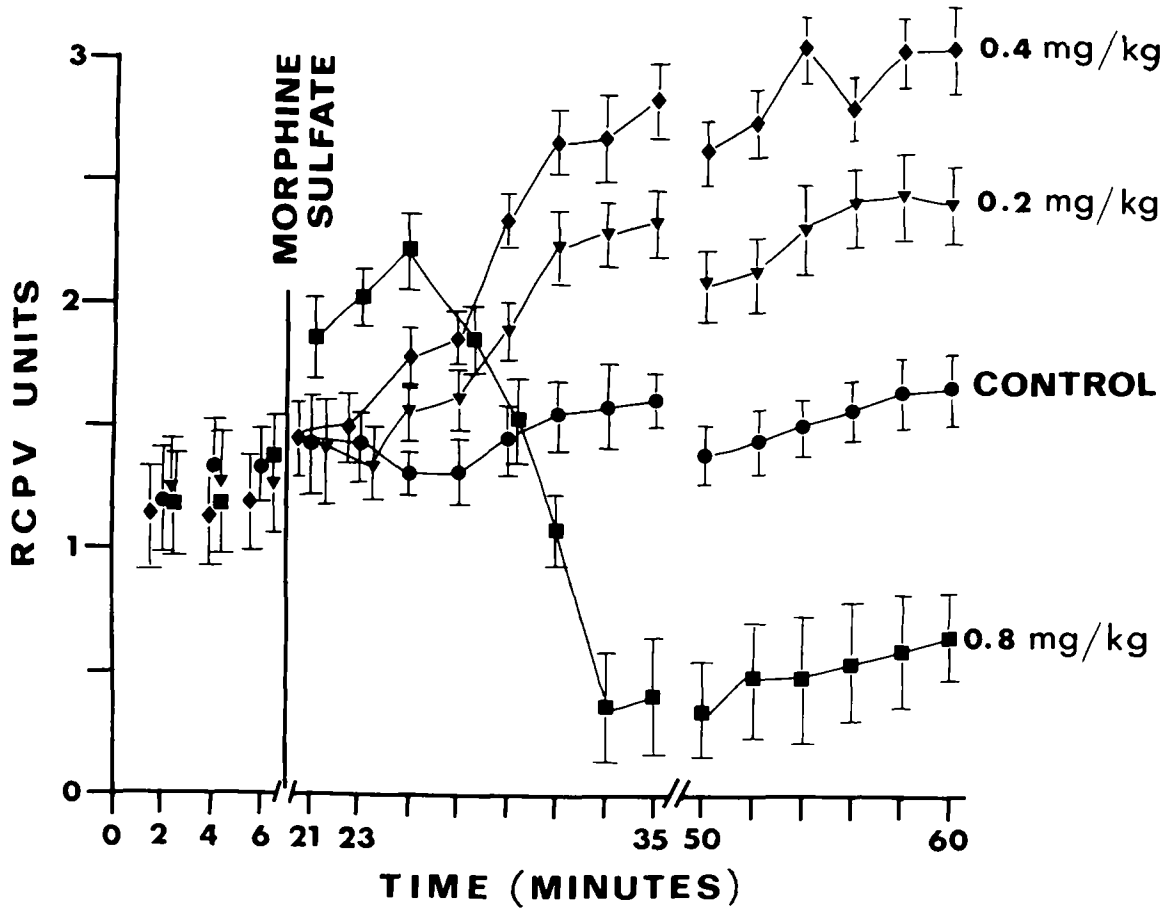

FIG. 3. A typical dose-dependent biphasic action of morphine sulfate on RCPV responses expressed in units (cat No. 5). Note a monophasic enhancement of RCPV after 0.2 and 0.4 mg/kg of morphine, and a biphasic effect of 0.8 mg/kg. Each average (and standard deviation) is based on 10 RCPVs.

