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Full-length article

Clonidine attenuates morphine withdrawal and subsequent drug sensitization in rhesus monkeys[1]

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Key words

cocaine; withdrawal; drug challenge; cross-sensitization; recurrence; monkeys

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Abstract

Aim: Clonidine is an α_2 adrenoceptor agonist that is frequently used to reduce withdrawal symptoms during opioid detoxification in humans. The long-term effects of clonidine on withdrawal symptoms and its effects on subsequent drug exposure have not been thoroughly documented. The aim of the study was to determine if clonidine administered during morphine withdrawal in rhesus monkeys produces long-lasting effects on withdrawal symptoms and alters the effects of subsequently taken drugs of abuse. **Methods**: Adult male rhesus monkeys were treated with increasing doses of morphine for 90 d to induce opiate (narcotic) dependence. The immediate and long-lasting effects of 1 week's administration of clonidine were measured *via* the recording of morphine withdrawal signs and the subsequent effects of challenge injections of morphine or cocaine. **Results:** Monkeys chronically treated with morphine displayed withdrawal signs that lasted 2 weeks after cessation of morphine administration and displayed sensitized responses to subsequent morphine and cocaine injections. Clonidine significantly reduced certain morphine withdrawal signs and overall withdrawal score, but these effects did not persist upon cessation of clonidine treatment. Sensitization to the effects of morphine and cocaine were significantly reduced in monkeys previously treated with clonidine. **Conclusion:** Our results suggest that in addition to its short-term alleviating effect on morphine withdrawal signs, clonidine may reduce subsequent effects of drugs of abuse after prolonged abstinence.

Introduction

Drug dependence is a chronic, relapsing disorder in which compulsive drug-seeking and drug-taking behavior persists despite serious negative consequences[1]. Addictive substances, such as opioids, induce pleasant states or relieve distress, effects that contribute to their recreational use. After repeated exposure, adaptive changes occur in the central nervous system that lead to drug dependence[1–3]. Although the intrinsic rewarding properties of addictive drugs such as heroin are important in the acquisition of drug self-administration, compulsive drug-seeking and drug-taking by addicts is not readily explained in terms of simple reward or positive reinforcement processes alone[4]. Abstinence from the drug in dependent subjects induces aversive withdrawal symptoms that are thought to contribute to the compulsive nature of drug self-administration in addiction.

The exact role of withdrawal in heroin addiction is debatable. It has been proposed that a drug addict may self-administer heroin to escape from abstinence symptoms (avoidance theory)[5,6], but also that withdrawal from heroin functions as a motivational state that enhances the incentive value of the drug (incentive-motivational theory)[4,7]. Other integrative reward theories of addiction, such as the incentive salience-sensitization theory[8], propose that drug-addicts are sensitized to some motivational effects of drugs of abuse. Indeed, it has been shown that repeated morphine administration produces hypersensitivity to subsequent

doses of morphine ("behavioral sensitization") and to other drugs of abuse ("cross-sensitization"). Sensitization and cross-sensitization have been extensively studied in rodents[9–11], but there is little evidence for such phenomenon in primates. Since drug priming effectively reinstates extinguished drug self-administration behavior in animals[12–14], it is likely that enhanced reactivity to the effects of drug of abuse may facilitate relapse in drug addicts. The fact that low doses of morphine or cocaine can cause hyperactivity or drug-seeking in drug reinstatement rodent models supports this notion[15,16]. Therefore, there is a great interest in developing medications that may block sensitization processes in order to reduce relapse in humans.

It is well established that noradrenergic pathways are implicated in morphine withdrawal. Activity of central adrenergic neurons is inhibited by opiates[17] and increased firing of the noradrenergic neurons in the locus coeruleus has been clearly demonstrated during opiate withdrawal[18]. Clonidine, an α_2 adrenoreceptor agonist, reduces this increased firing in morphine-dependent rats[18], an effect that is thought to mediate the drug's ability to reduce morphine withdrawal symptoms in animals and humans[19,20]. α_2 adrenoceptor agonists, such as clonidine and lofexidine, are used to reduce withdrawal syndromes during the initial phase of opioid abstinence in humans[21–23]. Typically, these α_2 adrenoreceptor agonists are used to control opioid withdrawal on a tapered dosing schedule for the first week of drug abstinence. Most of the studies conducted in animals have evaluated the effects of clonidine on opiate withdrawal symptoms and little is known about the long-term effects of α_2 adrenoceptor agonists after their administration.

Recent evidence suggests that noradrenergic pathways are involved not only in withdrawal states, but also in other aspects of drug dependence such as drug-seeking behavior[24] and behavioral sensitization[25]. The α_2 adrenoceptor agonist lofexidine attenuates stress-induced reinstatement of alcohol-seeking and also decreases alcohol self-administration[26]. The α_2 adrenoceptor antagonist, yohimbine, induces reinstatement in animal models of abuse of alcohol[26], methamphetamine[27], cocaine[28], heroin[29], and a mixture of cocaine and heroin (speedball)[30]. There is a strong correlation between increases in cortical extracellular norepinephrine levels and the expression of behavioral sensitization to amphetamine[25]; cortical α1-adrenergic receptors are critically involved in locomotor responses to amphetamine and morphine[31,32]. In spite of the widespread use of clonidine during the initial phase of opiate withdrawal in humans, the long-term effects of clonidine on subsequent motivational effects of drugs of abuse have not been explored.

