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Reassessing Morphine Effects in Cats: I. Specific Behavioral Responses in Intact and Unilaterally Brain-Lesioned Animals

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VILLABLANCA, J. R., C. M. HARRIS, J. W. BURGESS AND I. DE ANDRES. *Reassessing morphine effects in cats: I. Specific behavioral responses in intact and unilaterally brain-lesioned animals.* PHARMACOL BIOCHEM BEHAV **21**(6) 913–921, 1984.—Behavioral responses to single low doses of morphine (0.5–3.0 mg/kg IP) were measured in intact cats and in cats with removal of one cerebral hemisphere or one caudate nucleus. Responses were dose-dependent and formed 3 stages: (1) *autonomic stage* (0–15 min postdrug): with vocalization, salivation, licking, swallowing, retching and vomiting; (2) *quiet stage* (15–60 min postdrug): sitting, fixed gaze, mydriasis, and pricked pinnae; (3) *head movement stage* (from 30–60 min postdrug and decreasing by the 5th hr): fully aroused but mostly sitting; showing discrete, complex head movements of a visual-tracking type with pouncing/avoidance paw movements, and with irregular, dose-dependent bouts of rocking, pivoting, and backing. Sleep, grooming, micturition and defecation were suppressed. In hemispherectomized cats the frequency of head movements was increased only towards the side of the ablation, and there was a strong bias for body turning to that side together with a significant bias to move the ispilateral paw. None of these biases were significant in cats with a unilateral caudate ablation. We conclude that the cat is an excellent model for behavioral morphine studies when dose levels below those inducing "feline mania" are used. CNS sites underlying these responses are discussed.

Morphine Cats Behavioral effects Unilateral brain lesions Turning bias Stereotypies CNS site of actions

THIS is the first of a series of three papers reporting the results of our research on the neurological and behavioral effects of morphine in intact and brain-lesioned adult cats. The decision to perform this detailed analysis originated from our preliminary observations that relatively low doses of morphine can elicit a specific behavioral repertoire in the cat [6,14], and that the brain lesion method may be successfully used to disclose CNS sites of drug action involved in the expression of these behavioral effects [6,7]. Those observations contrasted with the prevalent concept that morphine elicits such strong excitatory effects in the cat that the inevitable result is "feline mania," a syndrome of limited interest for the study of discrete behavioral effects of morphine [9, 10, 18, 20, 25, 26]. This notion has so influenced research that relatively few behavioral studies of opiate effects have been conducted in cats ([10] see [34]). We expect that this attitude may rapidly change in view of the data on effects of relatively small doses of morphine presented in this series, as well as on the basis of recent behavioral reports reviewed in the Discussion.

Because the cat has been a classic subject for neuroscience research and, therefore, a vast body of neuroanatomical, neuropharmacological and behavioral data has already been accumulated which could be brought to bear on the issue of opiate actions, we felt that developing a model for the study of behavioral and neurological effects of morphine in this species could be particularly valuable for interfacing brain and behavioral mechanisms of opiate effects. Consequently, our current research has centered on a detailed analysis of behavioral effects of relatively small morphine doses [6, 8, 14, 33], and on efforts to define the CNS sites of action which can account for the key components of those manifestations [5, 6, 7, 32, 33].

In this paper, we present the result of quantitative behavioral studies of the effects of single doses of morphine in intact, unilateral caudate-nucleus lesioned, and hemispherectomized cats. The brain-lesioned cats were included to study directional biases in drug-induced movements which would be predicted in these animals by the nigrostriatal dopaminergic theory of rotational behavior [13,16]. Our

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use of lesioned animals can provide additional tests of this theory (which we have critiqued in previous work, [31], and may suggest other brain sites which could contribute to morphine-induced rotational behavior. In the second paper, we report sleep-wakefulness and EEG effects of a single dose of morphine, and in the third paper we describe the effects of chronic morphine administration, including naloxone-precipitated withdrawal, with an emphasis on the consequences of bilateral caudate nuclei lesions as well as the same unilateral lesions reported here [32,33].

Overall, our studies support the conclusion that morphine at low doses produces rich and specific behavioral effects in cats, suggesting that this animal is highly sensitive to morphine. Therefore, considering the other attributes of this species for neuroscience research, the cat may serve as an excellent model in which to understand the processes and sites of action involved in opiate effects. A number of clues for the latter are provided and discussed in these papers. Preliminary results in intact cats were previously presented [6,14].

METHOD

Subjects and Surgery

Twenty-eight male cats were obtained from the breeding facility at the U.C.L.A. Mental Retardation Research Center or from unclaimed animals at local shelters. Seven cats had removal of the left cerebral hemisphere (hereafter called hemispherectomized or HEMI cats), and 4 received an extensive unilateral lesion of the right caudate nucleus.

Hemisperectomy is a standard technique in our laboratory, with practically no mortality or morbidity [30,34]. Under aseptic conditions, pentobarbital anesthesia (35 mg/kg, IP) and hypothermia (30° to 33°C, rectal temperature) the calvarium over the left hemisphere was removed and the underlying dura was cut. Using blunt dissection with a suction pipette and under a fiber-optic headlight, the left hemisphere was separated from the thalamus at the level of the internal capsule and the caudate nucleus. After the pedicle of the middle cerebral artery was ligated and sectioned, the entire hemisphere was removed in a block. This ablation, shown in Fig. 1A, included the neocortex and medial walls of the hemisphere, the neostriatum and the hippocampus (except for its ventromedial portions, and ventromedial portions of the rhinencephalon). The diencephalon and mesencephalon were spared, with only occasional minor injury to the dorsal thalamus. At the end of surgery, the cranial defect was covered by the temporal muscle left intact at the beginning, and stitched back to the midline facia and/or bone.

