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Disruption of Diurnal Feeding Patterns of Rats by Heroin[1,2]

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THORNHILL, J. A., M. HIRST AND C. W. GOWDEY. *Disruption of diurnal feeding patterns of rats by heroin.* PHARMAC. BIOCHEM. BEHAV. 4(2) 129–135, 1976. – Adult male rats receiving 5 or 20 mg/kg heroin HCl by single injections (08:00 or 20:00 hr) or in 3 equal injections (8 hr intervals) showed a disruption in the normal diurnal pattern of behavior. Initially, heroin abolished feeding for several hr after the injection, reduced the total daily food consumption in a dose-related manner, due primarily to decreased night-time feeding, and prevented or slowed weight gain. Subsequent heroin injections led to a phase of vigorous feeding following the period of depression. Magnitude and duration of the depression decreased, but the stimulatory phase of feeding became more pronounced as tolerance developed. Total daily food intake and body weight returned towards control levels, but the proportion eaten during daylight hr became elevated. Sporadic feeding occurred on the first withdrawal day with abolition of the stimulatory phase which had followed each heroin injection. Subsequently, the normal diurnal pattern of behavior gradually returned. Close measurement of 24 hr food consumption may be a sensitive and valuable measure of the disruptive effects of narcotic analgesics.

Heroin – multiple injections Diurnal feeding patterns Depression Stimulation Withdrawal Tolerance

IT was reported in 1971 [7] that in the first 4 hr after large daily doses of morphine, eating, drinking and spontaneous activity were increased in morphine-tolerant rats, but in the remainder of the 24 hr these types of behavior were higher in control rats. Tolerant rats gained weight during the day and lost weight during the night, but their total daily food and water consumption was lower than that for the control rats.

The purpose of the present study was to determine the effects of 2 doses of heroin on the 24 hr feeding and growth patterns of rats given as single daily injections (in the morning or the evening) or as triple injections (given at 8 hr intervals) and the effects of withdrawal from heroin.

METHOD

Animals and Apparatus

Seventeen adult male Sprague-Dawley rats, weighing between 205 and 360 g at the beginning of the experiment, were trained to bar-press for food pellets on a continuous reinforcement schedule (one bar-press yielded one 45 mg Noyes food pellet). Rats were housed individually in a standard 1 lever Gerbrands' operant-conditioning chamber equipped with a rotary disc-feeder and water bottle within a shielded, temperature-controlled (26.0 ± 2.0°C), ventilated, sound-attenuated room, which had a fixed 12 hr on-off lighting schedule. Each of the test chambers was connected by cables to electromechanical equipment in an adjacent room where print-outs of the number of food pellets eaten during each half-hour of the day, 7 days a week, were recorded. To minimize any disruption in case of a power failure the feeders, clock and all the recording equipment were arranged so that they could be automatically powered by 12 volt batteries. Body weights were measured once daily for the single-injection rats and 3 times daily for the triple-injection rats, all prior to drug administration.

Procedure

After each rat had learned to bar-press, a 24 hr food consumption baseline was obtained for each animal over a 5-day saline control period. Close observation during day and night revealed that the rats almost invariably ate the food pellets immediately they were attained; therefore, food consumption was equated to pellet acquisition. Physiological saline (0.33 ml/100 g body weight) was injected subcutaneously at 08:00 hr or 20:00 hr every day or at 08:00, 16:00, and 24:00 hr for the triple-injection experiment, with the morning body weight being used to calculate the daily doses. Following this control period, 13 of the 14 single-injection rats began to receive heroin; the other rat was designated as a control and continued to receive daily injections of saline. Heroin hydrochloride, dissolved in saline, was injected subcutaneously in a volume of 0.33 ml/100 g body weight; 4 rats received 5 mg/kg and 5 received 20 mg/kg heroin HCl at 08:00 hr every day. In

[1] Supported by the Non Medical Use of Drugs Directorate of the Department of National Health and Welfare of Canada.
[2] Heroin HCl supplied by Mr. R. A. Graham, Chief, Scientific Services, Department of National Health and Welfare, Ottawa.

addition, 2 rats were injected with 5 mg/kg and 2 with 20 mg/kg heroin HCl once daily at 20:00 hr. In the triple-injection experiment, 2 rats received 1.67 mg/kg and 2 received 6.67 mg/kg heroin HCl three times daily (08:00, 16:00, and 24:00 hr). If on any day during the course of heroin injections a rat was able to surpass its mean 24 hr food consumption of the 5 day control period, it was considered to have become tolerant to heroin and withdrawal was induced by substituting injections of saline for the heroin. These saline injections were given 1 or 3 times daily for 2 weeks or more depending on previous heroin dosing regime. Rats which never achieved their former mean control level of food consumption were induced into withdrawal by saline injections after at least 2 weeks of heroin administration.

