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Quantifiable dose-dependent withdrawal after morphine discontinuation in a rat model

L. Langerman[a,*], B. Piscoun[a], M. Bansinath[a], Y. Shemesh[b], H. Turndorf[a], G.J. Grant[a]

[a]*The Department of Anesthesiology, New York University Medical Center, 550 First Ave., New York, NY 10016, USA*
[b]*The Intensive Care Unit, Barzelay Medical Center, Asqelon, Israel*

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Abstract

We evaluated the intensity of the withdrawal symptoms after the discontinuation of the morphine infusion in rats. Opiate addiction was induced by progressively increasing intraperitoneal morphine infusion rates. The control group (Group 1) received normal saline. The initial morphine rates were 1, 4, and 16 mg kg^{-1} h for Groups 2, 3, and 4, respectively. Infusion rates were gradually increased by a factor of 1.4, 2, 2.8, and 4 on the second, third, fourth, and fifth days, respectively. The last rate was used for 48 h and then infusions were disconnected. Weight reduction, food consumption, and water intake were used for evaluation of withdrawal. All morphine groups showed a significant reduction of body weight during the 4 postdiscontinuation days and a decline in food and water intake on the first postdiscontinuation day. All changes were dependent on the morphine infusion concentration. No changes were observed in the control group. We suggest that the rat model used in this study may be utilized for quantification of spontaneous withdrawal.

Keywords: Morphine; Addiction; Withdrawal; Rat

1. Introduction

Chronic abuse of opiates causes a number of adaptive changes in the central nervous system (CNS) accompanied by behavioral alterations related to drug dependence (Nestler, 1992). Abrupt discontinuation of opiate consumption after prolonged abuse is followed by a series of morbid symptoms defined as withdrawal syndrome. The latter is a significant factor deterring a drug-dependent patient from going through rehabilitation (Stewart et al., 1984).

Various animal models were suggested for the investigation of the opioid dependence and withdrawal. Opiate dependence was induced using single or repeated injections (Han and Zhang, 1993; Miyamoto and Takemori, 1993), adding drug to drink and food (Schoenbaum et al., 1990; Van-der-Laan et al., 1991), implantation of osmotic minipumps (Huffman et al., 1985; Van-der-Laan et al., 1991), and continuous drug delivery to different sites through indwelling catheters (Huffman et al., 1985; Teiger, 1974).

The lack of uniformity in the drug delivery methods, dosages, techniques, and indicators used for the evaluation of withdrawal intensity substantially complicate the comparison between the studies. To standardize the dependence induction, the pellet for a slow release morphine formulation was invented (St-Leger et al., 1983; Yoburn et al., 1985). Such a pellet was shown to effectively produce an opiate dependence in mice and rats. To intensify the dependence, in some cases more than one pellet was implanted simultaneously or at different time intervals (Bhargava and Gulati, 1990; Yoburn et al., 1985).

In most animal models, the withdrawal was induced by the opiate antagonists precipitating a complex of symptoms. The latter included behavioral abnormalities, somatic signs, reinforced behavioral, electrophysiological, and biochemical changes (Baldwin and Koob, 1993; Fdez-Espejo et al., 1995; Gellert and Holtzman, 1978; Guitart and Nestler, 1993; Nestler, 1990; Stinus et al., 1998). Some of the symptoms could be easily quantified, while the others were

* Corresponding author. c/o Eliahu Golomb, Department of Pathology, Sackler School of Medicine, P.O. Box 39040, Ramat Aviv, Tel Aviv 69978, Israel. Tel.: +972-2-623-67-24; fax: +972-2-623-67-25.

E-mail address: levl@hotmail.com

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classified, graded and used for the rating scale (Fdez-Espejo et al., 1995; Gellert and Holtzman, 1978; Schulteis et al., 1994). The antagonist-precipitated withdrawal proved to be an effective tool for investigating multiple aspects of the opioid dependence. It revealed a clear correlation between the withdrawal intensity and antagonist dose or an amount of the opioid used to induce the dependence (Miyamoto and Takemori, 1993; Yoburn et al., 1985).