For ablation of the caudate nucleus (Fig. 1B), the bone of the midline calvarium was removed, sparing the sagittal sinus, and the dura was opened on the right side. A 5-mm-wide spatula was stereotaxically positioned between the hemispheres and was used both to indicate the anterior margin of the underlying head of the caudate and to gently retract the hemisphere. Under visual control, a small opening was made in the gyrus cinguli (area 24) with a thin suction pipette. The corpus callosum was penetrated at this site, gaining access to the lateral ventricle and exposing the dorsomedial aspects of the caudate. At this point, the softer consistency of the caudate allowed reduction of the suction strength to a level sufficient for aspirating the nucleus without affecting tougher neighboring fiber structures e.g., the internal capsule. At the end of surgery, the skull bone defect was sealed with cranioplastic material and the skin was sutured.

Only routine postoperative care was necessary for all surgical animals including prophylactic treatment with penicillin-G for 3 days as well as monitoring the states of hydration and grooming for the first postsurgical days. For further details on the surgical and maintenance techniques, see [30,34] for hemispherectomy and [29] for caudate nuclei ablation.

Procedures

Intact cats (N=17) received IP injection of saline placebo and/or morphine sulphate in the following doses: 0.5 (N=5), 1.0 (N=5), 2.0 (N=6), or 3.0 (N=10) mg/kg. No animal received more than 2 doses and injections were greater than 15 days apart. Brain-lesioned cats were allowed to recover from surgery for at least 30 days before testing: HEMI and caudate lesioned cats all received the 2.0 or 30. mg/kg morphine dose only. In separate experiments, naloxone (1.0 mg/kg IV) was administered 2 hr after morphine (N=7); these later scores were not included in the statistical analysis of morphine effect.

The following protocol was used. On the day of drug administration, cats were first adapted for 1 hr to a sound-attenuated chamber where observations could be made through a 1-way mirror. Following adaptation, a low-light video camera was used to record 2-min behavioral samples at 15 min intervals between 1 hr before and 5 hr after injection. An additional 4–6 min sample was taken immediately after injection to determine immediate drug effects. Rectal temperature was measured at the beginning and end of each experiment (Yellow Spring Instr., Model 43A telethermometer, and Model 401 thermoprobe).

These samples were later played back and scored on a 12-channel Esterline-Angus event recorder, according to 3 broad behavioral categories: (a) Postural and motor activity: walking, standing, sitting, crouching, lying-down, jumping, rolling or rearing. (b) Discrete behaviors: movements of the head (including up, down right and left), paws, tail and pinnae; as well as licking, grooming and sniffing. (c) Autonomic and miscellaneous events: retching, vomiting, defecation, micturation, chewing, vocalization, tachypnea/panting, drowsiness/sleeping, and pupil size. Informal notes made during the experiments were also used for behavioral analysis.

From the event recorder strip-chart we then computed the percentage time per sample each animal spent in each of the body postures and the per sample frequency of discrete movements and miscellaneous events. Videotaping, scoring and data computation were all performed by investigators unaware of the animals' conditions or treatment. As an additional check, videotapes and strip-charts were rescored by