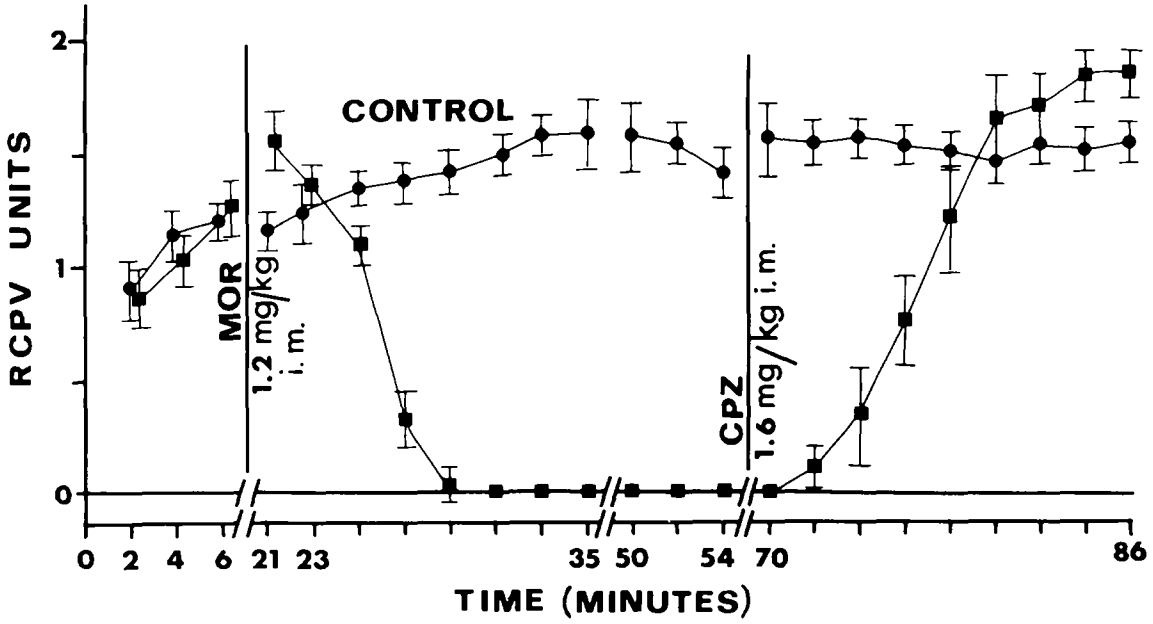

FIG. 4. A typical time course of depression of RCPV responses after a dose of 1.2 mg/kg of morphine sulfate, and a reversal following administration of chlorpromazine (1.6 mg/kg, IM) (cat No. 6).