Although the administration of opioid antagonist in the drug-dependent animals precipitates rapid and intensive withdrawal, in most clinical situations, opioid withdrawal occurs as a result of drug cessation (spontaneous withdrawal), which develops relatively slower and is far less intensive (Teiger, 1974). Therefore, the induction of quantifiable dose-dependent spontaneous withdrawal requires comparatively high doses for the development of the dependence. The simple way to induce the opioid dependence is to admix the drug to drink or food. The oral ingestion of narcotics has been widely studied in rats. The opiates dissolved in water induced the dose-dependent antagonist-precipitated withdrawal (Khavari and Risner, 1973; McMillan et al., 1976). However, a rat may digest only the limited amount of drug in the drinking solution. Therefore, this method does not suit for administering high morphine concentrations. To overcome this problem, high amounts of morphine was added to solid food or food–water admixture. The high concentrations of morphine resulted in notable spontaneous withdrawal (Van-der-Laan et al., 1991; Zeuchner et al., 1981). Furthermore, clear dependence of weight change and reduction in food consumption on the amount of ingested morphine was shown in a study of spontaneous withdrawal that used morphine admixture in a fluid diet (Ronnback et al., 1987).

Other studies using nonoral methods also showed dose-dependent spontaneous withdrawal. Intraventricular infusion of morphine in varying concentrations triggered dose-dependent withdrawal symptoms: jumping, launching, teeth chattering, body shakes, and vocalization (Huffman et al., 1985). The dose-dependent changes of cardiovascular parameters (heart rate, systolic and diastolic blood pressure) were shown after infusion of various morphine concentrations through osmotic minipumps (Chan et al., 1999). Another study quantitatively monitored the locomotor activity and sleep disorders (Stinus et al., 1998). All these studies used relatively low amounts of drug for induction of opioid dependence. However, they required complex implantation techniques and sophisticated monitoring equipment.

The aim of our study was to develop a simple animal model for evaluation of the dose-dependent opioid withdrawal. For this purpose, we induced an opiate dependence in rats using an escalating morphine regimen with a wide range of concentrations. To assess withdrawal intensity after the disconnection of morphine infusion, we monitored three quantifiable parameters: weight loss, food consumption, and water intake.

2. Methods

2.1. Animals

After Institutional Laboratory Animal Care Committee approval, Sprague–Dawley male rats weighing 330–430 g were housed individually and given free access to food and water. The room was kept on a 12 h light–dark cycle at $22 \pm 1^{\circ}C$. The animals were habituated to the environmental conditions 7 days before the experiments.

2.2. Catheter implantation and infusion setup

Under the pentobarbital anesthesia (50 mg kg^{-1}), a polyethylene catheter (PE-10, Clay Adams, Parsippany, NJ) was introduced intraperitoneally through an 18 G needle in the left flank area. The catheter was secured subcutaneously with an ethylene #6 suture. The free outer end was exteriorized through a subcutaneous tunnel in the dorsal cervical region and affixed to a subcutaneously implanted button. Drug administration was controlled by an electronic infusion pump (H-44, Harvard apparatus, South Natic, MA). Polyethylene tubing was used to connect the infusion set to the animals. The tubing was protected by a flexible metal sheath (Instech, Plymouth Meeting, PA) and was connected to a rotating swivel (Instech). This arrangement afforded the animals free movement in their cages during the entire experimental period.

2.3. Drug regimens

The rats were randomly assigned to four equal groups of six animals each. The intraperitoneal infusion of normal saline (Group 1) or morphine sulfate (Common Brothers, NY) at 1, 4, and 16 mg kg^{-1} h for Groups 2, 3, and 4, respectively, was started immediately after catheter implantation and continued for 24 h (0.05 ml h). Thereafter, the initial rate was progressively escalated $\times 1.4$, $\times 2$, $\times 2.8$, and 4-fold on the postoperative days: 2, 3, 4, and 5, respectively. The last rate (4, 16, and 64 mg kg^{-1} h for Groups 2, 3, and 4, respectively) was administered during 48 h, then the infusion was disconnected.

2.4. Withdrawal monitoring

Three easily quantifiable parameters were used to grade the withdrawal intensity: the animals' weight, food consumption, and water intake. The measurements were taken daily at 9:00–10:00 a.m.

2.5. Statistical analysis

To analyze the changes in weight, food, and drink after the morphine infusion, the baseline was set at 100% and the discontinuation values (DV) were expressed as a percentage of the baseline.