Significance of the results was calculated using Student's *t* test for paired or unpaired data, as appropriate.

RESULTS

Figure 1 shows for 3 of the experimental days the number of food pellets acquired by 2 typical rats injected with 5 or 20 mg/kg heroin HCl at 08:00 hr each day. From the figure it was calculated that on the fifth control (saline) day rat No. 57 ate 66% of its total daily food intake of 777 pellets (35 g) during the period of darkness (20:00 to 08:00 hr). After the initial injection of 5 mg/kg heroin HCl, the rat obtained no pellets for approximately 2 hr, but over the next 90 min it acquired 162 pellets. Although little additional food was taken over the remainder of the 12 hr light period, during the night hours the pattern of food intake was comparable to that on the previous control day. The total 24 hr food consumption was 643 pellets (29.9 g). On the 7th heroin day, the rat began to eat sooner and more often after the heroin injection than it had on the 1st heroin day. Again, the night-time feeding pattern was similar to that in the control period, but the total daily intake was further reduced (575 pellets – 24.5 g).

Rat No. 60 ate a total of 531 food pellets (23.9 g) on the 5th saline day, 72% of which was eaten during the night hours. On the 1st heroin day no feeding occurred for 4 hr after the injection at 08:00 hr of 20 mg/kg heroin HCl and very little food was taken over the rest of the daylight period. Night-time feeding was markedly reduced (205 pellets) compared to that following the saline injection on the previous day (382 pellets) and the total 24 hr intake was only 239 pellets (10.8 g). In contrast, on the 7th heroin day feeding was blocked for less than 90 min after the injection and over the next 2 hr the rat obtained 99 pellets. More feeding took place in the daylight hours than had occurred on either the 1st heroin or the control days and the 24 hr consumption of 462 pellets (20.8 g) approached the former control level.

Figure 2 illustrates the disruption in the patterns of feeding of 3 rats by 20 mg/kg/day heroin HCl given at different times. On the 5th saline (control) day the total food intake of Rat No. 3 was 673 pellets (30.3 g), of which 82% was taken at night. On the 1st day that the rat received heroin (at 08:00 hr) feeding was markedly reduced both during the daylight hours and at night, the 24 hr intake being only 465 pellets (20.9 g). On the 7th day of this heroin regime, feeding occurred sooner after the injection; it was increased during daylight hours but decreased during the night and the total food consumption fell to 366 pellets (16.5 g).