The present study was designed to evaluate the immediate and long-term effects of clonidine administered during the initial phase of morphine withdrawal in rhesus monkeys. First, to induce dependence, rhesus monkeys received an escalading morphine dosage regimen over 90 d. To induce morphine withdrawal, morphine treatment was abruptly stopped. To evaluate the immediate and long-term effects of clonidine on withdrawal signs, the monkeys received clonidine for 1 week and the withdrawal signs were measured daily during a period of 21 d. Finally, the effects of clonidine on the challenge injection of morphine or cocaine were evaluated after prolonged morphine abstinence.

Materials and methods

Drugs Morphine hydrochloride and cocaine phosphate were purchased from Qinghai Pharmaceutical Factory Co Ltd (Xi'ning, China). Solutions of morphine and cocaine were prepared with saline (0.9% sodium chloride) and delivered *via* sc injection. Clonidine is a commercial agent for human use, given ig.

Animals Laboratory-reared, male rhesus monkeys (*Macaca mulatta*), weight between 3.5 and 5.5 kg (2–3 years old), were purchased from the Beijing Xierxing Institute of Biological Resources (Beijing, China). The monkeys were housed individually in 80 cm (height)×70 cm (width)×70 cm (length) metal cages. The monkeys were allowed free access to water, and restricted food and fresh fruit access was available at 09:00 h and 15:00 h. The animals were maintained according to the Guide for the Care and Use of Laboratory Animals (National Institute of Health, 1996). The experimental protocol was approved by Institutional Animal Care and Use Committee of National Institute on Drug Dependence, Peking University.

Morphine dependence induction The experiment consisted of 3 phases: morphine dependence induction (90 d), morphine withdrawal (21 d), and drug challenge (7 d). To ensure consistent drug administration and to reduce stress on and increase the cooperativeness of the monkeys, all drug administrations were performed by the same experimenters, who were blind to the animals' group assignment.

During the 90 d period of morphine dependence induction, the monkeys were housed in an environment similar to that in which they were reared. Eighteen monkeys were randomly divided into 3 groups of 6 monkeys per group (3 groups: Sal-Sal, Mor-Sal, and Mor-Clo). Table 1 summarizes the details of the experimental procedure. Morphine dependence was induced by repeated administration of morphine at increasing dosages for 90 d. Every day, the monkeys

Table 1. Summary of experimental procedure.

Group	Dependence induction (sc, 3 times/d, 90 d)	Withdrawal intervention (ig, twice/d, d 1 – d 7)	Challenge 1 (sc, morphine)	Challenge 2 (sc, cocaine)
Sal-Sal	saline	saline	5 mg/kg	5 mg/kg
Mor-Sal	morphine (3–15 mg/kg)	saline	5 mg/kg	5 mg/kg
Mor-Clo	morphine (3–15 mg/kg)	clonidine (0.02 mg/kg)	5 mg/kg	5 mg/kg

received sc injections in their back legs (08:00 h, 13:00 h, and 20:00 h). The Sal-Sal group was given 0.5 mL/kg of saline. The Mor-Sal and Mor-Clo groups were given morphine on a dose schedule of 3 mg/kg (d 1–7), 6 mg/kg (d 8–14), 9 mg/kg (d 15–21), 12 mg/kg (d 22–28), 15 mg/kg (d 29–90). Each monkey in the Mor-Clo and Mor-Sal groups received a total of 3420 mg/kg morphine over 90 d.

Morphine withdrawal and clonidine treatment During the 21 d period of morphine withdrawal, none of the groups were given morphine. In the first week of withdrawal, the monkeys in the Mor-Clo group received 0.02 mg/kg (ig) clonidine twice per day just prior to feeding (09:00 h, 15:00 h), while the monkeys in the Sal-Sal and Mor-Sal groups received an equal volume of saline (ig). In the second and third weeks of withdrawal, all the monkeys were given saline injections twice per day just prior to feeding. Withdrawal signs, including holding the abdomen, tremor, spasm, grimacing, face flush, eye closing, dysphoric facial expressions, and provoked screams, were assessed immediately after each injection.

Drug challenge During the 7 d period of drug challenge, all the monkeys received a saline injection daily in the first 3 d, a challenge injection of 5 mg/kg morphine (sc) on the fourth day, a saline injection on the fifth and sixth days, and a challenge injection of 5 mg/kg cocaine (sc) on the seventh day. The monkeys' response activity, including locomotor activity, irritability, vocalization and grooming, were assessed immediately after the challenge injection.