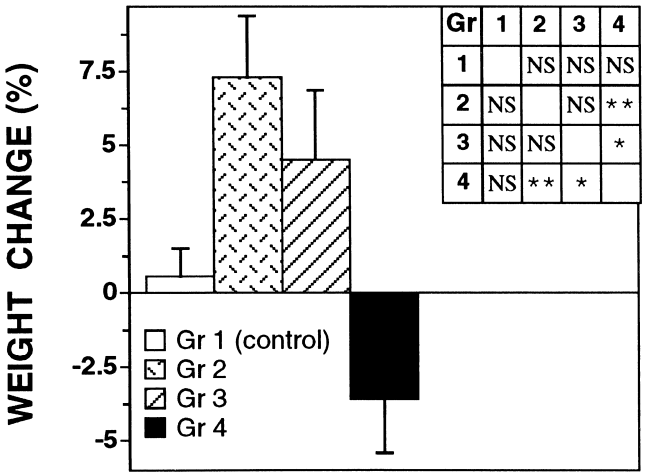

Fig. 1. Weight changes produced by morphine infusion in rats. Group 1 (control) received normal saline. The initial morphine rates were 1, 4, and 16 mg kg^{-1} h for Groups 2, 3, and 4, respectively. The infusion rates were gradually increased by 1.4, 2, 2.8, and 4 on days: 2, 3, and 4. The last regimen (4, 16, and 64 mg kg^{-1} h for Groups 2, 3, and 4, respectively) was continued for 48 h. The bars show the percentage of weight changes after six infusion days as compared to the baseline values. Data are means ± S.E.M. Intergroup differences were carried out with ANOVA followed by a post hoc SNK test. The significance of the comparisons between the groups is listed in the table. * $P<.05$; ** $P<.001$; NS: nonsignificant.

To analyze the changes during the withdrawal period, the DV were assumed to be 100%, and the changes were expressed as a percentage of DV.

The intergroup results were compared using the analysis of variance (ANOVA) followed by the Student–Newman–

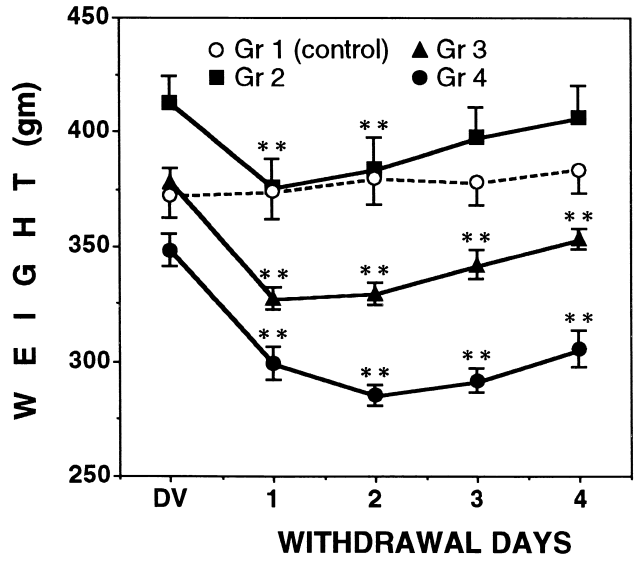

Fig. 2. Weight changes in rats on days 1, 2, 3, and 4 after discontinuation of morphine infusion. Group 1 received normal saline. The morphine rates before the discontinuation were 4, 16, and 64 mg kg^{-1} h for Groups 2, 3, and 4 respectively. Data are means ± S.E.M. Weight changes within the groups after infusion discharge were compared to the DV and analyzed using repeated measures ANOVA followed by Dunnett's test. * $P<.05$; ** $P<.001$.

