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Effects of Narcotic Abstinence on Schedule-Controlled Behavior in Dependent Rats[1]

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STEINFELS, G. F. AND G. A. YOUNG. *Effects of narcotic abstinence on schedule-controlled behavior in dependent rats.* PHARMAC. BIOCHEM. BEHAV. 14(3) 393–395, 1981.—Female Sprague-Dawley rats were trained to lever press for food on a variable interval one minute schedule of reinforcement. Four of these rats were then made tolerant to and physically dependent on morphine by a series of automatic IV injections. Four other rats were made tolerant to and physically dependent on LAAM. During the dependence state behavioral tolerance was exhibited to the suppressant effect of morphine on lever pressing, but not to the suppressant effect of LAAM. Abstinence was induced by discontinuation of injections. During both the morphine and LAAM abstinence states from the fourth through twelfth days mean lever presses per session were significantly higher than pre-drug control values. However, there were quantitative differences. Mean lever presses per session were significantly higher during morphine abstinence than during LAAM abstinence. This difference in degree of increased lever pressing observed during morphine and LAAM abstinence in this study extends our previous reports which demonstrated that in the rat morphine abstinence was associated with more severe behavioral disruptions than LAAM abstinence.

Morphine LAAM Abstinence Lever pressing

THE purpose of the present study was to compare the effects of morphine and l-alpha-acetylmethadol (LAAM) abstinence on operant lever pressing for food pellets in dependent rats. Previous studies have shown that changes in schedule-controlled behavior may be used as an index of tolerance development to the behavioral effects of narcotics [1, 2, 3, 4, 5]. In some of these same studies there were some indications of behavioral effects during narcotic abstinence [1, 2, 3, 4]. We have reported on several differences in behavioral correlates between morphine and LAAM abstinence in the rat [17, 19, 20]. In general, LAAM abstinence was found to be associated with fewer indications of behavioral disruption than morphine abstinence. It was therefore of interest to assess possible differences in the effects of morphine and LAAM abstinence on schedule-controlled behavior.

METHOD

Eight female Sprague-Dawley rats weighing between 300 and 325 g were used. During the experiment the rats were maintained at 80% of their free-feeding weights. In addition, each rat was prepared with an indwelling intravenous cannula for drug administration [15] and a miniature Continental connector which was attached to the skull with stainless steel screws and dental acrylate [7]. Each rat was housed in an individual cage equipped with an infusion swivel and a motor-driven syringe for drug injections. Rats were connected to the swivel system via the Continental connectors and flexible cables.

Daily experimental sessions were conducted in a BRS-LVE Test Cage (Model 143-21) which was enclosed in a sound-attenuated box with continuous white-noise in the background. Twenty to 25 g of force was required to operate the lever. Programming was accomplished with solid-state equipment. Data were collected with Gerbrands cumulative recorders and mechanical counters.

Lever pressing was reinforced by deliveries of 45 mg Noyes food pellets on a variable interval one min schedule of reinforcement. Daily sessions were 45 min in duration. After lever pressing patterns for food reinforcement had stabilized (four weeks), rats were divided into two groups of four each for establishment of tolerance to and phsyical dependence on morphine or LAAM. Four rats were made tolerant to and physically dependent on morphine sulphate (dissolved in 0.9% saline) by a series of hourly automatic IV injections. During the first day the rats received 1.25 mg/kg/hr of morphine (0.05 ml over 3 sec). The dose was increased to 2.5, 5.0 and 10.0 mg/kg/hr on successive days. On the fifth day the rats received 10 mg/kg of morphine every three hr and were maintained on this injection schedule for the next three

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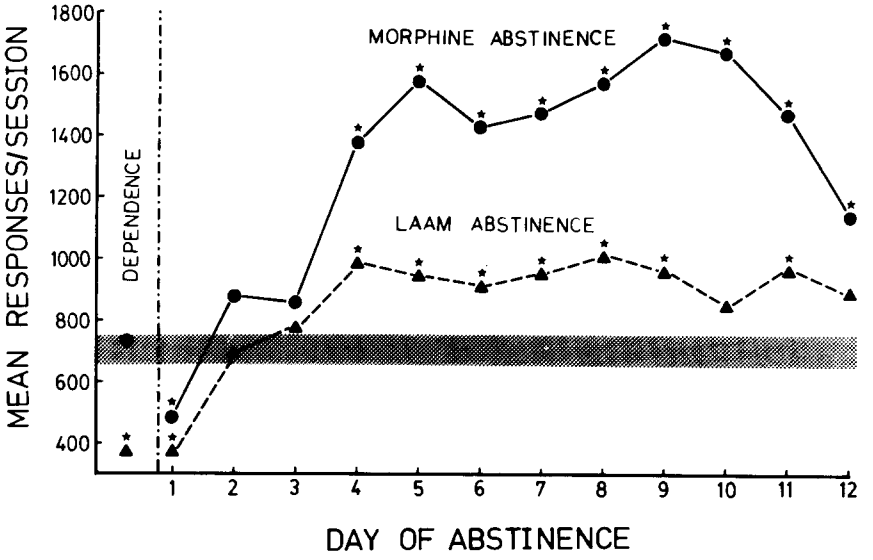