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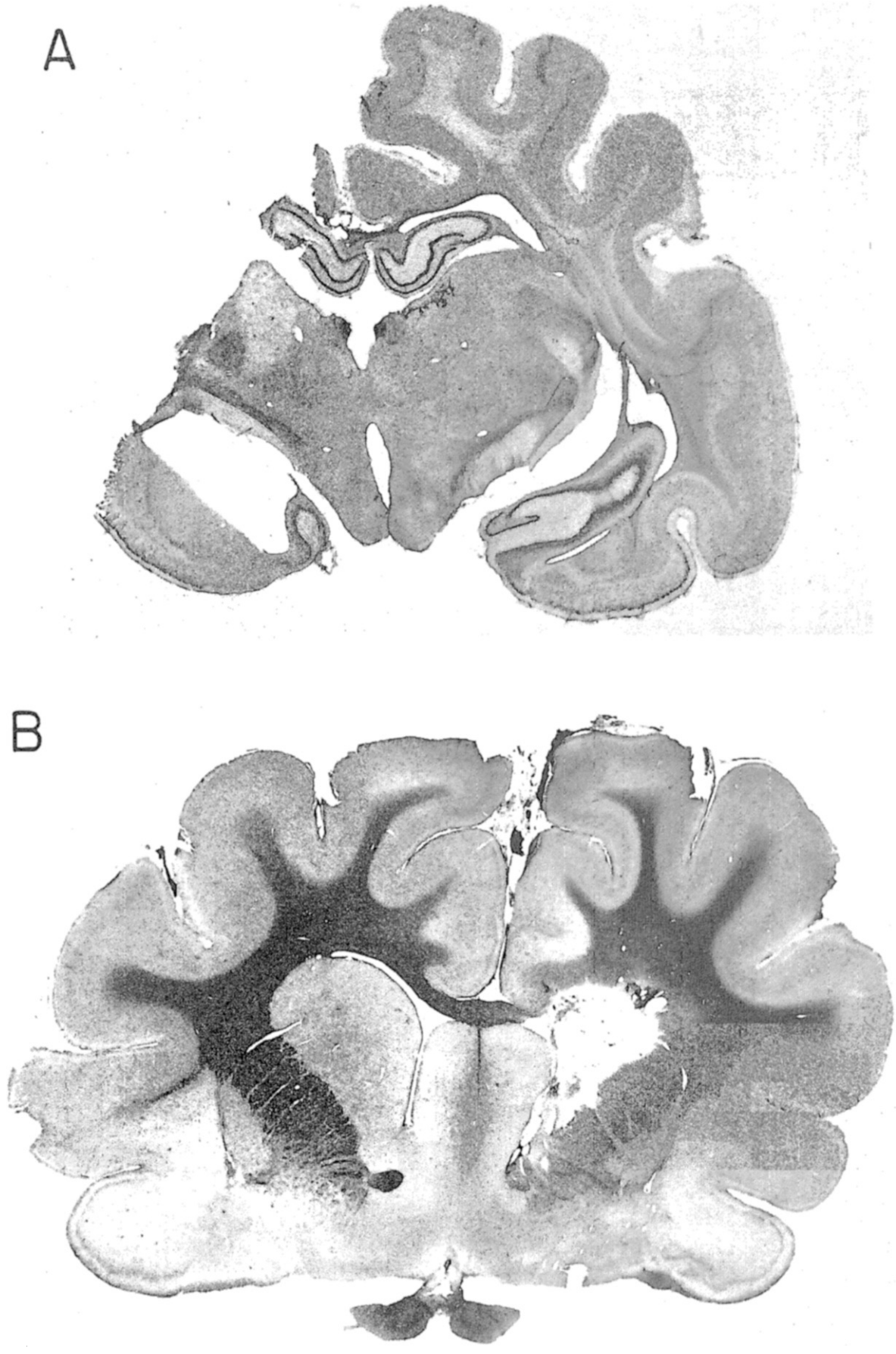
FIG. 1. A. Coronal section at approximately A8.0 [20] of the brain of a cat with removal of the left cerebral hemisphere to show extent of a typical ablation. Note considerable atrophy of the left thalamus. Nissl stain. B. Coronal section at approximately A16.0 [20] of the brain of a cat with a unilateral virtually complete removal of the caudate nucleus. Weil stain.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the same or another investigator. Inter-rater reliability for our scoring procedure was tested by having two raters score the same video records. Following the methods of Winer [35], reliability coefficients for the postures and movements tested showed a range of r=0.993 to r=0.817. These high reliability scores, combined with the above precautions, minimized the likelihood of experimenter biases.

To assess turning behavior, the number of body turns greater than 90° were counted during the video recording sessions of cats receiving the 2.0 or 3.0 mg/kg doses and their directionality (towards the right or the left side) was noted. Because the turns were minimal during the first hr post morphine, ("quiet stage" see Results), only data from the hr 2 through 5 are reported in Results.

Statistical Analysis

Observations from the 4–6 min immediately after drug administration, from the 1st hr, and from the remaining 5 hr were treated separately. Immediately after injection when intense autonomic effects dominated discrete behavior, qualitative observations from written notes and videotape records were tabulated and summarized. During the 1st hr, when behavioral changes proceeded rapidly, drug comparisons were based on 4 samples taken at 15-min intervals. During the remaining 4 hr, each 2 consecutive samples were averaged within each of eight 30-min periods through the remaining experiment.

To determine direction of head movements, right head movement was expressed as a percentage of total lateral head movements (Right/Right + Left × 100=% Right Head) and up head movement was expressed as a percentage of total vertical head movements (Up/Up + Down × 100=% Up Head). A similar computation was done to calculate percentage of right versus left forepaw movements (at the 3.0 mg/kg dose) and of right versus left body turns (at the 2.0–3.0 mg/kg dose). All percent scores were examined for normality before analysis; transformation was not necessary in these measures [24]. Each animal contributed one score, computed as the average of scores between 2–5 hr postdrug. To determine bias, these scores were compared to 50% (i.e., equal movements to both sides) using the *t*-test for comparisons of a sample to an estimated population mean [24].

Parametric statistics were used to determine drug-related behavioral effects after morphine. Dose comparisons between intact cats were made with 2-factor, repeated measures analysis of variance (dose × (samples × subjects)); using Winer's [35] unweighted means model. Planned comparisons were tested using the appropriate group error term for drug vs. saline placebo controls at each dose level, according to the procedures of Games [12]. To assess changes in rectal temperature, an analysis of covariance was used [35] to separate drug-related effects from individual pre-injection baseline differences.

Determination of lesion effects on drug response was based on 2-factor, repeated measures ANOVA (lesions × (samples × subjects)) [35], using the model described above. The two lesioned groups were compared both with the group of all intact cats receiving equivalent dose and with the saline baseline score.

Histology

Following the experiments, the brain-lesioned cats were terminated with a barbiturate overdose. The brains were perfused with 10% buffered formalin, subjected to gross examination, and then sectioned and stained with Weil and Nissl stains. To evaluate the extent of the hemispherectomy as well as any possible damage to non-caudate structures in caudate lesioned cats, the atlas of Reinoso-Suarez [23] was used, since it provides complete frontal sections through the forebrain. To calculate the amount of caudate tissue removed, the lesions in each brain were reconstructed at 5 regularly spaced anterior-posterior planes through the lesioned areas. Reconstructions were drawn on a grid of 10 mm squares superimposed on appropriate plates from the atlas of Snider and Niemer [23]. The percentage of caudate tissue removed was then computed by counting the number of squares covered by each lesion.