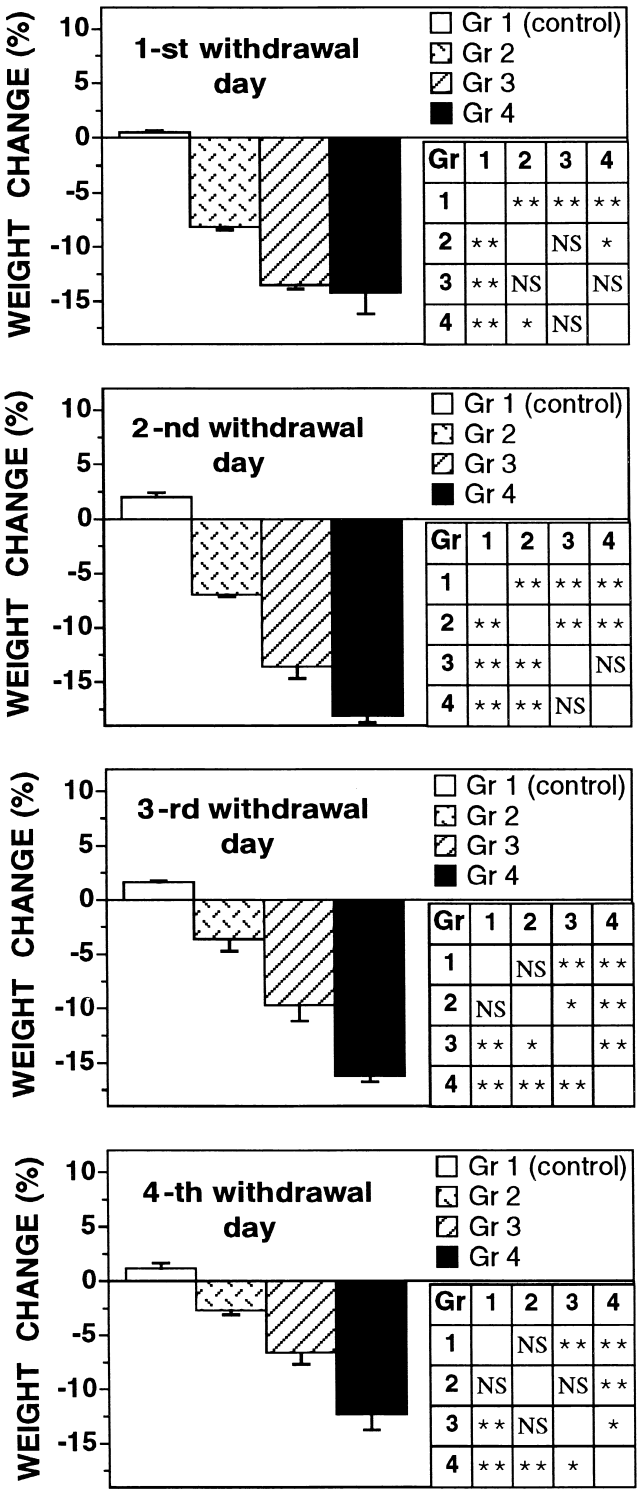

Fig. 3. Weight changes in rats compared between the groups on days 1, 2, 3, and 4 after discontinuation of morphine infusion. Group 1 received normal saline. The morphine infusion rates before the discontinuation were 4, 16, and 64 mg kg^{-1} h for Groups 2, 3, and 4, respectively. The weight changes presented in the percentage of DV. Data are means ± S.E.M. Intergroup differences were carried out with ANOVA followed by a post hoc SNK test. The significance of the comparisons is listed in the table. * $P<.05$; ** $P<.001$; NS: nonsignificant.

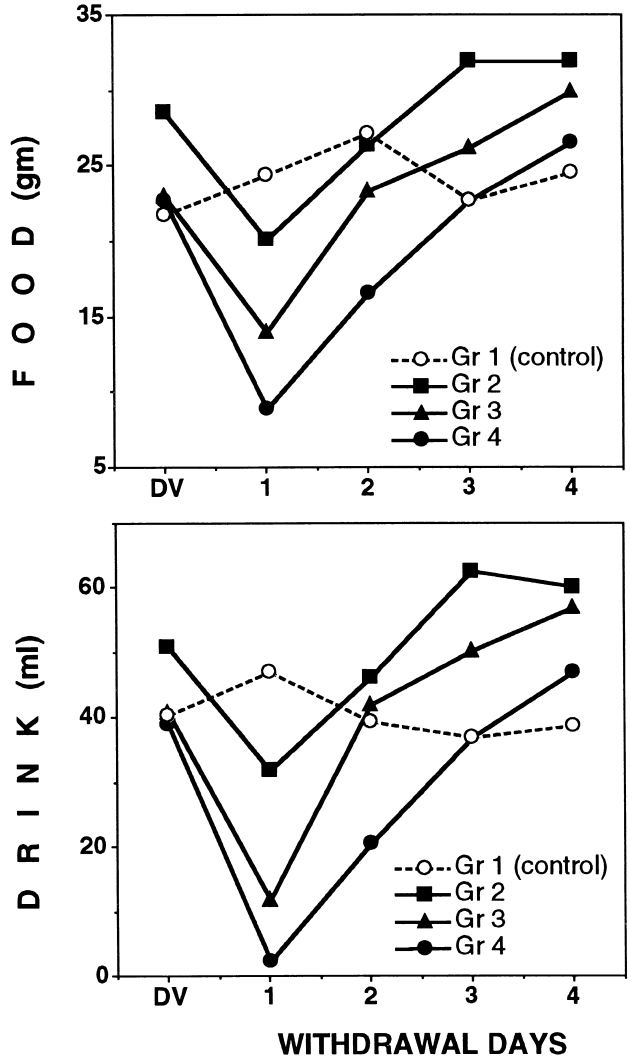

Fig. 4. Food and drink consumption in rats on days 1, 2, 3, and 4 after discontinuation of morphine infusion. Group 1 received normal saline. The morphine rates before the discontinuation were 4, 16, and 64 mg kg^{-1} h for Groups 2, 3, and 4 respectively. DV: discontinuation values.