FIG. 1. Mean responses per session for food reinforcement are shown during 12 days of narcotic abstinence. The shaded horizontal bar represents mean responses per session ± SEM for five days prior to any drug administration (control). Mean responses per session during the last five days of dependence are shown in the left of the graph. *Significantly different from pre-drug control, $p<0.01$.

weeks. This final injection schedule was chosen so that the total daily morphine intake of these rats approximated the morphine intake that has been reported for rats during self-maintained dependence [11]. The other four rats were made tolerant to and physically dependent on LAAM hydrochloride by a series of automatic IV injections. During the first three days the rats received 0.125 mg/kg/3 hr of LAAM [9]. The LAAM dose was doubled on the fourth day and thereafter every two days until a level of 1 mg/kg/3 hr was achieved. The rats were maintained on this 1 mg/kg/3 hr LAAM injection schedule for the next three weeks. This final injection schedule was chosen so that the total daily LAAM intake of these rats approximated the LAAM intake that has been reported for rats during self-maintained dependence [11]. During maintenance on morphine or LAAM rats were tested as usual in daily food reinforcement sessions. Since the interinjection intervals were three hr in duration, each rat was tested on different days during different thirds of the three hr interinjection interval. Abstinence from morphine and LAAM was then induced by discontinuation of automatic IV injections. Rats were tested in daily food reinforcement sessions during the first 12 days of abstinence.

Data were studied with analyses of variance. In some cases when significant F tests were found, means during experimental conditions were compared to the pre-drug mean by using the approach of Dunnett [16].

RESULTS

Mean lever presses per session ± SEM during the last five sessions prior to drug administration for all eight rats are represented by the shaded horizontal bar in Fig. 1. There was no significant difference in pre-drug mean lever presses per session between the four rats maintained on morphine and the four rats maintained on LAAM. During the last five sessions of morphine dependence on the 10 mg/kg/3 hr injection schedule mean responses per session were not significantly different from pre-drug control values. In the case of LAAM dependence, mean responses per session during the last five days were significantly lower than pre-drug control values. Since during dependence there were no apparent differences in amount of lever pressing when rats were tested during different thirds of the three hr interinjection intervals, the data were pooled in this respect for both the morphine and LAAM maintained groups. During morphine abstinence mean responses per session were significantly decreased on the first day compared to pre-drug values, were not significantly different from pre-drug values on the second and third days, and, finally, were significantly increased from the fourth through twelfth days. The same general pattern of changes in mean responses per session occurred during LAAM abstinence. However, changes in mean responses per session were significantly higher in magnitude during morphine abstinence than during LAAM abstinence, $F(1,11)=23.50$, $p<0.01$.

DISCUSSION

We found that morphine and LAAM abstinence had qualitatively similar effects on operant lever pressing for food pellets. In both cases when mean responses per session during abstinence were compared with pre-drug values, responses per session were significantly decreased on the first day. Furthermore, during the second and third days of abstinence mean lever presses per session were not significantly different from pre-drug values, while from the fourth through twelfth days of abstinence mean lever presses per session were for the most part significantly increased above pre-drug values. However, there were quantitative differences. Overall, mean responses per session were significantly higher during morphine abstinence than during LAAM abstinence. This difference in degree of increased lever pressing during morphine and LAAM abstinence extends our previous findings which demonstrated that in the rat mor-

phine abstinence was associated with more severe behavioral disruptions than LAAM abstinence [17, 19, 20].

One may speculate, based on the above data, that the LAAM abstinence syndrome was relatively milder than the morphine abstinence syndrome because the level of dependence during LAAM dependence was lower than that with morphine. However, in a previous study when morphine and LAAM dependent rats were administered hourly automatic IV injections of naloxone to precipitate abstinence, the degree and duration of REM sleep suppression was analogous in the two groups [17]. This suggests that the level of dependence in the morphine and LAAM dependent rats was also analogous. It is more likely that the relatively milder abstinence syndrome seen with LAAM is related to the long plasma half-lives of the pharmacologically active N-demethylated LAAM metabolites, nor-LAAM and dinor-LAAM [6].

We also found that tolerance developed to the suppressant effect of morphine on lever pressing during the dependence state. Other investigators have reported a similar finding [3,4]. In contrast, we observed no behavioral tolerance to the suppressant effect of LAAM on lever pressing. The observation of lack of development of tolerance to LAAM has also been reported by others [1,8], as well as to methadone [10]. It has been suggested that this lack of behavioral tolerance to the suppressant effect of LAAM may be due to an accumulation of pharmacologically active LAAM metabolites [1] which may eventually produce "behavioral toxicity" [8]. However, in our laboratory we have not observed any toxic behavioral effects during three to four weeks of stabilized LAAM self-administration in rats [18]. Moreover, if active LAAM metabolites were accumulating to a sufficient degree in this self-administration situation, the rats would have presumably decreased their daily LAAM intake; this did not occur. As an alternative explanation for the lack of behavioral tolerance to LAAM, we wish to propose that associative processes related to learning may be involved. It has been shown that certain aspects of tolerance development to morphine are affected by Pavlovian conditioning [12, 13, 14]. In the case of LAAM, for example, if the development of behavioral tolerance requires that an association be made between the onset of LAAM effect and the daily food reinforcement session, then the fact that the onset of LAAM effect is most likely delayed until the proper accumulation of LAAM metabolites might make such an association difficult to establish.