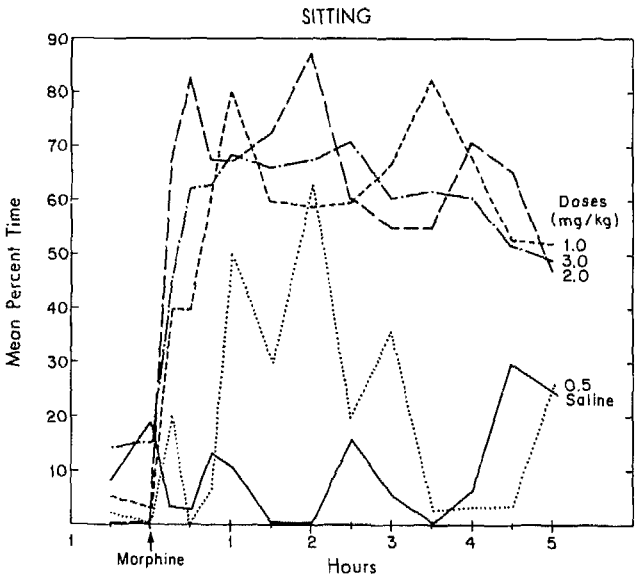

FIG. 2. Dose-response curves for the time spent in the sitting posture by intact cats (N=10 to N=15). Mean percent time: average percents for 2 consecutive 2-min video samples taken every 15 min within each 1/2 hr indicated in the abscissa.

RESULTS

Prior to the injection, most animals slept. Except for slight differences in the rate of adaptation to the chamber, there was no significant difference among the groups on any of the predrug behavioral indices. After morphine injections (0.5 to 3.0 mg/kg, IP) the behavioral events followed a regular, typical 3-stage sequence which will be described below separately for intact and brain-lesioned cats.

Autonomic Stage

Immediately after morphine injection, all cats displayed the strong autonomic effects for which this stage is named. Following a brief episode of vocalization and walking about the chamber, the animals exhibited salivation, licking and swallowing. Cats began to retch in 69.5% of the 62 experiments, at an average of 2.5 min after injection (range=0.5–14 min). Vomiting followed in 50.0% of the experiments. The latency of retching showed a significant dose-dependency (r=0.27; $t(60)=2.22$; $p=0.03$). Mydriasis began during this period, but its time-course was not determined.

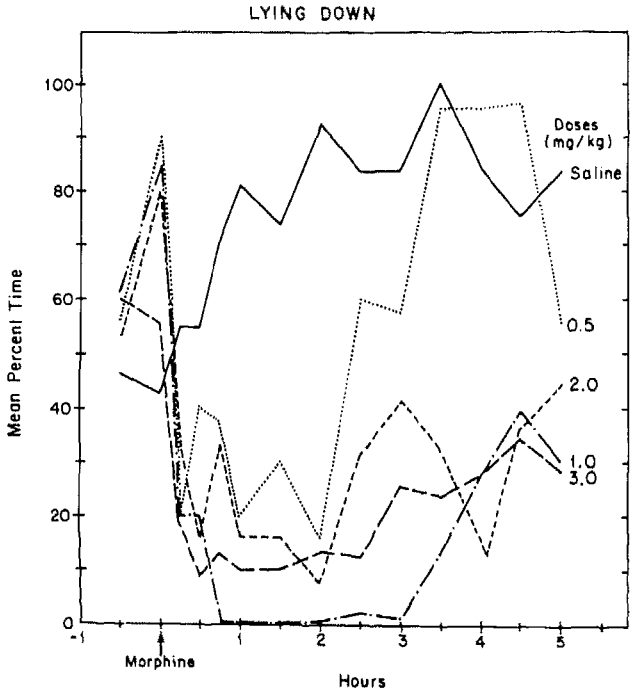

FIG. 3. Dose-response curves for the time spent in the lying posture by intact cats (N=10 to N=15). Mean percent time: as in Fig. 2.

Quiet Stage (First Hr Postmorphine)

This stage was characterized by quiet sitting as the dominant behavior (hence the label Quiet Stage). It also was a transition period for the rapid development of behaviors which peaked later.

Intact Cats

Posture and activity. While the placebo animals continued lying down or crouching, mostly asleep, morphine cats had pinnae pricked and appeared alert and attentive. As shown in Fig. 2, morphine animals spent over 50% of their time quietly sitting; significantly more than with placebo except at the lowest dose (1.0 mg/kg: $F(1,29)=5.58$, $p=0.02$; 2.0 mg/kg: $F(1,29)=11.02$, $p=0.002$; and 3.0 mg/kg: $F(1,29)=9.91$, $p=0.004$). Conversely, the percentage of time spent lying down (Fig. 3) was reduced by more than half at the same doses, relative to placebo (1.0 mg/kg: $F(1,29)=9.49$; $p=0.004$; 2.0 mg/kg: $F(1,29)=5.60$; and 3 mg/kg: $F(1,29)=6.88$, $p=0.01$). Since walking, standing and crouching were rare during this period, no further significant drug-related changes were apparent in these postures.