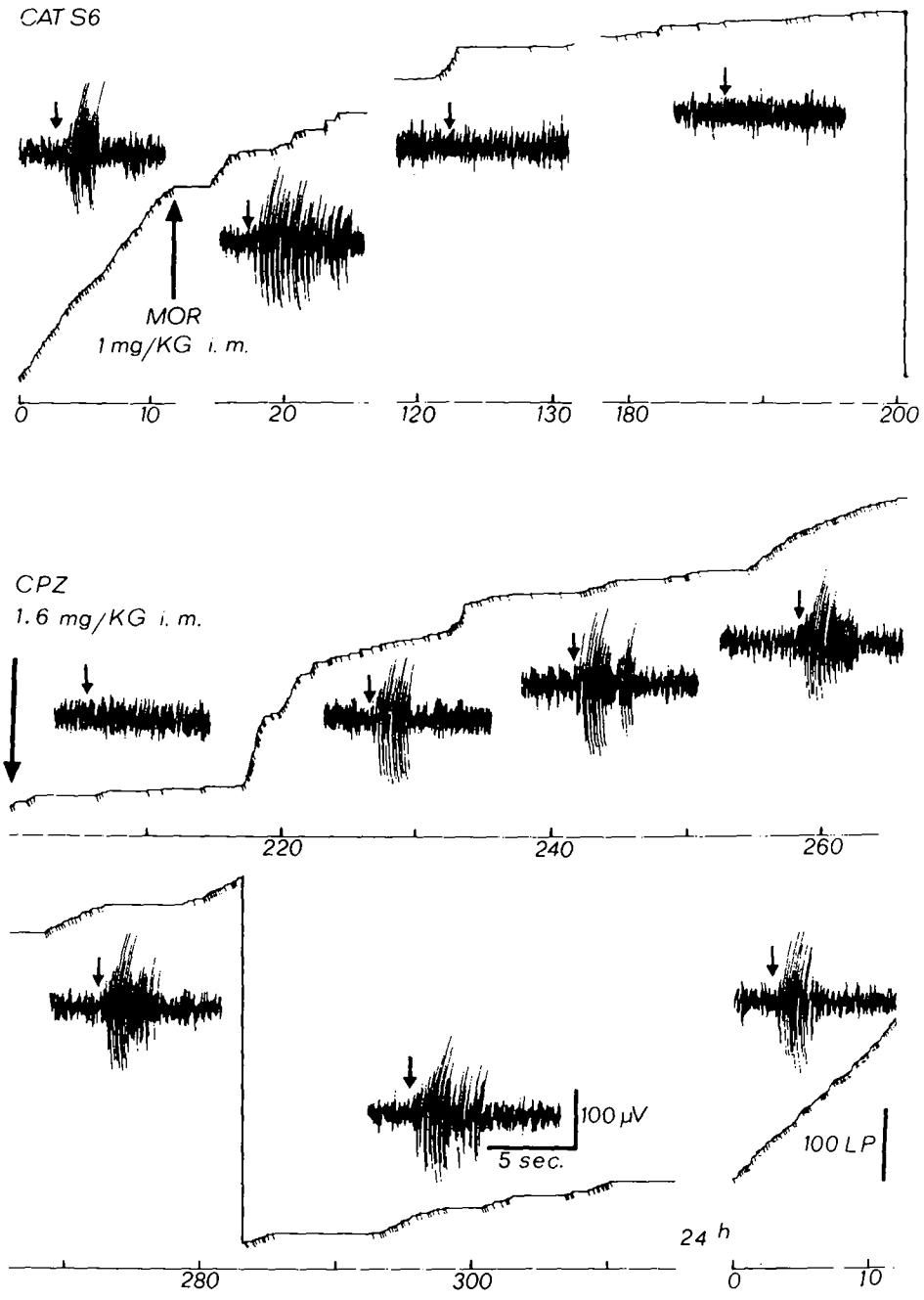

FIG. 5. A typical bar pressing performance and PRS responses during a development of a suppresssant effect of morphine, and the reversal caused by chlorpromazine (cat No. 6).

not studied in a systematic fashion, both groups appeared to perform similarly in control and during various doses of morphine.

The Effect of Light on PRS-RCPV Responses

In the absence of light, the well-timed as well as the dissociated PRS-RCPV responses were suppressed (Fig. 1), and morphine in all tested doses, in contrast to LSD-25 [13], was not able to restore them. Since approximately three hundred consummatory responses in the dark were recorded after various doses of morphine, the negative results can be considered as significant.

Frequency of PRS Rhythm

After 2 -4 weeks of training and with the emergence of well-developed PRS, it became apparent that a particular frequency of the PRS rhythm is a stable characteristic of an animal and an electrode. Across all 6 animals, the lowest control PRS rhythm was 7.5 c/sec (animal No. 4) and the highest, 11.7 c/sec (animal No. 2), as shown in Fig. 7, left.