Keuls (SNK) test for multiple comparisons when ANOVA was significant at $P<.05$.

The weight, food and water consumption within the groups during the withdrawal were compared to DV using repeated measures ANOVA followed by Dunnett's test if ANOVA was significant at $P<.05$.

3. Results

3.1. Weight

The rats' weight ranged from 332 to 419 g and was 370.4±9.6, 363.0±9.5, 362.6±12.5, and 385.2±9.4, for Groups 1, 2, 3, and 4, respectively. The mean weight did not differ significantly between the groups.

The changes in the animal weight among the groups resulting from the morphine administration are given in Fig. 1. The mean weight in Groups 1, 2, and 3 increased by 0.6%, 7.4%, and 4.5%, respectively, whereas in Group 4 it decreased by 3.6%. The weight changes of Groups 2 and 3 differed significantly as compared to Group 4 ($P<.01$ and $P<.05$, respectively). Other differences between the groups were not significant.

The rats' weight during the withdrawal is shown in Fig. 2. On the disconnection day, it was 372.4±9.5, 413±11.8, 378.6±3.1, 348±7.3, for Groups 1, 2, 3, and 4, respectively. The weight values significantly varied between Groups 1 and 2 ($P<.001$), 1 and 4 ($P<.05$), 2 and 3 ($P<.05$), and Groups 2 and 4 ($P<.001$). The weight difference between Groups 1 and 3 was not significant.

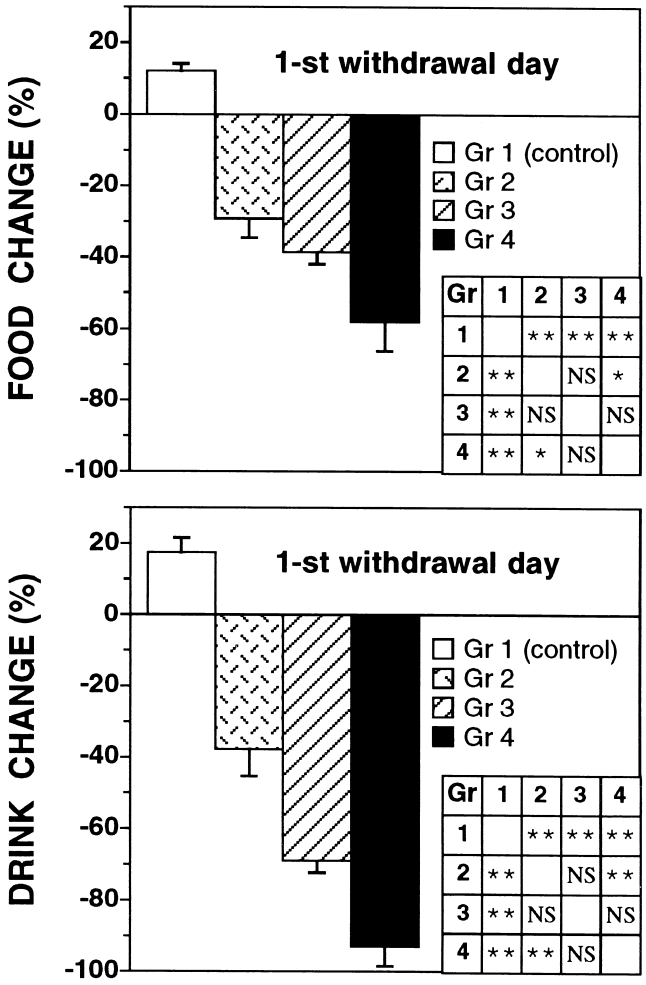

Fig. 5. Food and drink consumption changes between the groups 24 h after discontinuation of morphine infusion. Group 1 received normal saline. The morphine infusion rates before the discontinuation were 4, 16, and 64 mg kg^{-1} h for Groups 2, 3, and 4 respectively. The bars show the percentage of changes after 24 h as compared to the discontinuation values. Data are means±S.E.M. Intergroup differences were analyzed using ANOVA followed by SNK test. The significance of the comparisons between the groups is listed in the table. $*P<.05$; $**P<.001$; NS: nonsignificant.