Discrete behaviors. A characteristic type of head movement began during this period, around 15 min postmorphine at the high doses and after 30 min at the lowest dose (Fig. 4). Although these movements are most clearly described in terms of visual behavior, their underlying mechanism is undetermined. Initially, the head movements were relatively simple and appeared as if the animals had spontaneously begun to look around, moving the head in any direction. By the end of the first hr, particularly in the animals with larger doses, the head movements had markedly increased in speed and complexity such that the cat appeared to be visually tracking an object moving rapidly in an unpredictable trajectory. The total frequency of these movements increased threefold over placebo levels at 2.0 mg/kg, $F(1,29)=5.25$, $p=0.03$, and by fourfold at 3.0 mg/kg, $F(1,29)=22.59$, $p<0.0001$; lower doses did not reach significance. There was no consistent bias for the lateral or vertical head movements except for a small but significant one at the 3.0 mg/kg dose. At this dose the movements to the right (65.8%) and upward (66.7%) dominated: $t(10)=2.84$, $p=0.02$ and $t(10)=2.49$, $p=0.02$ respectively. Except for a decrease in grooming at the highest dose (3.0 mg/kg: $F(1,29)=5.42$, $p=0.03$), no other drug-related changes in discrete behaviors were seen during this period.

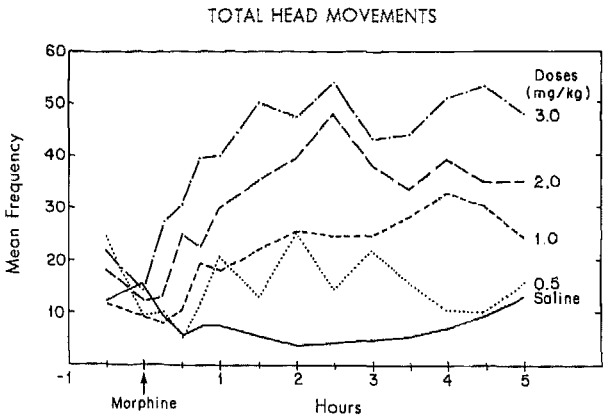

FIG. 4. Dose-response curves for the frequency of head movements in intact cats (N=10 to N=15). Mean frequency: average frequency for 2 consecutive 2-min video samples taken every 15 min within each 1/2 hr indicated on the abscissa.

Brain-Lesioned Cats

The posture/movement and discrete behaviors of these lesioned animals were qualitatively similar to those described above for intact cats receiving equivalent doses (2.0–3.0 mg/kg). The only quantitative difference during this period was in the direction of head movements in the HEMI cats. These animals showed a predominance (74.1%) of head movements ipsilateral to their brain lesion, i.e., towards the left side, $t(7)=2.51$, $p=0.4$.

Head Movement Stage (2–5 Hr Postmorphine): Intact Cats

Posture and activity. The morphine cats continued to be fully aroused throughout the remainder of the experiment. Postural trends seen during the first hour were accentuated during this later period, as shown in Fig. 2. Percent sitting increased fivefold at all but the lowest dose level, so that all cats receiving 1.0 mg/kg or more were found sitting most of the time (1.0 mg/kg: $F(1,26)=7.81$, $p=0.01$; 2.0 mg/kg: $F(1,26)=9.23$, $p=0.005$; and 3.0 mg/kg: $F(1,26)=9.84$, $p=0.004$). An inverse effect was seen for lying down (Fig. 3), which decreased markedly at the same dose levels (1.0 mg/kg: $F(1,26)=27.56$, $p<0.001$; 2.0 mg/kg: $F(1,26)=19.68$, $p=0.0001$; and 3.0 mg/kg: $F(1,26)=29.15$, $p<0.0001$). Both above changes were significantly dose-related but did not reach significance for the 0.5 mg/kg dose. The only other change of note was a modest increase in walking at the 2.0 mg/kg dose, $F(1,26)=4.66$, $p=0.04$. Nevertheless, walking remained very low for all levels (2.1% of overall time for the 2.0 mg/kg dose and <1.0% for all other doses). Under-

standably there were relatively few body turns during the 3 hr period (mean 14.9 N=9) and most were partial turns (just over 90°). Regarding their directionality, 4 animals had too few turns to be considerend (less than 4) and for the other 5 there was a non-significant bias towards the right (59.1%, $t(4)=1.76$, $p=0.15$).

Discrete behaviors. The characteristic morphine head movements described previously peaked during this later drug period (Fig. 4). Their frequency reached about 50 movements/sample for the two highest doses at 2.5 hr postdrug and either maintained that level (3.0 mg/kg) or slightly declined (2.0 mg/kg) thereafter. This represented more than a fourfold increase over control levels and reached significance for all but the lowest 0.5 mg/kg dose (1.0 mg/kg: $F(1,26)=7.99$, $p=0.01$; 2.0 mg/kg: $F(1,26)=5.68$, $p=0.02$ and 3.0 mg/kg: $F(1,26)=32.65$, $p<0.0001$). The direction of these head movements showed a modest, non-significant bias to the right (see Table 1) and a significant upward vertical bias at 0.5 mg/kg (77.2%, $t(4)=4.34$, $p=0.01$) and at 3.0 mg/kg doses (70.7%, $t(8)=3.31$, $p=0.01$).