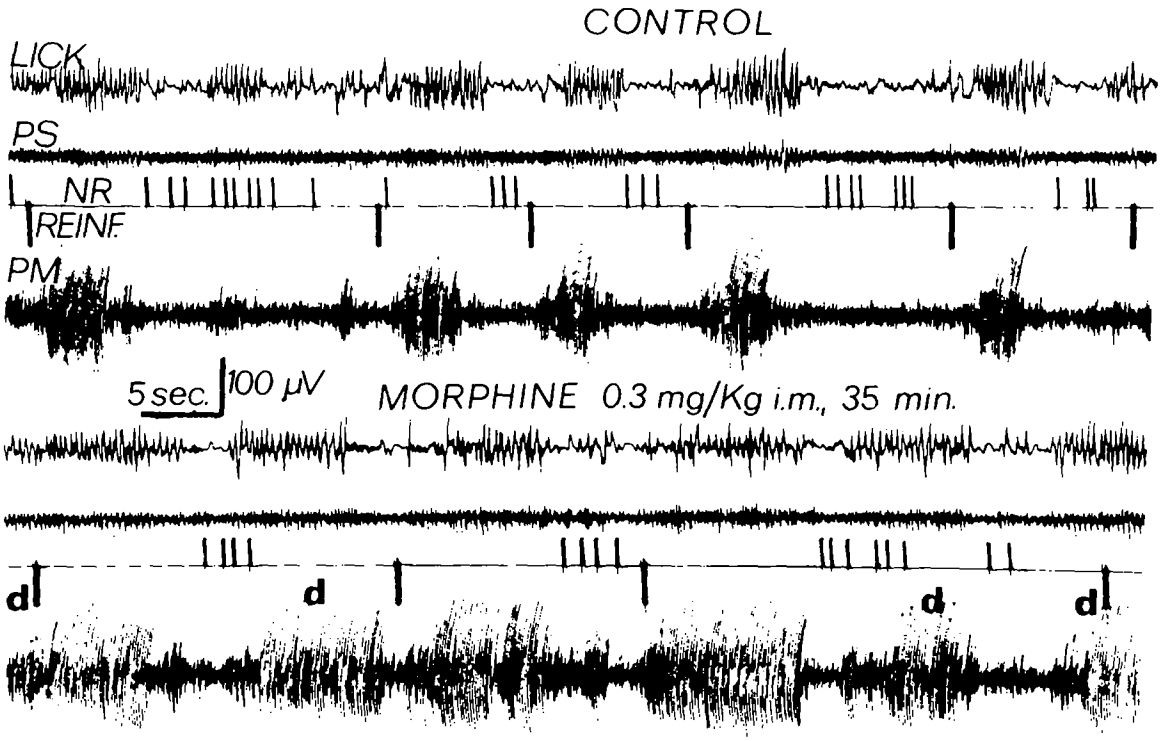

FIG. 6. Influence of milk consumption and the manipulanda (the lever and delivery cup) on the PRS phenomenon prior to and after morphine administration (cat No. 3). Top: Control PRS responses (milk reward delivered on a VI-10 sec Schedule). LICK-potentials caused by licking the empty cup or lapping. PS-posterior sigmoid gyrus; PM-posterior marginal gyrus; recording of the EEG with reference to the anterior ectosylvian gyrus. NR-nonrewarded bar press; REINF-rewarded bar press. Note that PRS over the PM occurs only during consumption, and PRS does not develop when the animal, during and after several nonrewarded bar presses, licks the empty cup between the first and second reward. Bottom: During the peak effect of morphine sulfate (0.3 mg/kg, IM) the dissociated PRS responses (d) occurring after nonrewarded or prior to the rewarded bar press when the animal licks the empty cup.

TABLE 2

THE EFFECT OF MORPHINE SULFATE ON THE OCCURRENCE OF DISSOCIATED EEG RESPONSES AND THE AVERAGE PRS RHYTHM IN TWO GROUPS OF ANIMALS

Dose of morphine sulfate mg/kg, IM	Group A Animals No. 2, 3 and 5		Group B Animals No. 1, 4 and 6	
	Mean % of dissociated EEG responses and standard deviation	Mean PRS rhythm c/sec	Mean % of dissociated EEG responses and standard deviation	Mean PRS rhythm c/sec
0	2.7 ± 0.1 (NS)	10.4 ± 1.0 (S)	2.6 ± 0.2 (NS)	7.9 ± 0.4 (S)
0.1	5.1 ± 4.2 (NS)	10.2 ± 1.0 (S)	2.8 ± 0.3 (NS)	7.7 ± 0.4 (S)
0.2	30.2 ± 9.3 (NS)	9.6 ± 1.1 (NS)	7.0 ± 6.8* (NS)	7.7 ± 0.4 (NS)
0.3	58.0 ± 10.5*(S)	7.8 ± 0.6*(NS)	19.5 ± 10.0*(S)	7.1 ± 0.3 (NS)
0.4	88.3 ± 11.0*(S)	7.4 ± 0.2*(NS)	19.8 ± 10.5*(S)	7.0 ± 0.3 (NS)
0.5	77.0 ± 14.0*(S)	7.6 ± 0.3*(NS)	26.5 ± 10.3*(S)	6.8 ± 0.2*(NS)
0.6	54.3 ± 20.2*(NS)	7.8 ± 0.3*(NS)	26.3 ± 11.5*(NS)	7.1 ± 0.1*(NS)
0.7	46.3 ± 17.1*(S)(R)	7.8 ± 0.4*(NS)	12.8 ± 8.2 (S)(R)	7.4 ± 0.4 (NS)
0.8	30.1 ± 18.5 (S)(R)	8.9 ± 0.8 (NS)	6.1 ± 6.2 (S)(R)	7.9 ± 0.7 (NS)

*= Significant change from control level ($p<0.01$).