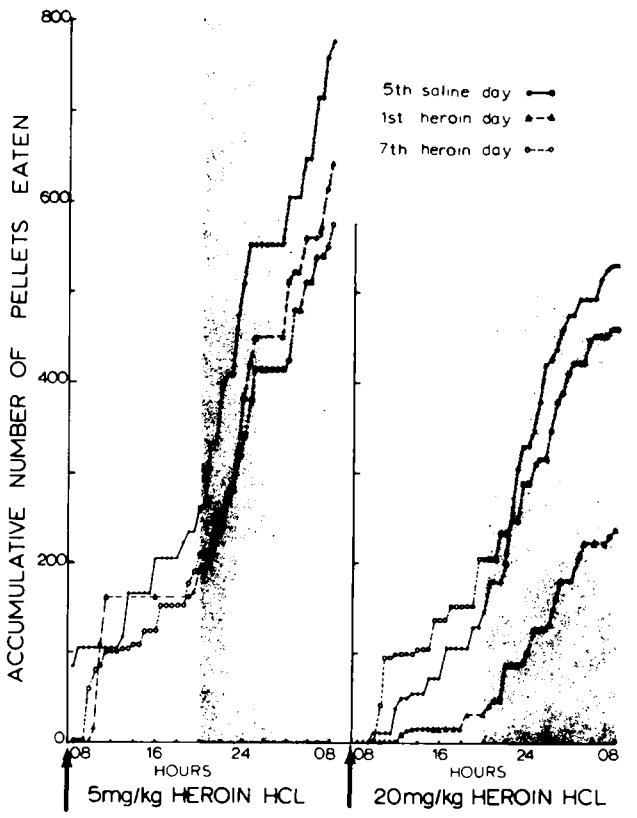

FIG. 1. Number of food pellets accumulated each half-hour over the 24 hr on the 5th saline day (control), 1st and 7th heroin days for 2 typical rats administered 5 mg/kg (Rat No. 57) or 20 mg/kg (Rat No. 60) heroin HCl at 08:00 hr daily. Shaded areas denote the 12 hr period of darkness. Arrows indicate the time of the injection of heroin.

Rat No. 34 obtained 90% of its total intake of 537 pellets (24.2 g) during the night-time period on the 5th saline day. On the 1st day of heroin administration the daylight feeding pattern was, of course, similar to that on the 5th saline day, but following the injection of 20 mg/kg heroin HCl at 20:00 hr feeding was blocked for 4 hr and the total intake that day was reduced to 290 pellets (13.1 g). On the 7th heroin day rapid feeding began some 2.5 hr after the injection and the intake during the daylight hours surpassed that in the preheroin control period; the total 24 hr consumption of 462 pellets (20.8 g) approached the control level.

The rat which received 3 injections per day at 8 hr intervals obtained 586 food pellets (26.4 g) in the 24 hours of the 5th control day, 88% of its intake being during the night-time hours. After the initial 6.67 mg/kg heroin HCl at 08:00 hr only 9 pellets were received before the next heroin injection at 16:00 hr and only a few more from then until the lights were extinguished. A more-or-less normal pattern of night-time feeding then occurred until it was interrupted by the third injection of heroin at 24:00 hr which blocked feeding for approximately 2 hr. A period of vigorous feeding followed, so that during the 1st heroin day the total food consumption was 376 pellets (16.9 g), of which 87% was consumed during the dark hours. By the 7th heroin day, vigorous feeding occurred after each of the

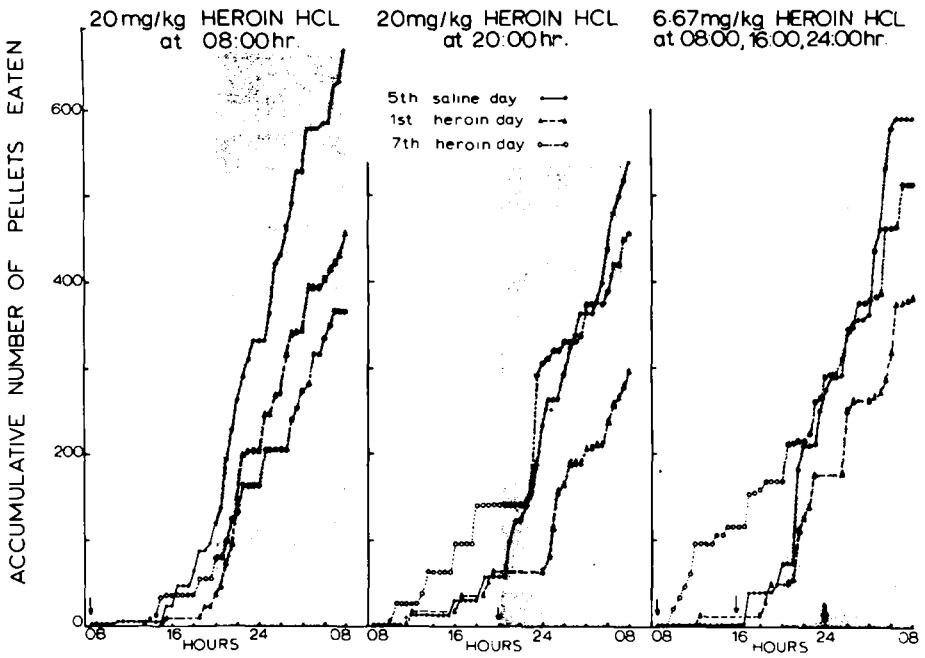

FIG. 2. Number of food pellets accumulated each half-hour over the 24 hr on the 5th saline day (control), 1st and 7th heroin days for 3 typical rats administered 20 mg/kg heroin HCl every day. Rat No. 3 received a single injection at 08:00 hr; Rat No. 34 a single injection at 20:00 hr; and Rat No. 103 received 3 doses of 6.67 mg/kg at 08:00, 16:00 and 24:00 hr. Shaded areas denote the 12 hr period of darkness. Arrows indicate the times of heroin injections.

