Pharmacology Biochemistry & Behavior, Vol. 21, pp. 929–936, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Reassessing Morphine Effects in Cats: III. Responses of Intact, Caudate Nuclei-Lesioned and Hemispherectomized Animals Following Chronic Administration and Precipitated Withdrawal.

CYNTHIA M. HARRIS,[1] JAIME R. VILLABLANCA,[2] J. WESLEY BURGESS AND ISABEL DE ANDRES

Mental Retardation Research Center, Departments of Psychiatry and Anatomy UCLA School of Medicine, Los Angeles, CA 90024 and Departamento de Morfología, Universidad Autónoma, Madrid, Spain (I. de A.).

Received 21 February 1984

HARRIS, C. M., J. R. VILLABLANCA, J. W. BURGESS AND I. DE ANDRES. *Reassessing morphine effects in cats: III. Responses of intact, caudate nuclei-lesioned and hemispherectomized animals following chronic administration and precipitated withdrawal.* PHARMACOL BIOCHEM BEHAV **21**(6) 929–936, 1984.—Behavioral response to low doses of morphine (2.0 to 3.0 mg/kg, IP) administered for up to 15 days, and responses to subsequent naloxone challenges, were measured in intact, unilaterally and bilaterally caudate-lesioned (acaudate) cats, and in hemispherectomized cats using a video time-sampling method. For all groups minor tolerance to posture and movement activation patterns was seen, with a reciprocal increase in motor relaxation, which was somewhat more marked for acaudate cats. In contrast to this weak tolerance, all cats showed strong, typical withdrawal manifestations at the beginning of abstinence and a "mini withdrawal" could still be precipitated 15–30 days later when morphine was no longer detectable in the blood. The cats with the unilateral lesions showed whole body turning toward the lesioned side after morphine and away from the lesioned side following naloxone. Only hemispherectomized and acaudate animals showed significant physical deterioration (e.g., weight loss, decreased activity). The comparisons between weak tolerance development versus strong physical dependence and the possible mechanisms involved in shifting the turning biases are discussed. The potential of the cat as a model for studying opiate effects is stressed.

Morphine Cats Tolerance Dependence Brain lesions Neostriatum Turning Bias

IN our previous work we have defined a range of single morphine doses (0.5 to 3.0 mg/kg) which produce a syndrome of typical, reliable, dose-dependent behavioral responses in adult cats [5, 9, 26, 30]. We have also shown that extensive, bilateral lesions of the caudate nuclei dramatically change those responses [30]. This latter finding, together with our preliminary results [4] demonstrating that local cerebral glucose utilization increases in the basal ganglia areas after cats receive a single dose of morphine, strongly suggest a participation of the neostriatum in the expression of the behavioral effects of morphine.

The present experiments were designed to test the hypothesis that striatal lesions might also modify responses to a complete cycle of chronic morphine addiction. For the cat there is no literature data pertinent to this problem. In rats, Glick *et al.* [8] reported that small electrolytic lesions of the caudate nuclei reduce the rate of morphine self-administration and attenuate naloxone-precipitated withdrawal. However, Linseman could not replicate the withdrawal attenuation with larger caudate lesions [17].

These experiments also examine the more general problems of development of tolerance and morphine dependence in the cat. As is the case for effects of a single dose [26], investigators have mainly examined the effects of repeated administration of *large* doses and, therefore, were able to assess tolerance effects only upon "feline mania" [11,16]. We know of only one study reporting quantitative effects of doses within the lower range used here; this examined only

[1]Present address: Department of Pharmacology, Texas College of Osteopathic Medicine, Forth Worth, TX 76107.

[2]Requests for reprints should be addressed to J. R. Villablanca, Mental Retardation Research Center, UCLA Medical School-NPI (Room 58-258), 760 Westwood Plaza, Los Angeles, CA 90024.

intact cats for a shorter period of administration [7]. Therefore, the question of whether feline reponses to a cycle of morphine at the present dose level are similar to those in other species remains unanswered, and the issue of the cat's suitability as a model for studying addictive properties of opiates remains open.

Finally, in our experiments on single-dose administration in unilaterally brain-lesioned animals [26], we observed some morphine-typical movement asymmetries and turning biases. For this reason, cats with a unilateral ablation of the caudate nucleus and with the removal of one cerebral hemisphere were included in the present experiments to assess possible tolerance and precipitated withdrawal effects upon those behaviors. In addition, we wished to test the applicability of the popular nigrostriatal dopaminergic theory [1, 12, 13, 25] to explain the morphine- or naloxone-induced rotational biases in the present cats. In previous reports [26,29] we have presented reasons which cast doubts about the generality of that theory. Some preliminary finding's were published in abstract form [31].

METHOD

Subjects

We used 23 adult male cats (2.8–4.2 kg) obtained either from the breeding facility of the U.C.L.A. Mental Retardation Center or from unclaimed cats at animal shelters. Five cats had extensive bilateral lesion of the caudate nuclei (hereafter called acaudate cats), 4 received extensive lesion of the *right* caudate nucleus only and 4 animals had removal of the *left* cerebral hemisphere (called HEMI cats). Five intact cats were used for morphine injection and the other 5 were saline controls. The surgical procedures were described in detail in [26]. For the bilateral caudate nucleus ablation, the same procedure described for the unilateral caudate-lesioned cats was repeated on the contralateral side. A minimum of 15 days was allowed for recovery of the lesioned animals prior to any intervention.