S or NS= Significant or non-significant difference between the groups.

R = Restless behavior interfered with bar pressing performance; data could be collected only during a relatively short period of facilitatory effect of morphine.

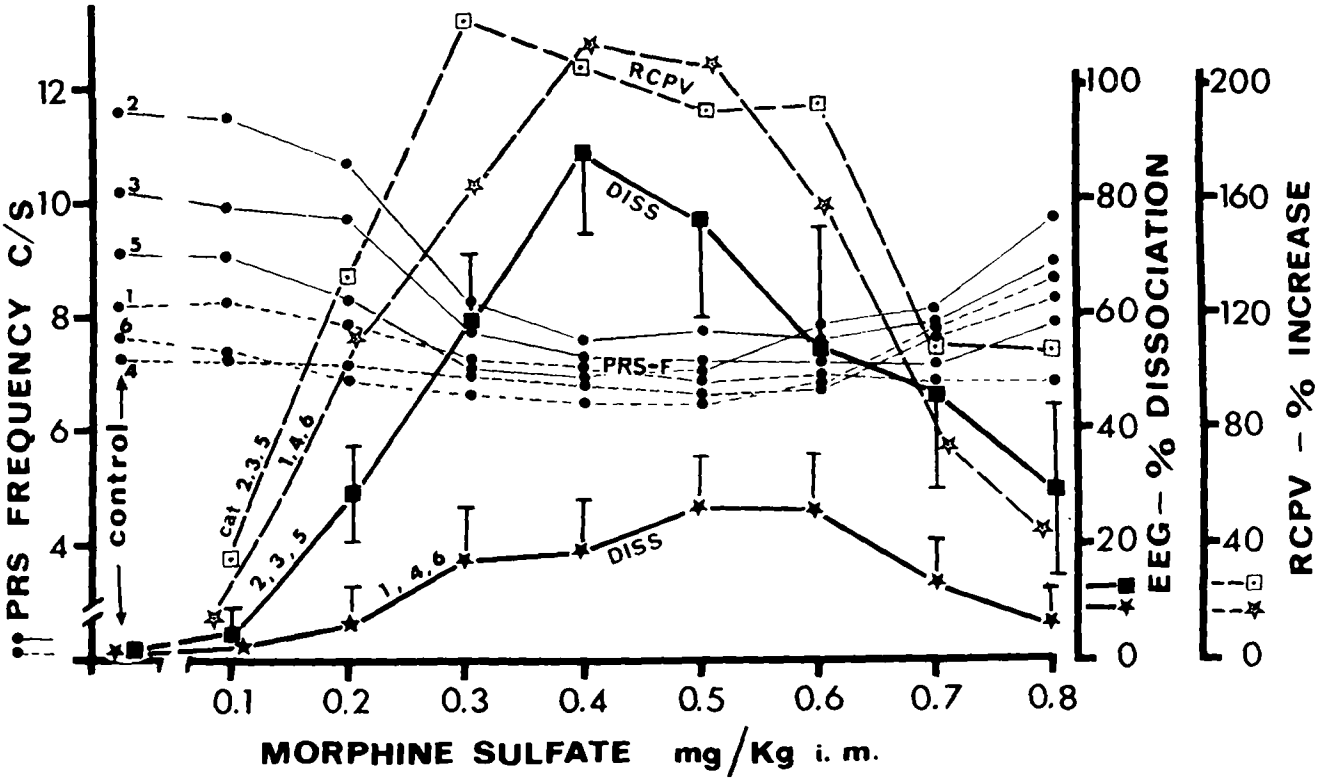

FIG. 7. Summary of the peak effects of increasing doses of morphine sulfate on 3 phenomena associated with bar pressing performance for milk reward: (a) the mean frequency of the PRS rhythm in animals Nos. 1 through 6 (each value based on the number of oscillations in 30 PRS bursts); (b) the mean per cent occurrence of dissociated PRS responses in the group of more "sensitive" (Nos. 2, 3, 5) and the group of less "sensitive" animals (Nos. 1, 4, 6); values are based on 50 responses from each animal counted during the peak effect of morphine; and (c) per cent increase of RCPV, as compared to control responses recorded during the peak effect of a particular dose of morphine in both groups of animals (the values and standard deviations are based on 20 responses from each animal). Note that both groups show similar enhancement of RCPV (open squares and stars). However, the more "sensitive" group, characterized by initially higher frequency of PRS rhythm, showed more conspicuous drop in the PRS rhythm and a much higher per cent occurrence of dissociated responses after doses of 0.2 through 0.7 mg/kg of morphine.

The changes in the average rhythm over a time period of 5–6 months did not exceed 7%. To emphasize the stability of the PRS rhythm during a typical control session lasting 40–90 min, it should be pointed out that the average rhythm during the first 5–10 min, when compared to that toward the end of a session, i.e., prior to satiation, showed no significant changes; if a tendency to a slower rhythm developed, it did not exceed 7% and was not significant ($p>0.05$).

As shown in Table 2 and Fig. 7, the animals have been divided into Group A that displayed a higher average PRS rhythm of 10.4 c/sec, and Group B with an average rhythm of 7.9 c/sec ($p>0.01$). Like the PRS-RCPV phenomenon, the stability of the PRS rhythm was affected by morphine in a dose-dependent and biphasic manner: lower doses (0.2–0.6 mg/kg IM) slowed the rhythm, whereas larger doses (0.7–0.8 mg/kg IM) were less effective in parallel with the supervening depression of PRS-RCPV responses and behavioral restlessness. As shown in Fig. 7, the effect of morphine on the PRS rhythm was inversely related to the potentiation of the PRS-RCPV phenomena, as well as to the occurrence of the dissociated EEG responses.