3 heroin injections and sooner than it had on the 1st heroin day. Total food consumption was 514 food pellets (23.3 g) of which only 67% was eaten during the night-time period.

Figure 3 shows the effect of withdrawal on the same 3 rats all of which had previously received 20 mg/kg heroin HCl/day. Rat No. 3, which was injected with saline once daily at 08:00 hr, ate sporadically and sparsely throughout the daylight as well as the dark hours of the 1st withdrawal day. The total food consumption on that day was 463 pellets (20.8 g), of which 61% occurred during the dark hours. By the 3rd withdrawal day, the daily food intake had increased to 628 pellets (28.3 g) as the night-time feeding pattern became more like that in the pre-heroin control period.

Rat No. 34, which received a daily injection of saline at 20:00 hr during the withdrawal period fed sporadically throughout the 1st withdrawal day. On the 3rd withdrawal day the food intake was similar to that in the preheroin control period.

Rat No. 103, which received 3 injections of saline at 8 hr intervals throughout the withdrawal period, decreased its 24 hr food consumption markedly on the 1st withdrawal day, but by the 3rd withdrawal day, the normal nocturnal feeding pattern had returned and total intake had increased to 551 pellets (24.8 g). Although not shown in these graphs, it was found that during the withdrawal period rats which had received the smaller total daily doses (5 mg/kg) of heroin HCl displayed feeding patterns and intakes which more closely resembled those in the control period.

Within the first week of withdrawal from either 5 or 20 mg/kg heroin HCl the 24 hr food consumption exceeded that in the control, preheroin peroid and was maintained. Moreover, the pattern of feeding gradually returned towards that in the control period so that some 70–85% of the total daily intake was consumed during the dark hours.

Table 1 is a summary of the effects of the various doses and dosage regimes of heroin and of withdrawal from heroin on daily food intake, day-night food consumption and body weight. The total food intake on the first few heroin days was reduced in all groups of rats compared to their former mean control levels. Analysis shows that on the 1st day of heroin administration 20 mg/kg caused a significantly greater decrease ($p<0.05$) in the 24 hr food consumption than 5 mg/kg when these doses were given at 08:00 hr. Similarly, 20 mg/kg heroin HCl given in 3 divided doses at 8 hr intervals produced significantly ($p<0.01$) more depression of feeding on the 1st heroin day than 5 mg/kg given in 3 divided doses. Although there was a tendency for the first dose of 5 mg/kg heroin HCl to be more depressing to the group receiving it at 20:00 hr than to those receiving it at 08:00 hr, the difference was not statistically significant; nor did the first 20 mg/kg dose have more effect when it was injected at 20:00 hr than at 08:00 hr.

As the heroin injections were continued, the total daily food consumption increased towards mean control levels in all groups of rats as tolerance developed, the rate being inversely related to the dose of heroin. Moreover, the triple-injected rats receiving either 1.67 or 6.67 mg/kg per injection returned towards their control levels of food consumption in fewer days than the single-injection rats receiving the same total daily doses.

The table also shows that the percentage of the 24 hr intake consumed during the daylight hours decreased in all groups of rats on the 1st heroin day compared to the 5th saline day except in those which were not injected until