Procedures

After a 2-week period of measurements to determine baseline rectal temperature and body weight, the cats received a 16-day course of 2.0–3.0 mg/kg/day, IP morphine sulphate. On day 16, the cats received the first challenge consisting of 1.0 mg/kg, IV naloxone given 2.5 hr after morphine injection. An identical challenge was repeated 14 to 30 days later.

On days 1, 5, 11 and 15, and during naloxone days, the cats were placed in a sound-attenuated chamber faced with a one-way mirror where they were studied for 2 hr before and for 5 hr after morphine administration. For behavioral analysis, we used the same video-time sampling technique described in [26]. In brief, two-min samples were taken at 15 min intervals starting 1 hr before and continuing for 5 hr after morphine (or 3 hr after naloxone) injection. Samples from the tapes were later scored on an event-recorder according to 3 behavioral categories: (1) postural and motor activities, (2) discrete movements of body parts, and (3) autonomic and miscellaneous events (for details, see [26]). From the event-recorder charts, we then computed the percent time per sample each animal spent in each of the body postures and the per sample frequency of discrete movements and miscellaneous events. Scoring precautions similar to those described for the single injection experiments were taken; these methods were shown to yield a high inter-observer reliability. The mean values per sample for each cat in selected behavioral events were calculated on each of the videotaping days and used for the analysis and figures. In addition, weight and vomiting were recorded daily just prior to or immediately after morphine injection respectively, and the rectal temperature was measured through day 11, before and after video sessions, and 45 min after naloxone (Yellow Spring Instr. Model 43 TA telethermometer and Model 401 thermoprobe).

For quantification of precipitated withdrawal, each cat was given a score ("severity score") for each of the behaviors seen during the first 30 min after naloxone injection; the mean of these scores for all cats and for each of the two naloxone challenges are shown in Fig. 2. The manifestations were scored on a 5-point scale individually constructed for each of the behaviors. A score of zero indicated absence of the behavior while a score of 4 was the average value for the 3 cats showing that manifestation with the maximum severity (interested investigators may request a copy of the scoring instrument). The measure for squinting (blinking to the light of the chamber), urination-defecation and spraying (shooting a stream of urine at the wall), was the percent of cats showing the behavior during the 30 min period.

Neurological and gross behavioral testing were conducted in all cats during the week prior to morphine administration and before the drug injection on days 11th and 15th of the addiction cycle, as well as 15th–20th days of the abstinence period. For this assessment we used the battery of tests described in [27].

A measure of motor activity was conducted for intact and acaudate cats on days 2, 7, and 14 of morphine administration, with several baseline sessions within 2 weeks before and after drug injections. On drug days the testing was performed once immediately before morphine (or saline) injection and again 3 hr after the injection. The test was conducted in a large open floor area in the middle of our vivarium (5.8×7.7×2.7 m) which houses about 48 male and female cats. The animal was released from his home cage and his spontaneous activity was measured as the number of whole body turns larger than 90° during the next 10 min period. The direction of body turns was also noted in order to assess changes in turning bias (see [26]). Six observers, 4 of whom were blind to the cats' drug status, were used.

Morphine and metabolites in plasma were determined by radioimmunoassay [3].

Statistical Analyses

For the comparisons, every 2 consecutive video samples (at 15 min intervals) were averaged throughout the experiments to provide repeated measures every 30 min. To detect changes in drug effects over 1–15 days of chronic morphine administration, simultaneous solution multiple regression techniques [33] were used, with drug days (1, 5, 11, and 15) as the dependent variable and behavioral values for each time sample (0–5 hr postinjection) as the independent variable. Thus, it was expected that strong tolerance effects would be revealed as significant positive or negative trends in the multiple correlation coefficient for the *entire* postdrug period, i.e., main effects corresponding to significant multiple regression coefficient ANOVAS; whereas weaker tolerance effects might only reach significance at one or more observation periods, i.e., simple effects corresponding to significant partial regression *t*-tests for single 30-min samples.

In assessing changes in autonomic responses and weight loss, an analysis of covariance [34] was used to separate drug-related changes in body weight and rectal body temperature and remove any correlation with individual cats' normal baseline differences. Percent vomiting per 4-day intervals and number of body turns per testing session were contrasted throughout drug administration with analysis of variance for repeated measures using an unweighted means model.

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 (Weil and Nissl). 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-Suárez [20] was used, since it provides complete frontal sections through the forebrain. The lesions in each brain were reconstructed at 5 regularly spaced anterior/posterior planes throughout the lesioned areas, 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.

RESULTS

Single Dose Effects

The behavioral effects seen following a single morphine injection in intact and in similar brain lesioned cats were described in [26] and [30]. Very briefly, in intact cats morphine induces a discrete syndrome of stereotyped head and paw movements with the animal mainly sitting but very aroused. The effect is similar for unilateral caudate-lesioned and hemispherectomized cats, although in the latter the head movements contralateral to the ablation side are markedly decreased. Acaudate cats show a much lower frequency of head movements than intacts, and the acaudate animal becomes hyperactive, as shown by a marked increase in walking.

Tolerance Effects

We were unable to show substantial behavioral tolerance effects for any of the groups. However, over the course of administration there was a modest decrease of the behaviors related to motor activation (e.g., walking), coupled with a reciprocal increase of those indicative of motor relaxation. This was best seen for the acaudate cats, and therefore, these results will be described first. As shown in Fig. 1, for the first morphine day acaudate cats spent about 30% of their time walking. This percentage slowly decreased during subsequent days, so that by day 5 no clear trend was seen; however, by administrations days 11 and 15, practically all walking values after the 2nd post-drug hr were less than those for day 1, suggesting development of tolerance. However, there were no statistically significant multiple correlation effects and a significant decrease (partial correlation) was found only for the walking values at 5 hr post-drug, $t(8)=2.450$, $p=0.03$. Mean walking time during day 1 remained around 5% for intacts (Fig. 1), unilateral-caudate-lesioned, and hemispherectomized groups and there was no clear tendency for change across the cycle of drug administration.