Other morphine-related discrete behaviors were observed during this period. Short-distance paw movements began near the end of the first hr and peaked at a frequency of about 10 movements/sample between 1.5–4 hr for the highest doses. These movements appeared to be of two types: (a) a pouncing/approaching movement, as if the cat was trying to catch a prey object, and (b) a more defensive-looking movement, as if the cat were trying to avoid an object about to hit the paw. We did not see any significant bias for movements of the right (54%) versus the left (46%) paw. These morphine behaviors reached significance at the highest dose level (3.0 mg/kg: $F(1,26)=6.48$, $p=0.02$). Abundant pinnae and tail movements were seen concomitantly.

Additional body behaviors occurred in the same period and at a frequency similar to pawing, including brief to-and-fro body rocking movements, brief backing movements, and quick pivoting on fixed hindlegs; these were observed at all but the 0.5 mg/kg dose. With the exception of rocking, these movements were rare at the 1.0 mg/kg dose, and were never seen before the second hr postmorphine. Frequent bouts of pinnae movements were also seen throughout this period at all dose levels. At the higher doses, all the behaviors occurring during this period seemed to be tied together in a complex behavioral pattern which gave us the impression that the cats may have been "hallucinating." Salivation, licking and sniffing were frequent at the higher doses.

Some behaviors were suppressed or markedly reduced after morphine. Sleep was totally suppressed (see next paper). Grooming was practically eliminated at all dose levels compared to controls (0.5 mg/kg: $F(1,26)=19.65$, $p=0.0001$); 1.0 mg/kg: $F(1,26)=17.06$, $p=0.0003$; 2.0 mg/kg: $F(1,26)=21.61$, $p<0.0001$; and 3.0 mg/kg: $F(1,26)=21.43$, $p<0.0001$). Of the total 62 experiments, defecation was seen in only 3 cases and micturation was totally suppressed.

Mean rectal temperature at the experiments' end (5 hr postmorphine) varied according to dose (saline=37.8°C; 0.5 mg/kg=38.5°C; 1.0 mg/kg=38.9°C; and 3.0 mg/kg=39.3°C). This increase was significantly dose-related ($r=.76$; $t(27)=6.03$, $p<0.0001$). The pre-injection value was 38.02°C.

Brain-Lesioned Cats

Posture and activity. There was a tendency for both morphine-injected HEMI and caudate lesioned cats to spend more time in the relaxed postures than intact cats receiving

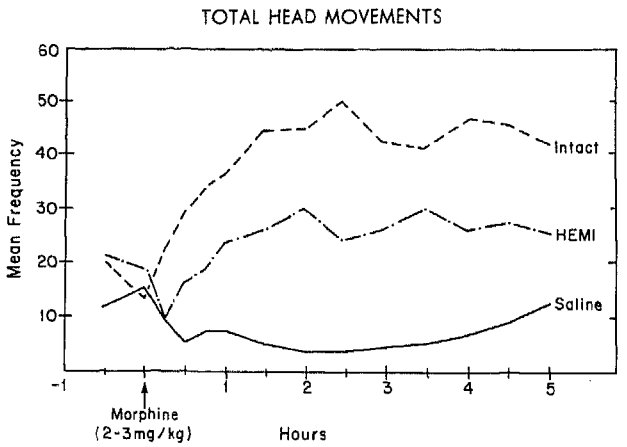

FIG. 5. Frequency of head movements in hemispherectomized (HEMI) cats as compared to intact cats with the same dose of morphine. Mean frequency as in Fig. 4.

the same dose. However, this decreased behavioral activation relative to intact cats receiving similar doses of morphine reached significance only for caudate lesioned cats' lying down, $F(1,17)=4.67$, $p=0.04$, in fact. In this measure, scores of the caudate lesioned cats after morphine were not statistically different from the saline placebo baseline of intact cats. There were non-significant changes for the 3 hr body turns counts for these cat (HEMI cat mean, 19.8; caudate lesioned cats mean, 11.2). A major lesion effect was a strong bias for the HEMI cats to turn toward the left or lesioned side (76.5%, $t(4)=372$, $p=0.02$), whereas in the caudate lesioned cats the frequency of body turns to the right or lesioned side (49.2%) was equivalent to the frequency of turning to the left.

Discrete behaviors. The major lesion effect was found in the head movements of the HEMI cats. Although they exceeded (Fig. 5) the saline baseline level, $F(1,10)=15.08$, $p=0.003$, HEMIs' total head movements were significantly fewer than in intact cats receiving a similar morphine dose, $F(1,19)=5.350$, $p=0.03$. This reduction resulted from a significant decrease of head movements to the right (Table 1). Thus, HEMI cats right head movements approached saline baseline levels, while head movements towards the left were statistically equivalent to those of intact cats receiving the same morphine dose. Hemispherectomized cats also showed a significant bias for paw movements of the left, ipsilateral, paw (61.8%, $t(5)=5.895$, $p=0.002$). In caudate lesioned cats, there was a nonsignificant decrease in the frequency of head movements compared to intact animals, particularly during the first post drug hr, but this was not accompanied by a change in directionality (Fig. 5 and Table 1). Movements of the right paw (ipsilateral to the lesion) were non-significantly decreased in these cats (42.3%, $t(5)=1.608$, $p=0.17$). Otherwise, lesioned animals' other discrete behaviors during this stage resembled those of intact cats given the same morphine dose.

As seen in Figs. 2, 3 and 4 the duration of the overall drug effect was generally shorter with the smallest dose.