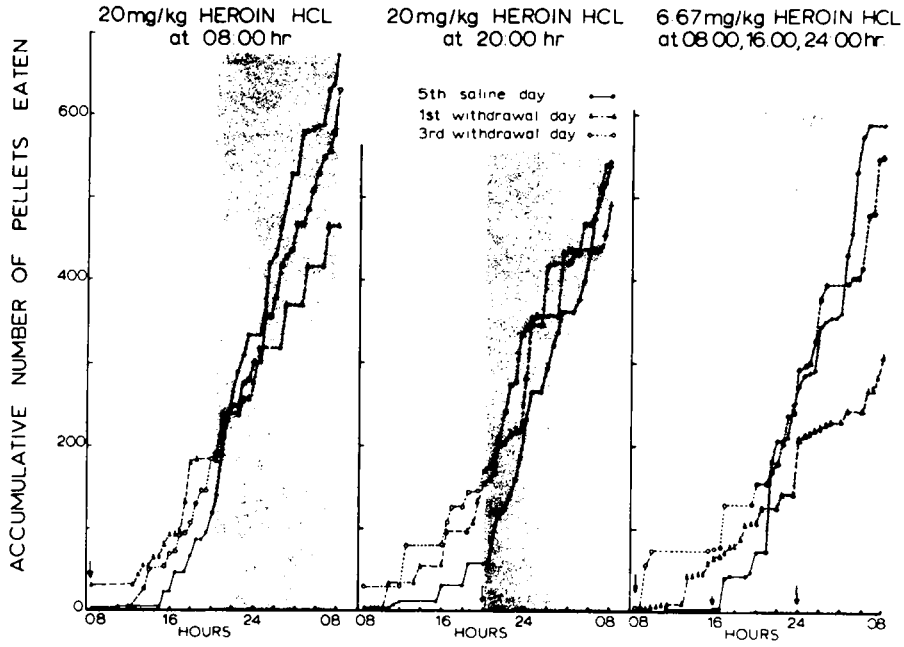

FIG. 3. Number of food pellets accumulated each half-hour over the 24 hr on the 5th saline day (control), and the 1st and 3rd withdrawal days when saline injections replaced the heroin for the same 3 rats as in Fig. 2. Arrows indicate the times of the saline injections (Rat No. 3 at 08:00; Rat No. 34 at 20:00, and Rat No. 103 at 08:00, 16:00, and 24:00 hr). Shaded areas denote the 12 hr period of darkness.

20:00 hr. As the heroin injections continued the percentage of daylight feeding increased to above control levels in all groups.

On withdrawal from heroin the food intake on the first day was less in the groups which had been receiving 20 mg/kg/day heroin HCl than in those which had received 5 mg/kg/day. As the withdrawal period continued most rats exceeded their preheroin levels of food intake and a more normal diurnal pattern was re-established: the night-time feeding increased and daylight feeding decreased. Table 1 also shows that as tolerance to heroin developed in the groups the mean body weights increased. By the end of the period of heroin injections the body weights had risen above the control levels by a mean of 7% for the group receiving 5 mg/kg heroin HCl at 08:00 hr (for an average of 11 days), by 11% for those administered 5 mg/kg at 20:00 hr (over 12 days), and by 19% for those receiving triple-injections of 1.67 mg/kg (over 11 days). On the 1st day of withdrawal there was a 1% decrease in the body weights of the rats which had been receiving the 5 mg/kg injection of heroin HCl at 20:00 hr, but the weights of the other two groups which had received the same daily dose at 08:00 or in triple injections increased slightly.

In all 3 groups receiving 20 mg/kg of heroin HCl per day the mean body weights decreased slightly on the 1st heroin day and remained lower than their control levels for about a week. On the 1st withdrawal day 7 of the 9 rats which had been receiving 20 mg/kg/day heroin HCl lost weight. Analysis revealed that the group which had received 20 mg/kg heroin HCl each morning lost significantly more ($p<0.025$) weight on the 1st withdrawal day than those which had received 5 mg/kg each morning.

The important effects of the different doses and dose regimes of heroin are summarized in Fig. 4 which shows the patterns of food intake by the groups over an 8 hr period following an injection. In the preheroin control period (5th saline day) a marked difference in feeding activity during the light and dark hours was present in all the groups. On the 1st day of heroin administration no feeding occurred for the first 2.5–4 hr following the injection and only small amounts were eaten over the rest of the 8 hr period. By the 5th heroin day, however, feeding occurred sooner after the heroin injection in all groups. In the withdrawal period the bout of feeding which had followed each injection of heroin was abolished. By the 5th withdrawal day the feeding patterns of all groups were similar to those in the control period.

The physical signs shown by the rats after an injection of heroin correlate with the initial abolition and subsequent stimulation of feeding activity described before. In the first few days of heroin administration when feeding was most affected, "lead-pipe" rigidity [5], muscle-incoordination, shallow breathing, bulging eyes, wheezing and apparent disorientation occurred within 3 min after the injection. These effects became less severe and of shorter duration as tolerance developed. A hyperactive phase during which feeding began became more pronounced as the heroin injections continued. The rats were also seen to chew the lever, food tray and bars of the cage floor and this behavior persisted throughout the heroin treatment.