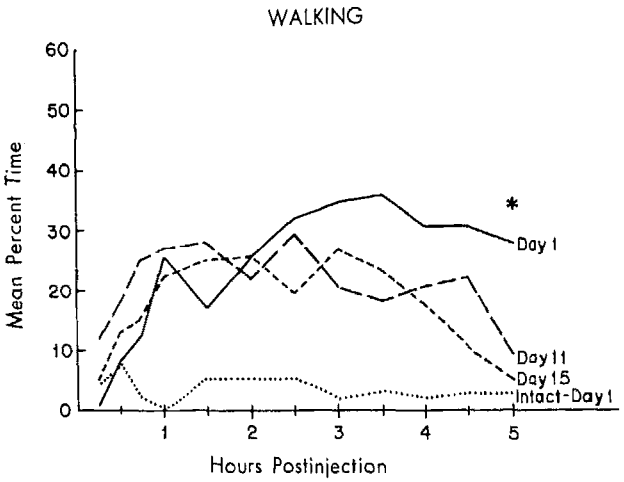

FIG. 1. Development of partial tolerance to the locomotion increasing effects of morphine in cats with extensive, bilateral caudate nuclei lesions (3 upper curves). The abscissa shows mean percent time: percents of recording time averaged for two 2 min video samples taken at 15 min intervals. On the ordinate are the hours postinjection on the days of chronic morphine administration shown. There was little change for this measure across days of administration for the other experimental groups since none of the cats walked much after morphine (see text); therefore, as a reference (and for simplicity) the mean percent of walking time is shown only for the first day of the intact cats. Significance levels:*$p=0.03$.

The trend toward motor relaxaion in caudate cats was shown by increases in crouching and in lying down. Crouching showed significant increases at 2.5 hr, $t(8)=2.47$, $p=0.02$, and at 4 hr postdrug, $t(8)=2.321$, $p=0.04$, and there was a tendency for increased lying down past the 3rd hr on days 11 and 15. In addition, in the acaudates there was a tendency to increased frequency of head movements from a mean of 29.7 per sample for day 1 to a frequency of 34.7, 38 and 34.4 per sample on days 5, 11 and 15 respectively; however, this change reached significance for only the 4th hr post injection, $t(8)=2.352$, $p=0.04$.

For intact cats the above tendencies were less marked and regular than for acaudates. They lay down more as reflected by increases reaching significance at 30 min postdrug, $t(15)=2.521$, $p=0.02$, and at 5 hr postdrug, $t(9)=2.600$, $p=0.03$. Significant increases in sitting were also seen in these cats at 30 min, $t(15)=2.805$, $p=0.01$, 45 min, $t(15)=2.262$, $p=0.04$, and 2.5 hr, $t(9)=2.409$, $p=0.04$, postdrug. No clear trends were seen for the frequency of head or paw movements.

The unilateral caudate lesioned cats showed a tendency for increased crouching which reached significance at 30 min postdrug, $t(6)=2.808$, $p=0.03$. These cats also showed a significant multiple correlation increase in discrete forepaw movements ($r=0.998$; $F(8,2)=55.323$, $p=0.02$) with significant partial correlation effects at all inclusive hours.

The minor effects demonstrated for the HEMI cats consisted of increases in crouching at 15 min post-injection, $t(11)=2.479$, $p=0.03$, and of increased frequency of head movements to the right side at 60 min post-injection, $t(11)=2.466$, $p=0.03$.

After morphine, only the HEMI cats showed a strong body turning bias (80% of turns were to the left side). The number of turns to the left was no different from those to the

TABLE 1

DEVELOPMENT OF TOLERANCE TO MORPHINE-INDUCED HYPERTHERMIA (°C)

Cat Groups		Baseline	Day 1	Day 5	Day 11	F(2,20)	*p*
Intact	mean	38.4°	39.6°	39.1°	38.8°	5.9	0.009
	(±SD)	(0.4)	(0.3)	(0.3)	(0.2)		
Bilateral Caudate*	mean	38.6°	39.9°	39.5°	39.2°	4.2	0.03
	(±SD)	(0.4)	(0.5)	(0.9)	(0.6)		
Unilateral Caudate*	mean	37.9°	39.5°	39.0°	38.4°	6.8	0.006
	(±SD)	(0.3)	(0.3)	(0.5)	(0.1)		
Hemispherectomy	mean	38.1°	39.9°	39.5°	39.2°	0.8	NS
	(±SD)	(0.2)	(0.5)	(0.9)	(0.6)		

*Indicates cats with bilateral or unilateral lesions of the caudate nucleus.

right for the 3 other groups (fluctuating around 50%). There were no significant changes for any group in the number of turns or in their direction at days 7 and 14 compared to day 2.

There was an attenuation of the hyperthermic response to morphine throughout the experiment. As shown on Table 1, this was significant for all groups except the HEMI cats. The baseline temperature of all groups was essentially identical as shown by a low within-groups correlation of baseline vs. postdrug values ($r=0.157$, $F(3,6)=0.5$, $p=$NS). The saline-treated cats did not show temperature changes. Vomiting after morphine injection was idiosyncratic for each cat and this characteristic had not changed by the end of the addiction cycle. Five cats vomited most times after injection, 5 animals seldom vomited and the remaining vomited roughly 50% of the time; these cats were equally interspersed within the 4 groups.