Naloxone (1 mg/kg IV) injected at the peak of the effects eliminated the morphine-induced behaviors in all groups within 2–4 min. After an initial period of vocalization, locomotor activity and increased respiratory rate, all cats became drowsy (at 15–45 min post naloxone) and later went to

TABLE 1

PERCENT RIGHT HEAD MOVEMENTS

Cat Groups	Dose (mg/kg)	Hours Post Morphine 2	3	4	5	Bias t	Significance df	p
Intact	3.0	59.3	57.1	56.4	50.9	1.84	8	0.10
	2.0	54.7	57.2	62.6	60.6	2.27	5	0.07
	1.0	46.3	48.1	53.7	46.5	0.32	4	0.77
	0.5	54.5	57.3	63.4	40.0	0.49	4	0.65
HEMI	2.0–3.0	28.0	27.2	30.5	29.5	7.58	5	<0.01
UAc	2.0–3.0	56.3	46.3	52.0	50.5	0.002	3	0.998

Right head movements are expressed as a percentage of total lateral head movements and the significance of the bias is calculated assuming that under no-drug conditions the movement to the right or left are 50% of the total. HEMI: cats with removal of the left cerebral hemisphere; UAc: cats with removal of the right caudate nucleus.

sleep. Cats urinated in 4 of 7 experiments and mean rectal temperature 3.5 hr post naloxone was 38.4°C. After about 2.5 hr, the naloxone-blocked behaviors reemerged. In experiments where naloxone was injected alone, we could detect no changes relative to saline controls.

Histology

The hemispherectomy lesion was as described in the Method section (Fig. 1A), except in 3 cases where moderate to slight damage was found in dorsolateral portions of the pulvinar and lateral geniculate nuclei on the side of the ablation. The brains of all 4 caudate lesioned animals were examined. Caudate tissue removal on the right side was 76.0, 79.6, 83.8 and 93.0% of the total nucleus respectively with no important additional damage except for the brain of the cat with the largest removal which had moderate internal capsule damage at about A 18–20 [22].

DISCUSSION

This study demonstrates that morphine induces in cats a typical behavioral response that follows a long and predictable time course when administered in the dose range used here. Although the behaviors displayed are relatively rich and complex and therefore difficult to reduce to numbers, we here provide what appears to be the first statistically verified measurement of discrete behavioral effects of morphine in cats. We have also shown that these behavioral manifestations are dose-dependent, with a threshold between 0.5–1.0 mg/kg for intraperitoneal administration.

To the extent that all the reported behaviors appeared relatively invariable across tested cats, they may be considered stereotypic. However, the present stereotypies appear far more complex than others that we have seen induced by psychoactive drugs including atropine [17,27], amphetamine [19], and harmaline [28], or which have been described for LSD [17]. At the peak of the drug's action the head movements give the impression of a "sensory hallucination" with a marked visual component and this impression is strengthened by the coordination of eye-body movements with concurrent paw and body approach-avoidance movements. Therefore, it appears that the head movements together with the accompanying discrete body movements in the context of a quiet posture, comprise the core of a highly specific behavioral profile induced by *low* doses of morphine in the cat.

The fact that the behavioral effects peaked at the 2–3 hr post drug period is interesting in view of the findings by Chernov and Woods [3] relative to the time-course of morphine plasma and brain levels in cats receiving a similar (2 mg/kg SC) dose of the drug. These authors demonstrated that at 2 hr the morphine concentration in cat plasma is over 66% of that at 30 min postinjection, and that by 4 hr the concentration is still about 30% of the peak value. Even more relevant is their finding that brain levels of the drug reach a peak at 2 hr and by 8 hr the brain levels are still more than 33% of the peak value. Therefore, the time course of our behavioral data are in striking agreement with the above pharmacokinetic results.

Some recent reports lend support to these findings. French and colleagues [11] found "that the prominent behavior was a prolonged sitting up" with "fixed staring," after intravenous morphine doses within the present range. They also noted head movements that "increased with equal distribution between vertical and horizontal direction," but did not attempt further description or measurement of these movements. Head movements appear to be the dominant behavior noted in the subjective description made during a one hr observation after 5.0 mg/kg IP morphine by Cools *et al.* [5]. These authors also suggest a 3-stage time-course comprising "depression, reorganization, and ritualization," which, assuming that we interpret them correctly, might correspond to the autonomic, quiet and head-movement stages we have measured.

Traditionally, the general conclusion to be drawn from the literature was that morphine in the cat induced a state of strong behavioral excitation, motor agitation and aggression [9, 10, 15, 18, 20, 25, 26]; this research was based on much larger doses of the drug (>5–10 mg/kg) than those used here. The term "feline mania" was coined to describe this state and, consequently, the cat was almost abandoned as a subject for investigation of discrete behavioral opiate effects. This is regretable, since the cat has an extensive behavioral repertoire and has long been a classical subject for neuroscientific studies. Thus, it would be advantageous if the large

body of knowledge accumulated for this species could be brought to bear on the problems of opiate research.

The present results suggest that "feline mania" might be the consequence of morphine overdose in an animal with a relatively low behavioral threshold for this drug. Even the 5.0 mg/kg dose used by Cools [5] may mark the upper limit for studying discrete behavioral effects, since at this level "sudden initiation" movements, running, jumping, and other manifestations of impending "mania" were already noticeable. Furthermore, even with the lower dose range which we use there is a trend for increasing motor activity with higher drug dosage.