Morphine-induced slowing of the PRS rhythm was more conspicuous in Group A whose average control rhythm of 10.4 was reduced to 7.8 c/sec by 0.3 mg/kg of morphine, whereas the "slower" Group B with the average of 7.9 c/sec showed a significant reduction to an average 6.8 c/sec only after a dose of 0.5 mg/kg (Table 2). Due to the greater slowing of the faster PRS rhythm in Group A, the initially significant difference between the means disappeared during the peak effect of 0.2 mg/kg of morphine sulfate.

DISCUSSION

The main observation that morphine in appropriate doses produces conspicuous enhancement of PRS-RCPV phenomena followed by suppression merits attention for three reasons: (a) this dual action can readily be quantified in the same animal, during the same and relatively short-lasting experimental session; (b) it appears that morphine modualtes the function of at least two neuronal systems influencing cortical EEG and gross behavior; and (c) using our experimental paradigm, further exploration and identification of these systems by pharmacologic and more refined electrophysiologic procedures, e.g. single unit recording, may shed light on the mode of action of morphine.

Enhancement of PRS-RCPV Responses

It is generally believed that the PRS is triggered by a short-lasting, almost quantum-like inhibition of the ascending reticular-activating system (ARAS) [4, 16, 25, 26]. Indeed, the striking similarity between patterns of evoked potentials to sensory stimuli recorded during burst of PRS and sleep onset [12], as well as the identical topographical distribution of the RCPV and the Sleep Onset Positive Variation (SOPV), support this contention [17,19]. Furthermore, flash stimuli at a frequency of PRS rhythm produce sleep in a relaxed cat and epicortical steady-potential shift whose topographical distribution is indistinguishable from that of the spontaneous PRS and SOPV [15].

It can be argued that the central complex and poorly understood mechanisms of morphine analgesia are partially linked to the synchronizing action. The thalamo-cortical alpha activity is based on widespread postsynaptic inhibitory phasing of neuronal activity; large populations of thalamic neurons of the specific and nonspecific sensory nuclei, during bursts of alpha waves, remain silent and hyperpolarized [1]. Hence, the transmission of sensory input to the cortex and the integration of nociceptive stimuli in the polymodality systems may be suppressed or even blocked. In man, morphine in analgesic doses enhances alpha activity [6] and slows its frequency [9].