Withdrawal from heroin led within 24 hr to transient piloerection, shivering, tremors, vocalization during handling and diarrhea, the degree of which was related to the dose of heroin the rats had been receiving.

DISCUSSION

Measurements of food consumption at 30 min intervals (around the clock, 7 days a week) before, during and after

TABLE 1

EFFECTS OF INJECTIONS OF, AND WITHDRAWAL FROM HEROIN ON DIALY FOOD INTAKE, % CONSUMED DURING DAYLIGHT HR, AND BODY WEIGHT

Group		Control	Heroin Day 1	Heroin Day 3	Heroin Day 5	Heroin Day 7	Withdrawal Day 1	Withdrawal Day 3	Withdrawal Day 7
5 mg/kg heroin HCl at 08:00 hr n = 4	A)*	100	78	92	91	86 (3)[a]	91	98	95
	B)†	26§	22	38	32	29	31	29	27
	C)‡	100	99	100	103	105	110	112	118
5 mg/kg heroin HCl at 20:00 hr n = 2	A)	100	60	74	86	78	96	110	116
	B)	20§	31	34	32	38	38	28	28
	C)	100	100	101	102	103	110	114	120
1.67 mg/kg heroin HCl ×3 at 08:00, 16:00, and 24:00 hr n = 2	A)	100	89	91	88	98	110	104	107
	B)	18§	16	24	22	25	20	17	22
	C)	100	100	102	108	111	121	129	139
20 mg/kg heroin HCl at 08:00 hr n = 5	A)	100	45	63	77	79	89	102	105
	B)	20§	9	35	38	29	29	29	18
	C)	100	99	99	99	100	105	110	116
20 mg/kg heroin HCl at 20:00 hr n = 2	A)	100	45	72	75	84	84	93	108
	B)	27§	44	39	41	52	27	29	22
	C)	100	97	99	98	100	99	103	109
6.67 mg/kg heroin HCl ×3 at 08:00, 16:00, and 24:00 hr n = 2	A)	100	53	80	93	80	73	99	117
	B)	12§	9	31	27	32	47	35	22
	C)	100	96	99	98	103	118	129	144

*Total food intake as % of mean 5 day control intake
†% of 24 hr intake consumed during daylight hr
‡Body weight as % of 5th saline day control
§Calculated for 5th saline day
[a]One rat had exceeded his control level of food intake and had begun withdrawal

doses of 5 and 20 mg/kg heroin HCl administered daily to rats in 1 injection or in 3 equal injections revealed a disruption in the normal diurnal pattern of behavior. The results indicated that in the early days of heroin administration a dose-dependent phase of muscle rigidity, unresponsiveness, respiratory depression and absence of feeding followed each injection. This effect is comparable to the dose-related reduction in eating which was reported [7] in non-tolerant rats injected with morphine. As the heroin injections were repeated, this phase became progressively shorter; that is, tolerance developed.