Therefore, it is interesting that relatively small doses of β-endorphin injected directly into the cerebral ventricles of cats seem to produce behavioral effects like those we describe after intraperitoneal injections of morphine. After 12.5–25.0 μg of intracerebroventricular morphine, Meglio *et al.* [21] described a behavioral syndrome where cats always appeared to have "visual hallucinations." In contrast, Beleslin *et al.* [1] administered 30–400 μg doses and found psychomotor excitation with a time-course similar to the present study, combined with irregular episodes of strong agitation, presumably at the higher doses.

The results presented in this study and in the two companion papers which follow, clearly establish the cat as a model for analysis of the behavioral effects of opiates. We are now attempting to understand the CNS sites of action which may be responsible for the basic components of the behavioral and neurological effects that we have described.

In experiments [7] with cats deprived of the entire telencephalon (diencephalic), and in pilot (unpublished) experiments in cats with a transection of the brain stem at the level of the mesencephalon (decerebrate), we have found the same protracted behavioral activation/arousal effect that we describe for intact cats here and in the following paper. One important difference is that, immediately after injection and corresponding in time to the onset of the "quiet stage" of intacts, diencephalic cats exhibit a pronounced inactive stage resembling nonREM sleep or catalepsy. In decerebrate cats, missing the diencephalon, this stage of inactivity is absent. These findings suggest that the lower brain stem is responsible for an overall background posture and arousal-activating drive, while the presence of the diencephalon contributes an inhibitory modulation that may form the neural basis for the "quiet stage" of the intact animal. In addition, the more complex, discrete behaviors (including characteristic head and paw movements) are absent in both the diencephalic and decerebrate preparations, indicating that the presence of the forebrain is necessary for the morphine-induced expression of these behaviors.

The present series of studies, combined with previous work, suggest two hypotheses to explain the neural basis of the morphine-induced head and paw movements. The first of these hypotheses is suggested by the morphine response of cats with extensive bilateral lesions of the caudate nuclei [29,33]. These animals show a marked decrease of these typical head and paw movements compared to intact cats, suggesting direct striatal control of the drug-induced expression of these behaviors.

However, the present research suggests that the cerebral cortex may also play a role. In fact, we here show that the morphine-induced head movements toward the side contralateral to the lesion are markedly curtailed in our HEMI animals which lack both the caudate and neocortex on that side. In cats which lack only the caudate but retain the neocortex (i.e., in our caudate lesioned cats), morphine-induced head movements are relatively unchanged from levels of intact animals. Therefore, the alternative hypothesis is that the discrete head movements might result from a basic neocortical influence which is only indirectly affected by the absence of the caudate nuclei (admittedly, limbic cortical areas are also included in the hemispherectomy but it is hardly likely that these areas might be involved in the motor response under discussion).

The relationship between cortical and striatal influences may be further clarified by the response of cats to chronic morphine administration, presented in the last paper of this series. We have previously noted that the reduction in morphine-induced head and paw movements in acaudate cats is accompanied by a marked increase in locomotor activity [31]. When locomotion diminishes with tolerance to chronic morphine (see last paper), there follows a concurrent trend for increasing head movements in acaudate cats, raising the possibility that the substrate for these head movements was always present but their expression was masked by the increased locomotion. This possibility is strengthened by the observation that cats missing the caudate on only one side show neither diminution of drug-induced head and paw movements, nor any increase in locomotion. The reduction of characteristic head movements in HEMI cats after morphine, and the fact that this reduction occurs toward the quasi-blind, visual field of the right eye in these animals, combined with the apparent visual component which accompanies these neurobehaviors, all strengthen the case for a possible cortical role in determining the discrete head movements.

In drug-naive adult-lesioned HEMI cats we have shown that there is a strong bias for ipsilateral body turning, i.e., to the left after left hemispherectomy [34]. It appears that this bias is not due simply to impairment of the visual field of the right eye in these cats [34], i.e., the animal does not just turn toward the side of the ablation because this is the only side he can see. In fact, hemispherectomized cats with the unimpaired eye blindfolded and kittens lesioned at a time when the eyelids are still closed, still show identical turning bias towards the ablated side [34].

The finding in our left HEMI cats that the head movements and the body turning to the left predominate after morphine, and that the left paw movements are more frequent, has a general interest not only for the understanding of drug-induced stereotyped behaviors, but it is also relevant to the site-of-action question raised above. In drug-naive, adult-lesioned HEMI cats we have demonstrated that there is also a bias for preferential usage of the forepaw contralateral to the intact hemisphere [2,34]. Because the later preference is seen in situations where the cats use the paw for "purposeful," directed tasks, the paw movement is most probably controlled by the intact telencephalon. Thus, if the morphine-induced movements are considered a stereotypy, (as we think they should be), it is clear that in our HEMI cat model a stereotypy results from activation by morphine of a group of coordinated movements which naturally occur in the animal and which probably depend on complex, preprogrammed neural circuitry.

In brief, from the preceding analysis it follows that a number of brain sites must contribute to the overall posture-movement behaviors induced by morphine. These include telencephalic sites (as proposed for the discrete head and paw movements) and brain stem areas (for general postures and the body turning bias). Asymmetries in the ni-

grostriatal system have been traditionally held responsible for rotational behavior [13,16] but, as it will be discussed in more detail in the last paper of these series, that model does not seem applicable to our findings.

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