The saline-treated animals showed no remarkable changes in any of our measures. Immediately after drug injection they showed some activity but thereafter they slept or crouched quietly for most of the remaining observation time.

Precipitated Withdrawal Effects

In contrast to the mild tolerance manifestations described above, all cats showed *strong* withdrawal signs precipitated by a naloxone injection following the last dose of morphine. As shown in Fig. 2, most of the precipitated abstinence signs described for other species were also displayed by these cats. The maximum expression of these behaviors (which provided the score of 4 for the evaluation scale; see the Method section and Fig. 2) typically was as follows: vocalization lasting about 15 min; pyloerection lasting 10 min or more; profuse salivation or drooling; escape behavior including tearing the floor and rearing; odd postures such as tail held out straight from the body, hunched-over posture, and catatonia (2 cases); about 35 shakes (whole body or body segment "wet dog shakes"); panting lasting for about 30 min; and tachypnea, with a respiratory frequency of 200/min or higher.

The time-course of these events for the challenge on day 16 of morphine were as follows. For about 10 min the cats appeared distressed, moving briskly, attempting to escape, vocalizing loudly and panting. The other manifestations lasted longer. For 20–40 min the animals showed salivation, photosensitivity (squinting), urination or spraying, continuing vocalization, total body or partial "wet dog shakes," and

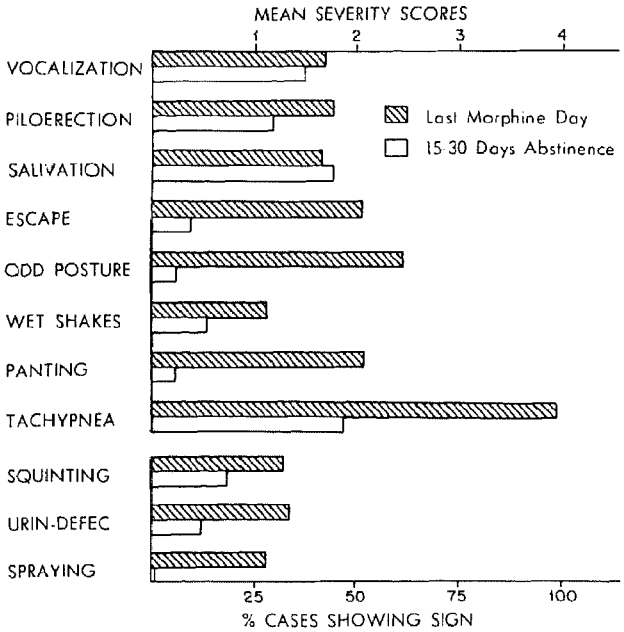

FIG. 2. Withdrawal manifestations shown by intact and brain-lesioned cats following injection of 1 mg/kg naloxone IV, on the days of the addiction cycle indicated. See text for explanation of scoring.

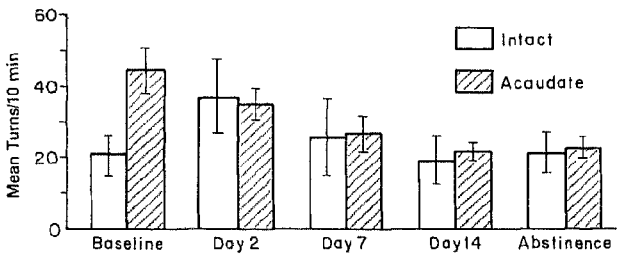

FIG. 3. Mean whole body turns (90° and over) in cats with extensive bilateral caudate nuclei lesions versus intact animals during the addiction cycle at times shown in the abscissa. On morphine days (2, 7 and 14) counts were taken immediately prior to drug administration. Note the increase and return to baseline levels in intact cats, whereas in acaudate, there was a significant progressive decline.