Physiological recording and behavioral scoring On the last day of the 90 d morphine induction period, the body temperature, breath rate, heart rate, and body weight of each monkey were recorded just before each feeding (09:00 h, 15: 00 h), and the average of the 2 values of each parameter was recorded as the pretest value. This measure was repeated on withdrawal day 1–21. While the measurements were taken, the monkeys were limited to a small corner of the cage by an apparatus designed by the experimenters. The monkeys were allowed to rest for at least 10 min prior to the recording. Withdrawal signs were observed from 08:30–09:00 and 14:30–13:00. If symptoms of withdrawal appeared in the 30 min observation, the monkey was given a score of 1, and observation periods in which symptoms were not shown were given 0. The average score (0, 0.5, or 1) of the 2 daily observations of each monkey was taken as their current day score value. On the challenge days (challenge d 4 and 7), the monkeys' behavioral changes were scored according to the following scale: -3 (apparently reduced), 0 (no change), 3 (few observed), 6 (many observed), and 10 (extremely changed). The observation span was 6 h after the challenge injection (09:00 h). All the recording of physiological signs and behavioral scoring were carried out by the same experimenters who were kept blind to the group assignment in order to ensure consistency.

Statistical analysis The data are expressed as mean± SEM. and were analyzed with SPSS 13 software (SPSS Inc, Chicago, Illinois, USA). Physiological signs and withdrawal score were analyzed using a repeated measure ANOVA followed by a mean comparison with the Bonferroni test. Behavioral scores from the challenge tests were analyzed with repeated measure ANOVA followed by a mean comparison with the Bonferroni test.

Results

After the 90 d morphine treatment, the monkeys exposed to morphine (Mor-Sal and Mor-Clo groups) showed body weight loss (3.88±0.14 kg before morphine treatment *vs* 3.61±0.11 kg thereafter, n=11) compared with the morphine-naive monkeys (Sal-Sal group, 3.87±0.23 kg before *vs* 4.08± 0.20 kg after, n=6, P<0.05). During the chronic morphine treatment, 1 monkey in the Mor-Clo group died at d 69 after receiving a total dosage of 2430 mg/kg morphine.

Effects of clonidine on morphine withdrawal signs During the 21 d withdrawal period, different weight loss trends was observed in the 3 groups ($F_{[2, 11]}$=3.341 P=0.074; Figure 1A). Most withdrawal signs were observed in the first week after cessation of morphine treatment; thus, the effects of clonidine on body weight were analyzed in the first, second,