In any event, a better understanding of the effects of LAAM dependence and abstinence on operant lever pressing might be accomplished by further understanding the effects of nor-LAAM and dinor-LAAM dependence and abstinence on lever pressing.

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REFERENCES

1. Aigner, T. G. and R. L. Balster. Behavioral effects of chronic oral administration of levo-alpha-acetylmethadol in the rat. *Pharmac. Biochem. Behav.* **8:** 593–596, 1978.
2. Babbini, M., M. Gairadi and M. Bartoletti. Changes in operant behavior as an index of a withdrawal state from morphine in rats. *Psychon. Sci.* **29:** 142–144, 1972.
3. Babbini, M., M. Gairadi and M. Bartoletti. Changes in fixed-interval behavior during chronic morphine treatment and morphine abstinence in rats. *Psychopharmacologia* **45:** 255–259, 1976.
4. Ford, R. D. and R. L. Balster. Schedule-controlled behavior in the morphine-dependent rat. *Pharmac. Biochem. Behav.* **4:** 569–573, 1976.
5. Heifetz, S. A. and D. E. McMillan. Development of behavioral tolerance to morphine and methadone using the schedule-controlled behavior of the pigeon. *Psychopharmacologia* **19:** 40–52, 1971.
6. Henderson, G. L., H. North-Root and S. H. Kattab. Metabolism and disposition of *l*-α-acetylmethadol in the rat. *Drug Metab. Disposit.* **5:** 321–328, 1977.
7. Khazan, N. The implication and significance of EEG and sleep-awake activity in the study of experimental drug dependence on morphine. In: *Methods in Narcotics Research (Modern Pharmacology-Toxicology, Vol. 5)*, edited by S. Ehrenpreis and A. Neidle. New York: Marcel Dekker, Inc., 1975, pp. 173–215.
8. Lichtblau, L., K. E. A. Burklund and S. B. Sparber. A method for determining behavioral toxicity after chronic oral administration of *l*-α-acetylmethadol to female rats. *Neurobehav. Toxicol.* **2:**13–19, 1980.
9. Lukas, S. E., J. E. Moreton and N. Khazan. Attenuation of the EEG, power spectral, and behavioral effects of morphine and methadone in the *l*-alpha-acetylmethadol (LAAM)-maintained rat. *Pharmacologist* **20:** 269, 1978.
10. McMillan, D. E., W. T. McGivney and W. O. Hardwick. Effects of drugs on behavior in rats maintained on morphine, methadone or pentabarbital. *J. Pharmac. exp. Ther.* **215:** 9–14, 1980.
11. Moreton, J. E., T. Roehrs and N. Khazan. Drug self-administration and sleep-awake activity in rats dependent on morphine, methadone, or l-alpha-acetylmethadol. *Psychopharmacology* **47:** 237–241, 1976.
12. Siegel, S. Evidence from rats that morphine tolerance is a learned response. *J. comp. physiol. Psychol.* **89:** 498–506, 1975.
13. Siegel, S. Morphine tolerance acquisition as an associative process. *J. exp. Psychol.: Anim. Behav. Proc.* **3:** 1–13, 1977.
14. Siegel, S., R. E. Hinson and M. D. Krank. The role of predrug signals in morphine analgesic tolerance: Support for a Pavlovian conditioning model of tolerance. *J. Exp. Psychol.: Anim. Behav. Proc.* **4:** 188–196, 1978.
15. Weeks, J. R. Long-term intravenous infusion. In: *Methods in Psychobiology, Vol. 2*, edited by R. D. Myers. New York: Academic Press, 1972, pp. 155–168.
16. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1962.
17. Young, G. A., G. F. Steinfels and N. Khazan. Spontaneous vs naloxone-induced abstinence in dependent rats self-administering *l*-alpha-acetylmethadol (LAAM) or morphine. *Pharmac. Biochem. Behav.* **10:** 585–589, 1979.
18. Young, G. A., G. F. Steinfels and N. Khazan. Pharmacodynamic profiles of *l*-α-acetylmethadol (LAAM) and its N-demethylated metabolites, nor-LAAM and dinor-LAAM, during self-administration in the dependent rat. *J. Pharmac. exp. Ther.* **210:** 453–457, 1979.
19. Young, G. A., G. F. Steinfels and N. Khazan. Narcotic abstinence in dependent rats: EEG and behavioral correlates. *Pharmac. Biochem. Behav.* **13:** 115–119, 1980.
20. Young, G. A., J. E. Moreton, L. T. Meltzer and N. Khazan. L-alpha-acetylmethadol (LAAM), methadone, and morphine abstinence in dependent rats: EEG and behavioral correlates. *Drug Alc. Depend.* **2:** 141–148, 1977.