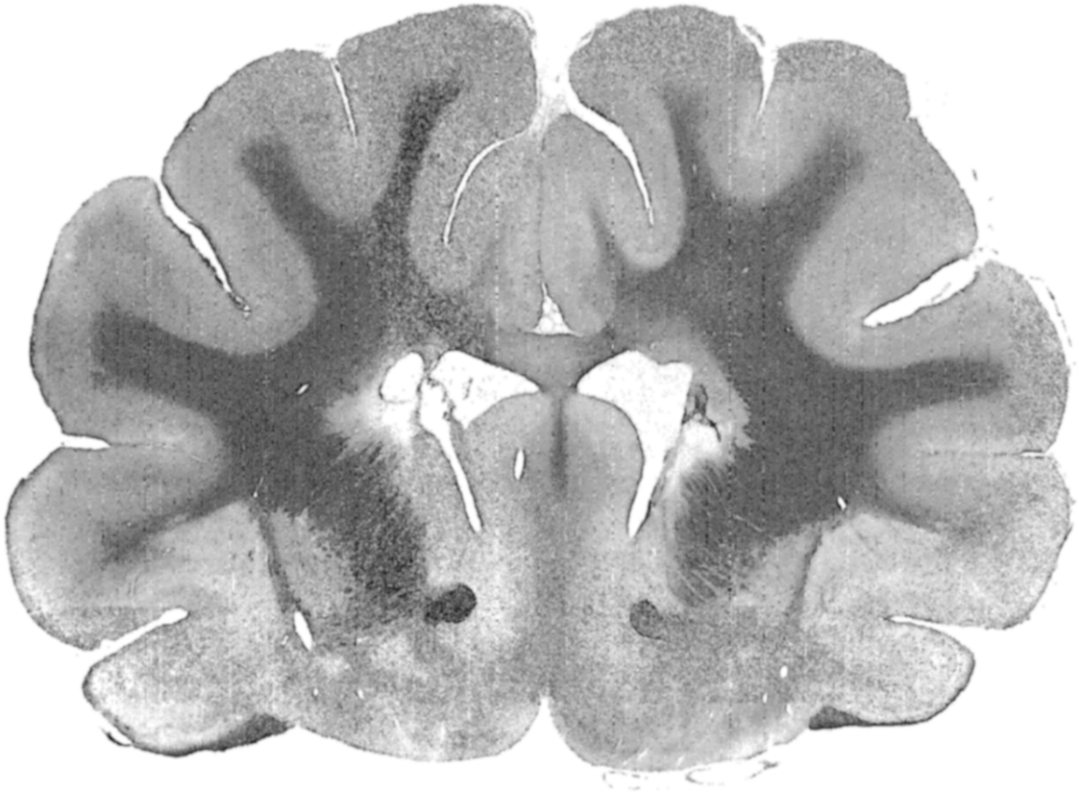

FIG. 4. Coronal section at approximately A 15.0 [23] of the brain of a cat with extensive bilateral caudate nuclei removal; Weil stain.

displayed odd postures including a hunched-over body and stiff tail (pointing straight up or directed caudally). Mydriasis and hyperthermia were blocked. After 10–15 min the animals became calm, sitting or crouching, but tachypnea continued for 60–100 min and drowsiness or sleep occurred only 1–2 hr after naloxone.

Similar but milder manifestations were seen after the later naloxone challenge (Fig. 2). These lasted for only about 20 min but were in clear contrast with the absence of manifestations in the saline-treated cats. In the latter, only sporadic vocalization and mild escape behavior were observed.

The brain-lesioned cats showed the same basic withdrawal manifestations described with exception of the cat with almost a total removal of the caudate nuclei (98.6%). This animal exhibited a dramatic episode of catatonia accompanied by *flexibilitaes cerea* lasting for about 20 min. Neurological testing, performed at about 45 min after naloxone, revealed that manifestations of the "compulsory approaching syndrome," previously described for acaudate cats [27,29], were absent. In the cats with unilateral brain lesion, practically all body turns seen during the 30 min following naloxone injection were to the side contralateral to the ablation; (i.e., to the *right* in the HEMI cats and towards the *left* in the unilateral caudate lesioned). After the naloxone effects wore off, the HEMI cats returned to their original bias of moving towards the side ipsilateral to the ablation, whereas the unilateral caudate lesioned did not show much bias at all.

Behavioral examinations during the abstinence period, revealed no changes in the neurological deficits and gross behaviors seen prior to morphine; this included the "compulsory approaching" syndrome of acaudate cats [27,29]. However, the acaudate and HEMI cats showed deterioration of grooming, apparent depression, and a lowered level of motor activity. For the acaudate cats decreased motor activity was documented (Fig. 3) by a significant reduction in the mean number of turns/min in the activity test of days 2, 7 and 14 (Lesions × days $(3,15)=3.43$, $p=0.04$). In pilot testing no clear trend was seen for HEMI cats. In addition, acaudate and HEMI cats experienced a significant weight loss: a 9.7% decrease for acaudates $F(1,26)=14.68$, $p=0.0001$, and a 9.5% decrease fo HEMI, $F(1,20)=21.12$, $p=0.0001$, cats. No such loss was seen for intact animals and unilateral caudate lesioned cats which lost a non-significant 3.6%. Therefore, there was an overall deterioration of the physical status of the cats with larger brain lesions, particularly the acaudates, but this did not affect self-feeding or basic health.

Plasma Morphine Levels

All blood samples taken prior to daily morphine administration contained morphine and/or morphine metabolites (ranges:<3.0 to 43 ng/ml, morphine; 27 to 5000 ng/ml, metabolites), whereas samples (n=9) from abstinent and control cats did not.

Histology

The amount of caudate tissue removed varied between 56–99% (bilaterally lesioned, Fig. 4) and 76–93% (unilaterally lesioned). Bilaterally lesioned brains also had septal (n=2) and internal capsule (n=1) damage on one side, whereas the brain with the largest unilateral caudate lesion also had slight

internal capsule damage. The hemispherectomy lesion was accurate (see the Method section) apart from 1 brain in which the pulvinar and lateral geniculate nucleus were slightly damaged. For illustrations of the unilateral lesions see Fig. 1 in [26].

DISCUSSION

The answers to the main research problems outlined in the Introduction are as follows:

(a) In our analyses of physical dependence and of tolerance, we saw a clear contrast between weak behavioral indicators of tolerance, and the demonstration of strong withdrawal manifestations following the naloxone challenge on day 16, including the first description of a "mini" withdrawal precipitated well into the abstinence period. These findings confirm suggestions in the literature [7,15] that cats show a dissociation between development of physical dependence vs. tolerance to repeated morphine administration.

(b) Previous studies of the behavioral effects of single doses of morphine revealed marked differences between intact cats and cats with extensive neostriatal lesion [30]. The hypothesis that differences might also occur across a cycle of the drug's administration, was not supported by the present experiments. Instead, we found that, with small exceptions, both tolerance and withdrawal manifestations of animals with virtually total ablation of the caudate nuclei were similar to intact cats.