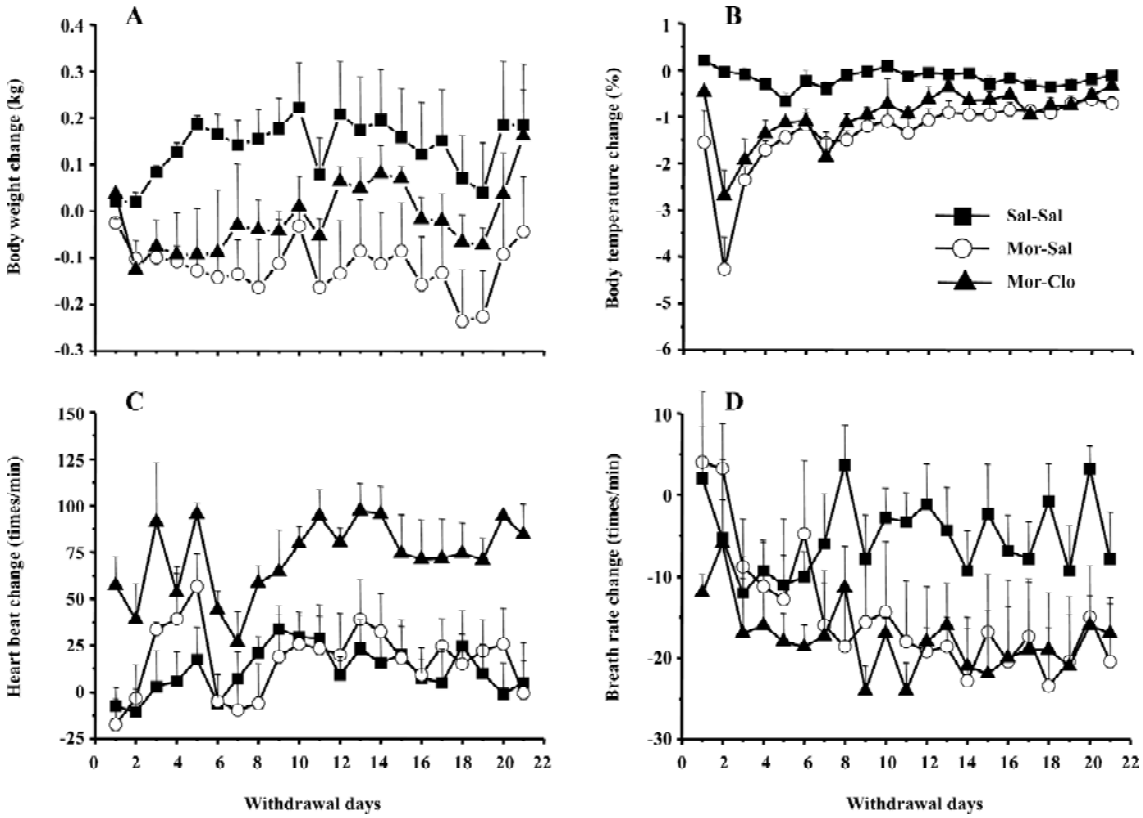

Figure 1. Effect of clonidine on physiological changes during 21 consecutive days following cessation of chronic morphine treatment. Rhesus monkeys received morphine for 90 d to induce morphine dependence (Mor-Sal, n=5 and Mor-Clo, n=4–5) and the control group received vehicle (Sal-Sal, n=6). On withdrawal d 1, the morphine treatment was stopped to induce withdrawal. Physiological parameters were measured from withdrawal day 1 to 21. The monkeys of the Mor-Clo group received clonidine treatment from withdrawal d 1 to 7, while the monkeys from the Sal-Sal and Mor-Sal groups received vehicle. Results were expressed as mean±SEM of change in bogy weight (in kg, A), body temperature (in C, B), heart rate (in times/min, C), breath rate (in times/min, D) compared to the baseline measure at day before withdrawal.

and third week separately. A significant difference was observed in the weight loss in the first ($F_{[2,11]}$=12.146, P< 0.01), but not the second or the third withdrawal weeks. However, clonidine had no effect on the body weight in the first week of morphine withdrawal (P>0.05).

During the 21 d withdrawal period, significant differences were found in the body temperatures ($F_{[2,11]}$=16.381, P<0.01; Figure 1B) of the 3 groups. As compared with the Sal-Sal group, morphine withdrawal (Mor-Sal) produced a significant decrease in body temperature (P<0.01), but clonidine treatment had no effect on these withdrawal-induced body temperature decreases. Significant differences were found between the 3 groups for body temperature during the first ($F_{[2,11]}$=20.6, P<0.01), second ($F_{[2,11]}$=11.8, P<0.005), and third ($F_{[2,11]}$=4.8, P<0.05) weeks of the withdrawal period.

During the 21 d withdrawal period, a significant difference in heart rate ($F_{[2,11]}$=4.722, P<0.05; Figure 1C) was observed in the 3 groups. Clonidine was found to increase the monkeys' heart rate in the first withdrawal week. No significant difference was found for breath rate (Figure 1D) in the 3 groups.

Cessation of morphine treatment produced obvious withdrawal signs, including holding the abdomen, tremor, spasm, grimacing, face flush, eye closing, dysphoric facial expressions, and provoked screams. During the first week of withdrawal, 1 monkey in the Mor-Sal group died at withdrawal d 7. A significant difference was only observed for the overall withdrawal score during the first 14 d withdrawal period ($F_{[2,13]}$=19.9, P<0.01); most withdrawal signs disappeared after the fourteenth day (Figure 2). In the 14 d observation, clonidine reduced the symptoms of withdrawal only during the first 4 withdrawal days (P<0.01, Mor-Sal *vs* Mal-Clo). Clonidine had no further effects on withdrawal symptoms after 4 d (P>0.05). The main behaviors that contributed to the total withdrawal score were holding the abdomen, tremor, spasm, grimacing, face flush, eye closing, dysphoric facial expressions, and provoked screams (Figure 3). Clonidine proved effective in controlling the withdrawal

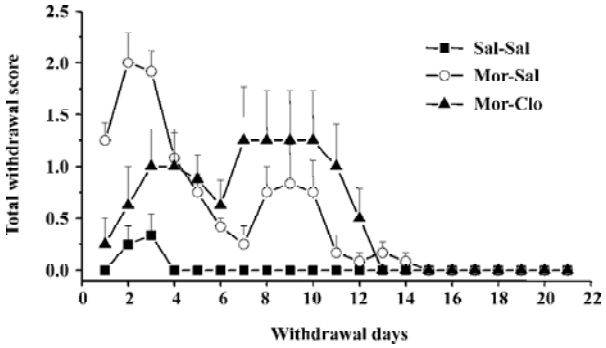

Figure 2. Effect of clonidine on morphine withdrawal signs during 21 consecutive days following cessation of chronic morphine treatment. Rhesus monkeys received morphine for 90 d to induce morphine dependence (Mor-Sal, *n*=5 and Mor-Clo, *n*=3–5) and the control group received vehicle (Sal-Sal, *n*=6). At withdrawal d 1, the morphine treatment was stopped to induce withdrawal. A composite withdrawal signs were measured daily in each monkey. The monkeys in the Mor-Clo group received clonidine treatment from withdrawal d 1 to 7, while the monkeys in the Sal-Sal and Mor-Sal groups received vehicle. Results were expressed as mean±SEM of the morphine withdrawal score.