The slowing of the PRS rhythm may be a crucial factor in augmenting the RCPV. When phasing of neuronal activity occurs at a frequency lower than 9 c/sec, the summation of sequential IPSPs in cortical pyramidal cells, and the associated surface positivity, may be enhanced since intervals of 120–135 msec between two IPSPs are optimal to obtain a cumulative effect [30]. Interestingly enough, this range of intervals corresponds to a lower rhythm of 7.4–8.3 c/sec which was characteristic of PRS bursts in most animals after doses of morphine that maximally enhanced the surface reward-induced positivity. In conclusion, it can be stated that morphine in lower doses appears to maximize, at the thalamic and cortical levels, the function of the phasing inhibitory mechanism triggered by biological reward.

Considering the possible neurohumoral mechanisms involved in the enhancement by morphine of the PRS-RCPV responses, one can marshall several arguments supporting the role of the 5-HT system. Firstly, during the last 10 years, a substantial body of data has accumulated, indicating that activation of the 5-HT system is responsible for suppression of the ARAS, EEG synchronization and slow-wave sleep [8,11]. These data, in conjunction with the aforementioned similarities between the RCPV and SOPV phenomena [17,19], appear particularly convincing. Secondly, numerous observations indicate that activation of the serotonin system plays an important role in the mechanism of morphine analgesia [24].

Dissociation of the EEG Responses from Consumption

The apparent correlation between the dose-dependent morphine-induced EEG dissociation and the slowing of the PRS rhythm is difficult to explain. Similar dissociation was observed after 15–30 μg/kg of LSD-25 [12,13]. The mechanism of action of LSD appears to be different from that of morphine. It should be recalled that, unlike the human alpha activity, the PRS depends on unpatterned light input, even in cats trained and fully habituated to perform in the dark [18]. LSD-25 is capable of restoring the PRS-RCPV responses in the dark [13], whereas morphine, despite the enhancement of PRS-RCPV in the presence of light, had no effect in the dark.

Suppression of the PRS-RCPV Responses

Larger doses of morphine (5–20 mg/kg, IM) are known to produce in cats a strong increase in sympathetic tone characterized by mydriasis, piloerection, restlessness, aggressiveness, violent motor responses to mild environmental stimuli, yowling, clawing and biting of "neutral" objects, i.e., a syndrome that has been likened to a manic reaction [5, 7, 23, 28, 31]. The suppression of PRS-RCPV and the emergence of behavioral restlessness after larger doses of morphine are likely to be caused by a common neurohumoral mechanism which may be dopaminergic in nature. Several pharmacologic observations in cats have implicated dopaminergic mechanism in morpine-induced behavioral excitement after doses higher than 5 mg/kg [5]: (a) catecholamine depletors such as reserpine and tetrabenazine, or central dopaminergic receptor blocking agents such as haloperidol or chlorpromazine prevent the behavioral and autonomic responses to morphine [5]; (b) alpha- and beta-adrenergic receptor blocking agents such as phenoxybenzamine and propranolol do not antagonize the morphine-mania [5]; and (c) anticholinergic drugs (atropine), anti-histaminic drugs (mephyramine), and antiserotonin agents such as LSD-25 do not present morphine-mania [5]. In our experiments, the observation that chlorpromazine was very effective in restoring the PRS-RCPV responses suppressed by morphine is compatible with the hypothesis [5] that morphine in larger doses activates the dopaminergic mechanisms which play an important role in the modulation of the ARAS and/or the thalamo-cortical synchronizing system. Since moderate doses of morphine had a biphasic action on the PRS-RCPV responses, it can be suggested that, in the second phase, the dopaminergic mechanisms override the sedative and synchronizing serotonergic influences.

The difference in sensitivity to morphine in our animals merits special attention, but before any plausible explanation can be offered a more extensive study must be carried out encompassing general behavioral characteristics, such as sleep-waking patterns, as well as turnover of brain monoamines prior to and after administration of morphine. Furthermore, the degree of analgesia in both groups after the same dose of morphine should shed some light on the nature of the dichotomy between the two groups of animals.

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