(c) Based on previous findings after acute administration [26] we examined the relationship between rotational behavior and chronic morphine on brain-lesioned cats. After naloxone, there was a reversal of the morphine-induced body turning bias in cats with a unilateral brain lesion. This finding confirms our previous suggestion [30] that the nigrostriatal dominergic theory does not explain the entire range of drug-induced rotational biases, and that lower brain stem areas must be taken into account to understand the mechanisms for this behavior.

Tolerance and Withdrawal

Overall, we have found that 15 daily morphine injections tended to increase the amount of time that animals spent in postural patterns indicative of motor relaxation (i.e., lying down or crouching) and to decrease the motor activation patterns of standing and walking (in acaudates). However, for none of the groups could we show significance for the *entire* drug period. Therefore, we conclude that cats did develop partial tolerance to the motor activation effects of the drug but that this effect was weak. In contrast, all cats (except for the HEMI animals) showed a robust tolerance to the hyperthermic effects of morphine which was clearly manifested by the 11th administration day.

This finding indicates that tolerance development to the behavioral effects of morphine takes longer in cats than in other species, particularly the rat. In examining a similarly broad spectrum of behaviors in intact cats but only for a 7 day drug period, French *et al.* [7] arrived at a similar conclusion. Older literature which explored the development of tolerance to "feline mania" occuring at higher doses of morphine [2, 11, 16], suggested that even tolerance to mania may be partial and late to appear. For example, Borrell and Borrell [2] found that cats receiving 10 mg/kg morphine daily still "showed some hyperexcitability after 30 days of morphine administration." Only with doses reaching lethal levels (9 to 58 mg/kg) could other authors [11,16] find a decrease in excitation by about 15 days. The reason why cats do not develop tolerance to morphine as readily as other animals is not clear at this time.

There are reports which propose that the cat is a poor subject to show morphine withdrawal, and hence physical dependence, to morphine. Anecdotal observations [6, 16, 24] appear to have formed the basis for the statement that "a very low grade physical dependence may occur in cat" [21]. More recently, Kilbey and Ellinwood [15] reported that naloxone (0.1 mg/kg, IM), produced only piloerection and vocalization in some of their cats receiving self-administered morphine for up to 14 days. In the present experiments there was no doubt that the strong classical signs of precipitated withdrawal seen in other species were demonstrated after 1.0 mg/kg naloxone, while the animals had measurable morphine in the blood. We were particularly impressed by "classical" signs like "wet dog shakes," stiff tail postures and catatonic-like behaviors. Practically identical results were reported by French *et al.* [7] in the only other comparable experiments that we have found. In Kilbey and Ellinwood's trials [15] the smaller dose of naloxone used might account for the weak signs of abstinence reported. In fact, their description is reminiscent of our results following the late naloxone challenge.

Definite naloxone-precipitated manifestations were still present 15 to 30 days after abstinence, when morphine could no longer be detected in the plasma. The presence of this "mini-withdrawal" syndrome was unexpected but not surprising in the light of recent reports. For example, Jones *et al.* [14] showed that rats excrete morphine in the urine up to the 3rd week of abstinence, when there was presumably no drug remaining in the plasma. The authors speculate that morphine could accumulate in body compartments or organs by tightly binding to tissue components and that the slow release of this morphine could have long-term physiological consequences. In the present experiments we documented the absence of morphine in the blood at the time of the "mini-withdrawal" and can not offer a better explanation for this finding. Further exploration of these long-lasting effects of morphine, both behavioral and metabolic, could be useful to increase our understanding of relapse susceptibility during compulsory morphine self-administration.

Bilateral Caudate Lesions

Both tolerance and withdrawal manifestations were stronger in acaudate cats but, in general, the behavioral responses to repeated morphine administration took the same form and time-course as in other groups. Therefore, it appears that extensive neostriatal lesions do not fundamentally change the behavioral effects of chronic morphine in cats.

However, two tolerance-related findings were of interest in acaudate cats. First, throughout the administration period this group showed the most marked tendency to attenuation of motor activation (as revealed by decreased walking). We have demonstrated [27] that undrugged acaudate cats are spontaneously hyperactive and attributed this effect to a lack of neostriatal inhibition upon a brain stem mechanism for motor activation and arousal [28,29]. We have also shown that a single dose of morphine markedly increases the hyperactivity of acaudate cats which, under the influence of the drug, walk most of the time [30]. Thus, the fact that walking decreases following repeated dosage indicated development of tolerance to a true excitatory morphine effect. This contrasts with suggestions in the literature that CNS tolerance effects can develop only to depressant effects of morphine [21]. Second, parallel to the reduction of motor

activity there was an increase in the frequency of head movements in acaudate cats and of paw movements in the unilateral caudate lesioned animals. This finding suggests that the reported decrease of morphine-induced head and paw movements in acaudate cats [26] might be the consequence of a masking phenomenon; i.e., the marked walking activity of acaudate cats under morphine may mask the discrete head-paw movement: as walking decreases due to tolerance, the discrete movements tend to reemerge.

This is the first published report on the consequence of caudate lesions on effects of chronic morphine in cats. In rats, Glick *et al.* found attenuation of withdrawal manifestations [8], but Linseman [17] could not replicate those results. Unfortunately, the rat is not an appropriate animal for striatal lesion studies since, in this species, most cortical fibers are interspersed with the cellular fields of the caudate putamen instead of forming a discrete internal capsule as in cats and man (see [29]). As a consequence, electrolytic lesions of the rat caudate may destroy cortico-subcortical fibers that may serve a role in the expression of morphine effects.