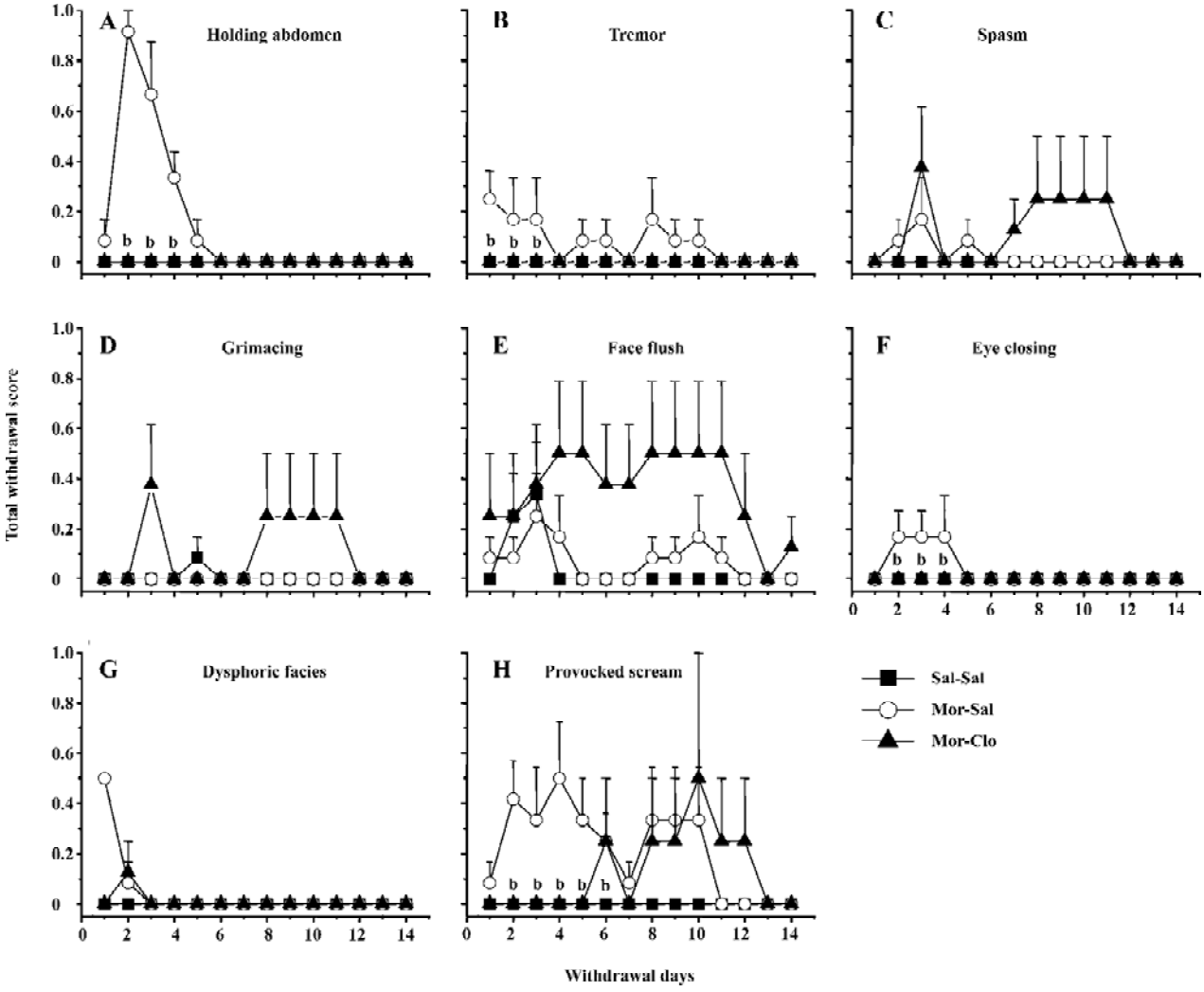

Figure 3. Effect of clonidine on specific withdrawal signs during 14 consecutive days following cessation of chronic morphine treatment. Rhesus monkeys received morphine for 90 d to induce morphine dependence (Mor-Sal, *n*=5 and Mor-Clo, *n*=3–5) and the control group received vehicle (Sal-Sal, *n*=6). At withdrawal d 1, the morphine treatment was stopped to induce withdrawal. Individual withdrawal scores were measured daily in each monkey for various signs or symptoms that were noted in the figure. The monkeys in the Mor-Clo monkeys received clonidine treatment from withdrawal d 1 to 7, while the monkeys from the Sal-Sal and Mor-Sal groups received vehicle. Results were expressed as mean±SEM of the morphine withdrawal score. $^{b}P<0.05$ in group comparisons.

signs of abdomen holding, tremor, eye closing, and provoked screams in the first 7 withdrawal days, but not all the signs of morphine withdrawal were eliminated by clonidine.

Effects of clonidine on the effect of cocaine and morphine challenge injection Body weight, body temperature, and heart and breath rate were also recorded 5 min before and 1 and 5 h after the challenge injection of morphine or cocaine. No apparent change was observed in the monkeys receiving morphine priming. The response activities to morphine or cocaine, including locomotor activity, vocalization, grooming, and irritability were reliably observed and used to discern the sensitization of the monkeys to the challenge of morphine and cocaine.