The weight of the control group during the withdrawal did not change significantly; all three morphine groups showed a significant reduction in the body weight. The maximum weight drop was after 24 h in Groups 2 and 3 and after 48 h in Group 4. After these nadirs, the animals put on their weight gradually. Group 2 reached the initial weight on the third withdrawal day. Groups 3 and 4 did not return to initial values during the 4-day observation period. Values of weight changes in percentage of DV and levels of significance between the groups are given in Fig. 3.

3.2. Food and drink

The patterns of the rats' food and drink changes during the withdrawal are shown in Fig. 4. The control group had no significant change in the food and drink consumption after ending the infusion; Groups 2, 3, and 4 significantly reduced the food and drink consumption. The drink reduction in Groups 2 and 3 remained significantly below the baseline for 24 h and in Group 4 for 48 h. Thereafter, the food and drink consumption returned to its initial levels.

The reduction in the food and drink consumption on the 1st postdiscontinuation day was significantly different between the groups and correlated with the morphine induction dose (Fig. 5). This difference was not significant on the 2nd and 3rd withdrawal day.

4. Discussion

The results of this study show a clear correlation between the drug concentration and the intensity of the symptoms during the spontaneous opioid withdrawal. This correlation was expressed for all the three parameters: weight, food, and drink.

We found that all the three criteria used in the withdrawal monitoring were significantly altered after disconnection of the morphine infusion. The animals weight reduction ranged from 5% to 20% and persisted for 2 and 4 days for lower and higher morphine concentrations, respectively. The food and drink consumption after morphine cessation went down profoundly (food: 40–80%, drink: 50–100%) but was briefer (1 day) than the weight reduction.

A dose-dependent intensity of withdrawal after an antagonist challenge has been previously reported (Schoenbaum et al., 1990; St-Leger et al., 1983; Teiger, 1974; Yoburn et al., 1985). The withdrawal induced by the drug termination is less intense. Therefore, the detection of the dose-dependence effect during the spontaneous withdrawal required the induction of dependence with high drug doses or the use of the skilled implantation techniques and sophisticated equipment. Our setup permitted the use of a wide range of morphine concentrations and a gradual escalation of the infusion rate. This escalation was necessary to develop tolerance to morphine and counteract the toxicity due to high initial drug concentration (Langerman et al., 1995). This regimen induced a strong opiate dependence in rats, which clearly correlated with the morphine induction dose. Our results agree with those of Ronnback et al. (1987), who demonstrated a correlation between the amount of digested morphine and the reduction in weight and in food consumption. We found that the water intake reduction also correlated with the amount of drug used for induction of opioid dependence. The assessment of withdrawal using the weight change and the food or water consumption was simple, reliable, and did not require any sophisticated equipment.

It may be argued that the available inexpensive antagonist challenge models obviate the need to study the withdrawal due to the drug cessation. However, in clinical practice, we deal with withdrawal after drug cessation and antagonist challenge may alter the nature of the withdrawal process. The opioid antagonist increases the intensity of the withdrawal, changes the timing and frequency of the symptoms, and triggers symptoms, atypical to the spontaneous withdrawal (Horan and Ho, 1991; Teiger, 1974). Moreover, certain withdrawal symptoms are related to different neural structures and opiate receptor subgroups (Howes, 1981; Jaw et al., 1993; Maldonado et al., 1992). Hence, the precipitated withdrawal may be dependent on the chemical structure of a particular antagonist (Howes, 1981). Therefore, in some cases, the spontaneous withdrawal may be preferred as a model for investigation of drug addiction. The setup used in this study was technically simple, effective, relatively inexpensive, and flexible for the drug administration regimens. We propose that this rat model be used as an appropriate tool for investigation of spontaneous withdrawal.

The evidence in favor of the dose dependence of the withdrawal intensity may have clinical implications. Most detoxification programs are pharmacotherapy-based (Guthrie, 1990; Kosten, 1990) and only a few include gradual reduction of opiate dose (Amass et al., 1994; Gold et al., 1988) prior to a complete break off. Some patients drop out of rehabilitation programs during detoxification due to their inability to endure the withdrawal crisis (Amass et al., 1994; Bouchez et al., 1998). The correlation between the dose and the crisis intensity suggests that gradual drug reduction prior to entire discontinuation may be an additional valuable method for avoiding drop out in these patients.

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