Turning Biases

In [26] we reported that, following morphine, only HEMI cats showed a strong significant body turning bias toward the side of the ablation. That observation was confirmed in the present study (and directionality of turning did not change with repeated dosage). In addition, here we showed an interesting manifestation of withdrawal in *all* unilaterally lesioned cats: immediately after naloxone the body turns were to the side contralateral to the ablation (i.e., naloxone reversed the morphine-induced turning bias).

It has been shown that rats with a unilateral substantia nigra lesion which receive morphine turn toward the side of the lesion [12]. Amphetamine, a dopamine (DA) agonist, induces a similar turning pattern in these animals [1, 12, 13], and a popular hypothesis is that this bias is due to preferential release of DA from intact nigrostriatal fibers such that lesioned rats circle away from the more DA-active striatum. On this basis it has been proposed that opiates enhance DA activity in the nigrostriatal pathway. Furthermore [12], naloxone injected in morphine-dependent rats induces turning contralateral to the side of the nigra lesion, and measurements of DA concentration in the neostriatum of these rats demonstrate an elevation of the neurotransmitter only on the side contralateral to the lesion. This was interpreted as a result of a temporary decrease in DA activity in that nigrostriatal pathway. Therefore, the authors imply that the reverse rotational bias during precipitated abstinence still follows the nigrostriatal theory, i.e., the rats still turn away from the relatively more DA active striatum, which is now ipsilateral to the nigra lesion.

The above hypothesis and interpretations would account for the strong ipsilateral turning bias induced by morphine in our HEMI cats, where the absence of neostriatum on the side of the ablation would result in an overwhelming dominance by the opposite side. However, this theory would predict a similarly strong bias in our cats with the unilateral caudate lesions: the fact that this was not seen challenges the theory. Our results on precipitated withdrawal pose an even stronger challenge to the nigrostriatal dopaminergic theory; the theory simply does not account for a turning bias away from the ablated side after naloxone in the unilaterally lesioned cats, since in these cats the entire neostriatum is virtually absent on one side. These discrepancies clearly show that the nigrostriatal DA explanation can not be applied to the present model.

In the past, we have criticised rotational models based exclusively in the nigrostriatal system [29]. Because the present turning bias was marked in the HEMI cats, and since in these animals there are no telencephalic structures to account for the reversal of the rotational behavior following naloxone in morphine-dependent animals, it follows that the brain stem should be considered as a possible site involved in asymmetric turning. Some of our past work has revealed persistant turning bias even in the absence of the diencephalon in cats and kittens with mesencephalic transections that were slightly asymmetrical in the right-left coronal plane (see [29]). Median raphe nucleus-reticular formation areas, where opiate neurotransmitter interactions may also occur, have been implicated in rotational behavior [10, 18, 19, 22]. According to these authors, these systems may be even more important for this behavior than the nigro-striatal projections, since rotation is more pronounced after lesions in this area. Thus, lower brainstem mechanisms clearly have the potential for initiating the rotational behaviors seen in this study.

We were impressed by the mild but clear physical deterioration in the HEMI and acaudate cats. In addition, in HEMI cats there was a subjective deterioration of the mild preexisting hemiparesis [32] during the peak hours of the morphine effects (i.e., a tendency to postural collapse of the right rear limb and a larger angle of abduction of both right limbs). This deterioration would suggest a negative interaction between opiates and brain lesions that, as far as we know, has not been described before.

Finally, considering the entire body of results on a wide range of behaviors which we have presented in here as well as in [5] and [26] relative to the effects of *single* or *multiple* doses of morphine, we propose that the cat emerges as a sensitive species to study the behavioral effects of opiates and the CNS site of actions which might be involved in these effects. The results reported in this paper demonstrate the apparently single main difference with other species used for morphine studies: a dissociation of tolerance and physical dependence. Instead of being detrimental, this property may render the cat model of particular value to disclose putative differential mechanisms involved in the two important processes [15].

ACKNOWLEDGEMENTS

The authors acknowledge the support of USPHS Grants DA-02518 and HD-04612 as well as the participation of Ch. E. Olmstead, Ph.D. in the early stages of this research. We wish to thank D. H. Catlin, M. D., UCLA Department of Pharmacology, for the measurement of plasma levels of morphine, and to I. Buckberg and S. Belkin for their assistance with the research and illustrations respectively.

REFERENCES

1. Anden, N. E., A. Dahlstrom, K. Fuxe and K. Larson. Functional role of the nigroneostrial dopamine neurons. *Acta Pharmacol Toxicol* **24**: 263–274, 1966.

2. Borrell, J. and S. Borrell. Chronic administration of morphine in cats: Effects on adrenal and urinary catecholamines. *Eur J Pharmacol* **32**: 337–343, 1975.