An injection of 5 mg/kg morphine caused a significant increase in locomotor activity ($F_{[2,11]}$=29.9, P<0.01) and irritability ($F_{[2,11]}$=24.8, P<0.01), but not in vocalization ($F_{[2,11]}$= 1.445, P>0.05) and grooming ($F_{[2,11]}$=1.445, P>0.05; Figure 4A). The monkeys naive to morphine (Sal-Sal) showed deceased locomotor activity and irritability, which may reflect the sedative effects of morphine[33–35]. Compared with the Sal-Sal group, the monkeys with a history of morphine treatment demonstrated increased locomotor activity and irritability (P<0.01, Sal-Sal *vs* Mor-Sal), and these behavioral responses were greatly attenuated in the monkeys that had previously received clonidine during the first week of morphine withdrawal (P<0.01, Mor-Clo *vs* Mor-Sal).

An injection of 5 mg/kg cocaine increased all the observed behaviors in the Mor-Sal group (Figure 4B): locomotor activity ($F_{[2,11]}$=8.941, P<0.01), vocalization ($F_{[2,11]}$=7.364, P<0.01), grooming ($F_{[2,11]}$=5.535, P<0.05), and irritability ($F_{[2,11]}$=5.914, P<0.05). The monkeys that received clonidine treatment during the first week of morphine withdrawal showed significantly decreased vocalization compared with no clonidine treatment after morphine withdrawal (P<0.05, Mor-Clo *vs* Mor-Sal), and enhanced grooming performance compared with saline control (P<0.05, Sal-Clo *vs* Sal-Sal). One monkey in the Mor-Sal group demonstrated a dramatic increase in locomotor activity (scored 10), irritability (scored 8), and vocalization (scored 6) 1 h after the cocaine injection. After 4 h, all the above behaviors declined, and 1 monkey died 13 h after the cocaine injection in the Mor-Sal group.

Discussion

In the present study, withdrawal symptoms were found to persist for 14 d after cessation of the 90 d morphine administration in the rhesus monkeys. Clonidine administration reduced morphine withdrawal symptoms. However, the effects of clonidine were not persistent and disappeared when clonidine administration was stopped. After prolonged

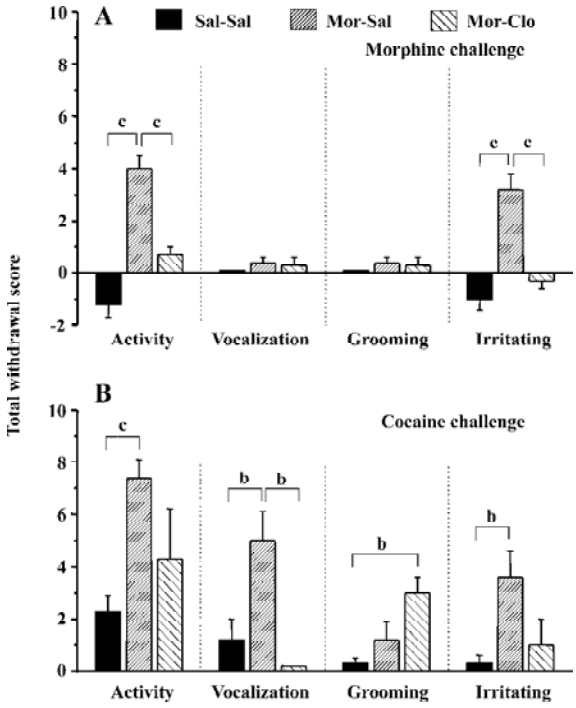

Figure 4. Effect of clonidine on the responses to challenge injection of morphine and cocaine after 21 d withdrawal. Rhesus monkeys received morphine for 90 d to induce morphine dependence (Mor-Sal, n=5 and Mor-Clo, n=3–5) and the control group received vehicle (Sal-Sal, n=6). The monkeys were given a challenge injection on different days with 5 mg/kg morphine (A) and 5 mg/kg cocaine (B) after 21 d of morphine withdrawal. The behavioral signs were measured after the challenge injection. Group Sal-Sal (n=6, control monkeys), group Mor-Sal (n=5, monkeys with history of morphine treatment), and group Mor-Clo (n=4–5, monkeys with history of morphine treatment and 7 d clonidine treatment during the first week of morphine withdrawal). ^{b}P<0.05, ^{c}P<0.01 using Bonferroni test *vs* control group.

abstinence from morphine, the morphine-dependent monkeys displayed an enhanced response to a challenge injection of morphine ("behavioral sensitization") and cocaine ("cross-sensitization"). Clonidine administration during the initial week of the withdrawal phase produced attenuation of subsequent behavioral sensitization and cross-sensitization.