Following the phase of depressed behavior a period of

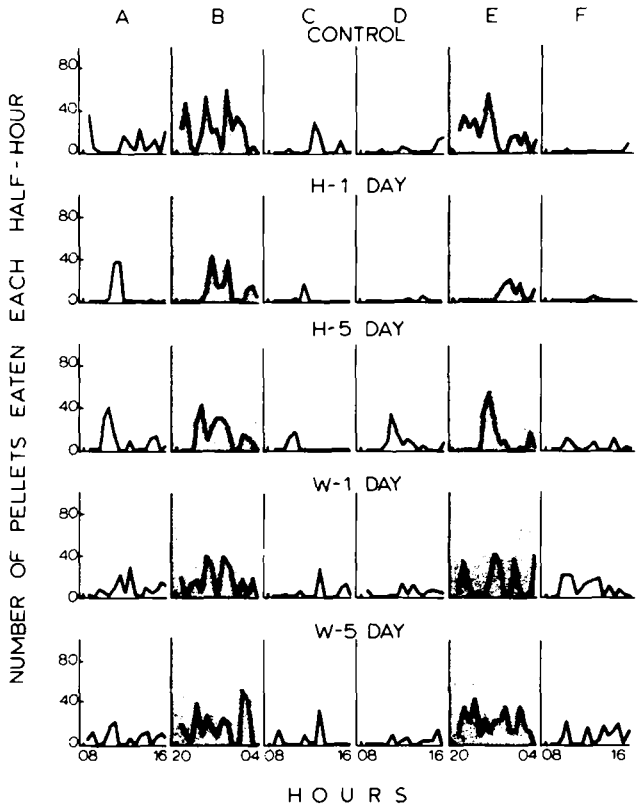

FIG. 4. Patterns of feeding in groups of rats for the 8 hr period following injections of saline or heroin. The mean number of food pellets obtained by each group is shown at each half-hour on the 5th saline day (control); 1st (H–1) and 5th (H–5) heroin days, and 1st (W–1) and 5th (W–5) withdrawal days. Three groups received 5 mg/kg heroin HCl every day: A at 08:00 hr, B at 20:00 hr and C received 1.67 mg/kg at 08:00, 16:00, and 24:00 hr. Three groups received 20 mg/kg every day: D at 08:00 hr, E at 20:00 hr and F received 6.67 mg/kg at 08:00, 16:00, and 24:00 hr. Shaded areas denote periods of darkness.

hyperactivity was observed which became more pronounced and occurred progressively sooner after the heroin injections as tolerance to the depressant effects developed. It is interesting that feeding always occurred during this phase of hyperactivity and was seen whether the heroin injections were made at 20:00 hr or at 08:00 hr when normally the rats would be fairly inactive. Moreover, this drive to eat was abolished when saline was substituted for the heroin injections. Kumar *et al.* [7] reported that maximum stimulation of eating, drinking and spontaneous activity occurred 2 3 hr after morphine injections in tolerant rats and they postulated that morphine might affect hypothalamic reward mechanisms. This stimulation of feeding following repeated injections of heroin is consistent with earlier reports [8,9] that with morphine tolerance develops more rapidly to the "depressant" than to the "stimulant" effects.

It is interesting that bilateral electrolytic lesions in the hypothalamus of rats have been shown to disrupt the naturally-occurring diurnal feeding [2, 3, 6]. In young rats lesions in the dorsomedial hypothalamic nuclei led to hypophagia and a subnormal growth rate; rats with lesions in the ventromedial nuclei were normophagic but feeding was equally distributed between the dark and light periods [3]. These experiments suggest that hypothalamic nuclei are involved in circadian feeding rhythms but whether the hypothalamic structures are primary or secondary in the modulation of feeding behavior is debatable [6].

A period of anorexia followed the hyperactive phase, especially after higher doses: only 3 of 9 rats receiving a total of 20 mg/kg/day heroin HCl had returned to their control levels of food consumption within 16 days and all 3 were in the group injected at 08:00 hr each day. This suggests that morning injections of heroin were less disruptive to feeding behavior in rats than injections at night or at 8 hr intervals.

The degree of disruption of the normal feeding pattern appeared to depend upon the total daily dose, dosing schedule (single or triple injections) and duration of heroin treatment. In the first few days heroin caused a marked reduction in food intake and a greater percentage of the total intake was consumed during the daylight period. With continued injections the total food consumption tended to return towards the preheroin control levels but the elevated level of daylight feeding persisted until heroin was withdrawn. These results are comparable to those of an earlier study [7] in which large doses of morphine, given at 10:00 hr each day, "disturbed, and to some extent even reversed, the characteristic diurnal patterns of the rats' behaviour," and the drug "seemed to have a complex, biphasic time course of action."

Rapid loss of body weight on withdrawal from opioids has been considered [1,4] to be the best index for quantification of physical dependence. In the present experiments withdrawal from heroin produced loss of weight only in the group which had received 20 mg/kg at 20:00 for 16 days. By the criterion of weight loss on withdrawal most of the rats in this study could not, therefore, be considered to have become physically dependent upon heroin. Yet heroin had disrupted the diurnal feeding pattern of all the rats and some degree of tolerance, as shown by increasing food intake and body weight and shorter periods of postinjection depresssion, had occurred in all groups.

This study suggests that careful, continual measurements of the amount and pattern of food consumption may be of value in an animal model as a sensitive indicator of the disruptive effects of narcotic analgesic substances such as heroin.

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