3. Catlin, D. H. Pharmacokinetics of morphine by radioimmunoassay; the influence of immunochemical factors. *J Pharmacol Exp Ther* **200:** 224–235, 1977.
4. Chugani, H. T., J. R. Villablanca, C. M. Harris and J. W. Burgess. Effects of a single, small dose of morphine on local cerebral glucose utilization (LCGU) in cats. *Soc Neurosci Abstr* **10:** 588, 1984.
5. de Andrés, 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:** 923–928, 1984.
6. Eddy, N. B., and C. K. Himmelsbach. Experiments on the tolerance and addiction potentialities of dihydrodesoxymorphine-d(Desomorphine). *Public Health Rep* **118:** 1–33, 1936.
7. French, E. D., S. A. Vásquez and R. George. Behavioral changes produced in the cat by acute and chronic morphine injection and naloxone precipitated withdrawal. *Eur J Pharmacol* **57:** 387–397, 1979.
8. Glick, S. D., R. Drawbaugh, M. Haynes and A. M. Crane. Changes in morphine self-administration and morphine dependence after lesions of the caudate nucleus in rats. *Psychopharmacologia* **41:** 219–224, 1975.
9. Harris, C. M., J. R. Villablanca, I. de Andrés and J. W. Burgess. Morphine induces dose-dependent increases in stereotyped head and paw movements in cats. *Physiologist* **25:** 333, 1982.
10. Hodge, C. G. and L. L. Butcher. Role of pars compacta of the substantia nigra in circling behavior. *Pharmacol Biochem Behav* **10:** 695–709, 1979.
11. Huidobro, F. and J. Lewin. Tolerance and physical dependence to morphine in the cat. *Acta Physiol Lat Am* **19:** 345–350, 1969.
12. Iwamoto, E. T., H. H. Hoh and E. L. Way. Circling behavior after narcotics drugs and during naloxone-precipitated abstinence in rats with unilateral nigral lesions. *J Pharmacol Exp Ther* **197:** 503–516, 1976.
13. Iwamoto, E. T. and E. L. Way. Circling behavior and stereotype induced by intranigral opiate microinjections. *J Pharmacol Exp Ther* **203:** 347–359, 1977.
14. Jones, A. W., A. Neri and E. Anggard. Prolonged excretion of morphine in rats during protracted abstinence: Lack of effect of naltrexone. *J Pharmacol Exp Ther* **224:** 419–423, 1983.
15. Kilbey, M. M. and E. H. Ellinwood. Self-administration of morphine in the cat. *Int J Addict* **15:** 447–460, 1980.
16. Labrecque, G. and E. F. Domino. Tolerance to and physical dependence on morphine: Relation to neocortical acetylcholine release in the cat. *J Pharmacol Exp Ther* **191:** 189–200, 1974.
17. Linseman, M. A. Effects of lesions of the caudate nucleus on morphine dependence in the rat. *Pharmacol Biochem Behav* **28:** 465–472, 1976.
18. Miliaressis, E. Refractoriness of neurons subserving circling following stimulation of the median raphe region in the rat. *Physiol Behav* **16:** 709–713, 1981.
19. Porceddu, M. L., A. Imperato, M. R. Melis and G. D. Chiara. Role of ventral mesencephalic reticular formation and related noradrenergic and serotonergic bundles in turning behavior as investigated by means of kainate, 6-hydroxydopamine and 5,7-dihydoxytryptamine lesions. *Brain Res* **262:** 187–200, 1983.
20. Reinoso-Suárez, F. *Topograpischer Hirnatlas der Katze.* Darmstadt: Merck AG, 1961.
21. Seevers, M. H. and G. A. Deneau. Physiological aspects of tolerance and physical dependence, In: *Physiological Pharmacology,* vol 1, edited by W. S. Root and F. G. Hofmann. New York: Academic Press, 1963, pp. 565–640.
22. Slater, P. Effect of morphine on circling behavior in rats with 6-hydrodopamine striatal lesions and electrolesions of the raphe nucleus. *Pharmacol Biochem Behav* **14:** 625–630, 1981.
23. Snider, R. S. and W. T. Niemer. *A Stereotaxic Atlas of the Cat Brain.* Chicago: University of Chicago Press, 1961.
24. Tatum, A. L., M. J. Seevers and K. H. Collins. Morphine addiction and its physiological interpretation based on experimental evidences. *J Pharmacol Exp Ther* **36:**447–475, 1929.
25. Ungerstedt, U., L. L. Butcher, S. G. Butcher, N. E. Anden and K. Fuxe. Direct chemical stimulation of dopaminergic mechanisms in the neostriatum of the rat. *Brain Res* **14:** 461–471, 1969.
26. Villablanca, J. R., C. M. Harris and J. W. Burgess. Reassessing morphine effects in cats: I. Specific behavioral responses in intact and unilaterally brain-lesioned animals. *Pharmacol Biochem Behav* **21:** 913–921, 1984.
27. Villablanca, J., R. J. Marcus and C. E. Olmstead. Effects of caudate nuclei or frontal cortical ablations in cats. I. Neurology and gross behavior. *Exp Neurol* **52:** 389–420, 1976.
28. Villablanca, J. R., R. J. Marcus and C. E. Olmstead. Effects of caudate nuclei or frontal cortical ablations in cats. II. Sleep-wakefulness, EEG and motor activity. *Exp Neurol* **53:** 31–50, 1976.
29. Villablanca, J. R. and C. E. Olmstead. The striatum: A fine tuner of the brain. *Acta Neurobiol Exp* **42:** 225–297, 1982.
30. Villablanca, J. R., C. E. Olmstead and I. de Andrés. Striatal lesions change the behavioral effects of morphine in cats. *Brain Res* **248:** 159–167, 1982.
31. Villablanca, J. R., C. M. Harris, J. W. Burgess and I. de Andrés. Neurobehavioral responses of intact and caudate nuclei lesioned cats during a cycle of addiction to low doses of morphine. *Soc Neurosci Abstr* **9:** 892, 1983.
32. Villablanca, J. R., J. W. Burgess and B. J. Sonnier. Neonatal cerebral hemispherectomy: A model for post-lesion reorganization of the brain. In: *The Behavioral Biology of Early Brain Damage,* vol 2, edited by C. R. Almli and S. Finger. New York: Academic Press, 1984, pp. 179–210.
33. Wesolowski, J. L. *Multiple Regression and Analysis of Variance.* New York: John Wiley, 1976.
34. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw Hill, 1972.