After a period of chronic opiate administration, failure to continue periodic administration of the drug to an animal resulted in severe physiological and behavioral disturbances several hours after the last dose of the drug. This complex of signs and symptoms, termed opiate abstinence syndrome, indicates that the organism has become physically dependent on the opiate[36]. Here, the withdrawal symptoms were measured daily for 21 d to determine precisely the time-course of appearance and disappearance of the symptoms in rhesus monkeys as compared with the symptoms previously docu-

mented[37,38]. Few reports are available on the time-course of the various opiate withdrawal symptoms following spontaneous abstinence in rhesus monkeys. Here we found the peak of withdrawal signs at day 2 and 3 following the cessation of the morphine injections (Figure 2). Morphine withdrawal was associated with significant weight loss and decrease of body temperature (Figure 1A, 1B), but there was no significant change in heart rate. Although breath rates decreased in morphine-dependent monkeys compared to control group, this difference was not significant (Figure 1D).

The various signs of morphine withdrawal that were scored during the withdrawal phase were highly specific, since control monkeys did not exhibit these signs (see however Figure 3 the presence of face flush in some control monkeys). After 14 d of withdrawal, nearly all of the observed withdrawal signs disappeared (Figures 2, 3). It should be noted that withdrawal in this study was not induced by injections of opiate antagonist, but with the cessation of morphine treatment, a situation that mimics that of human addicts that stop taking drugs. In contrast, many previous investigators have used opioids antagonists, such as naloxone or nalorphine, to induce morphine withdrawal syndrome in monkeys[39] and rodents[40–46] (see ref 47 for a comparison of studies). The symptoms of withdrawal are often more pronounced when provoked by an injection of an opioid antagonist than provoked by cessation of morphine injections. Evidence suggests that the motivational signs of withdrawal appear first in a situation of mild withdrawal, whereas physical signs are seen in a situation of severe withdrawal[46–48]. However, some physical signs of withdrawal were also relatively severe during the spontaneous withdrawal. This present finding was strengthened by the fact that 1 monkey died during the period of morphine withdrawal (see Results). Interestingly, the withdrawal syndrome created upon the cessation of access to opioids in rats previously trained to self-administer morphine caused many of the signs we reported here in monkeys, such as weight loss, tremor, hypersensitivity, agitation, soft stools, and increased respiration[49].

Administration of clonidine, an α_2 adrenoceptor agonist, significantly decreased morphine withdrawal symptoms during the first week of withdrawal. This finding is in agreement with previous reports on rhesus monkeys[38], rodents[47,50–58], and humans[21,22,59] that clearly demonstrate that clonidine effectively attenuates some opiate withdrawal signs and symptoms. Here, clonidine was able to reduce some, but not all, symptoms of morphine withdrawal (Figure 3). Notably, clonidine significantly reduced the overall withdrawal signs during the first week of withdrawal (Figure 2). Some signs, such as the provoked screams and holding of the abdomen in the monkeys, were totally abolished by clonidine administration. In contrast, clonidine had no or limited effects on other symptoms such as face flush or grimacing. Previous reports had shown that clonidine only affected a subset of symptoms in monkeys[38], rats[47], and humans[21,22,59]. The effects of clonidine on withdrawal symptoms were short-lasting, since the intensity of the morphine withdrawal signs were even higher at the cessation of the clonidine treatment (at week 2), suggesting a rebound phenomenon (Figure 2). It should be noted, that clonidine produced a significant effect on weight loss induced by withdrawal that was noted at the third week of morphine withdrawal. However, most of the withdrawal symptoms disappeared at d 14, regardless of the presence or absence of clonidine treatment during the first week of withdrawal, indicating that clonidine did not dramatically alter the time-course of the abstinence symptoms and is only able to attenuate its acute manifestations. Hypotension or sedation are 2 frequent side-effects induced by clonidine treatment in opiate addicts[60,61]. The increase of heart rate found in the group of monkeys receiving clonidine treatment may reflect a compensatory mechanism to a hypotensive effect of clonidine. No particular sedation was noticed in the monkeys receiving clonidine treatment.

We have also investigated the effects of priming injections of morphine and cocaine after prolonged abstinence of morphine. The monkeys with a history of morphine treatment displayed enhanced locomotor responses to 5 mg/kg cocaine and to 5 mg/kg morphine, as compared with naive control monkeys (Figure 4). These enhanced responses likely reflect “behavioral sensitization” to morphine and “cross-sensitization” to cocaine. It is also possible that this behavioral sensitization to morphine may have been facilitated by the development of tolerance to the sedative or depressing effect of morphine, since morphine administration decreased locomotor activity in naive monkeys. However, since these experiments have been performed following prolonged withdrawal from morphine and it is well known that extended abstinence strongly decreases the tolerance to the effects of opiate, it is likely that the increased response to morphine in the monkey with a history of morphine administration reflects the development of behavioral sensitization.

Although many investigators have demonstrated that repeated administration of morphine can produce long-lasting behavioral sensitization in rodents[62], very limited evidence has been published so far that this phenomenon is also observed in humans or monkeys. Therefore, this is (to

our knowledge) the first evidence that monkeys with history of morphine treatment displayed both sensitized responses to morphine and cross-sensitization to psychostimulants. These findings were in agreement with previous experiments performed on rodents which showed that heroin exposure facilitates subsequent locomotor and drug-seeking responses to cocaine in rats[10,63], and enhanced locomotion to ethanol in mice[64]. In addition, animals with a history of heroin self-administration displayed locomotor sensitization to amphetamine[15].

It may seem surprising that a sensitized response to cocaine administration has been observed on the locomotor activity, but not grooming behavior following cocaine administration, since grooming is also a behavior mediated by dopamine transmission and notably by the stimulation of the dopamine D_1 receptor in rodents[65–68]. Since locomotor activity is mediated by both the D_1 and D_2 receptor stimulation, this dissociation may reflect a preferential activation of the D_1 receptor. However, the fact that morphine administration does not produce vocalization or grooming behavior, that are known to reflect stress in monkeys[28], strongly suggests that cocaine may have produced some aversive effects in these monkeys that morphine dose not produce. It is well known that cocaine, like other drugs of abuse[69], produces both positive and aversive effects[70,71]. It appears that opiates produce less aversive effects compared to cocaine[70,71], which may explain the absence of grooming and vocalization induced by the morphine challenge in these monkeys. This hypothesis can be tested in subsequent studies using doses of hormones that approximate the level of stress in these monkeys following drug administration.

Interestingly, some responses to a challenge injection of cocaine and morphine were not affected by the previous exposure to morphine (see Results). Notably, the cardiovascular response to 5 mg/kg cocaine and 5 mg/kg morphine was identical in all groups. Unfortunately, despite a considerable research effort in the last 2 decades, the causes of cardiovascular response to cocaine are still poorly understood[72]. Many investigators are convinced that the cardiovascular changes are mediated by changes in catecholamines levels at the periphery[72], but this interpretation is debatable. Opiates are mainly known to reduce heart rate and blood pressure[73]. However, an initial cardiovascular stimulating effect of opiate has been noted in cats[74], dogs[75], and humans[76]. These effects may also involve catecholamine and histamine release[73]. Here, we extended these findings to rhesus monkeys, since we had found significant cardiovascular activation 1 h following morphine and cocaine administration. It was noteworthy that the monkeys were sensitized only to response mediated by the dopaminergic system, whereas other responses that were likely to reflect peripheral effects of drugs of abuse were not affected.

Early clonidine intervention during the first week of withdrawal reduced sensitized behavior responses. This effect was significant for locomotor activity and the irritation score for morphine and for the vocalization score for cocaine. A trend downward (a non-significant decrease) was also noted for the locomotor activity and irritation behavior in the monkeys. These findings suggest that clonidine treatment during the first week of withdrawal serves not only to reduce the acute withdrawal symptoms, but also to affect the subsequent effects of drug challenge. Drug priming is able to reinstate drug-seeking behavior in animals in a reinstatement paradigm and can produce relapses in humans. It is possible that clonidine administration has blocked some neurobiological adaptations that are involved in behavioral sensitization processes ("incentive salience-sensitization theory") or that the reduction of the intensity of the withdrawal symptoms accounts for the subsequent behavioral response to a drug priming injection ("incentive-motivational theory"). Further experiments are needed to delineate which of these hypotheses underlie the effects of clonidine. Since the noradrenergic structure mediates the expression of opioid abstinence, one possibility is that clonidine normalizes the firing of locus coeruleus neurons during withdrawal to produce its effects[53,77]. However, the recent and surprising finding that total neurochemical lesion of noradrenergic neurons of the locus coeruleus does not alter either naloxone-precipitated or spontaneous opiate withdrawal, nor influence ability of clonidine to reverse opiate withdrawal[78] suggests that other brain sites may be involved. It is noteworthy that a noradrenaline-rich subdivision has been recently identified in the human nucleus accumbens[79] and that behavioral sensitization processes to psychostimulants and opiates may involve sensitized responses of noradrenaline in frontal areas that project to the nucleus accumbens[25]. Recent evidence suggests that the blockade of noradrenaline release induced by clonidine during opiate withdrawal[57] may involve brain areas other than the locus coeruleus, and may implicate brain areas involved in the motivational control of drug-seeking behavior.

In conclusion, the present study demonstrates that morphine-dependent monkeys will not only present typical morphine withdrawal symptoms at the cessation of morphine administration, but will also display enhanced behavioral responses to challenge injections of both morphine and cocaine, providing evidence for both "behavioral sensitization" and "cross-sensitization" processes in non-human

primates. In agreement with previous findings in humans and laboratory animals, clonidine served to decrease morphine withdrawal symptoms. However, these effects were short-lasting. Clonidine produces long-lasting effects on subsequent morphine and cocaine challenge after prolonged abstinence. While these experiments do not allow us to determine if the effects of clonidine are mediated by an interaction with behavioral sensitization processes or with the possible link between morphine withdrawal and subsequent effects of drugs, these experiments indicate that active intervention in the first stage of withdrawal using an α_2 adrenoceptor agonist has a positive effect on reducing drug relapse in the overall therapeutic strategy.

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