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Correspondence and requests for materials should be addressed to X.L. (lixw701@sina.cn)

Differential effects of propranolol on conditioned hyperactivity and locomotor sensitization induced by morphine in rats

Shuguang Wei[1,2] & Xinwang Li[1]

[1]Beijing Key Laboratory of Learning and Cognition, Department of Psychology, Capital Normal University, Beijing 100048, China, [2]College of Education Science and Teacher Development, Henan Normal University, Xinxiang 453007, China.

According to memory reconsolidation theory, when long-term memory is reactivated by relevant clues, the memory traces become labile, which can be altered by pharmacological manipulations. Accumulating evidence reveals that memory related to drug abuse can be erased by disrupting reconsolidation process. We used an animal model that could simultaneously measure conditioned hyperactivity and locomotor sensitization induced by morphine. β-adrenoceptor antagonist propranolol or saline were administered following conditioned stimuli (CS) or a small dose of morphine reactivation. The results showed that the conditioned hyperactivity could be disrupted by propranolol treatment following CS reactivation. However, the expression of locomotor sensitization could not be disrupted by propranolol administration following CS or morphine reactivation. Furthermore, morphine injection and propranolol intervention enhanced the locomotor sensitization effect. These data suggest that blocking the reconsolidation process can disrupt the conditioned hyperactivity induced by environmental cues associated with morphine treatment, but not morphine-induced locomotor sensitization.

Drug addiction is a chronic relapsing disorder characterized by compulsive drug seeking and use. Over 80% of addicts relapse to drug seeking and use after a period of withdrawal and abstinence[1]. For instance, cocaine abusers exhibit strong conditioned craving when they are presented with stimuli previously associated to cocaine use in a laboratory setting[2]. Consequently, there are two major aims in preclinical research: one is to clarify the behavioral, environmental and neural mechanisms underlying relapse, and the other is to discover medications that can prevent relapse. A major contributor to relapse is exposure to environmental stimuli that have previously been associated regularly with drugs[3]. Many studies have shown that neutral clues can acquire excitatory locomotor (hyperactivity) effect in a drug free state when drug administration is repeatedly paired with those clues[4–7]. Locomotor sensitization refers to a progressive and persistent increase in the psychomotor activating effects of drugs (e.g. opioids and psychostimulants), which often occurs when drugs of abuse are given repeatedly and intermittently[4,8–10]. Sensitization-related neuroplasticity in brain reward systems may contribute to addiction[8–10].

The process of previously consolidated memories being recalled and actively consolidated is defined as the memory reconsolidation. During this process, memory traces become labile and can be altered by various pharmacological manipulations[11–13]. Increasing studies have begun to reveal that memory reconsolidation is mediated by various neural events, including receptors[14,15], signal transduction pathways[16,17], and proteins[18,19]. Using conditioned place preference (CPP)[20–24], self-administration[25] and conditioned approach[26] paradigms, it has been demonstrated that disruption of reconsolidation could impair the expression of drug-associated memory, which suggests that such a technique to target the reconsolidation process could be a prospective treatment for drug addiction. Evidence shows that the noradrenergic system is critically involved in memory reconsolidation. For example, the administration of β-adrenoceptor antagonist propranolol after the reactivation of cocaine-[27,28] or morphine-[29–31] induced CPP impairs the conditioning response.

There are only a few studies that have examined the reconsolidation of memories underlying drug-induced locomotor sensitization. Bernardi et al[32] have reported that systemic anisomycin treatment given immediately after a reactivation session in which rats are put into the cocaine-associated context blocks cocaine-induced locomotor sensitization. However, Valjent et al[24] found no effect with anisomycin using a similar paradigm. Exposure of animals to drug-conditioned context/cues (CS) in the absence of drug administration (unconditioned stimuli, US) has frequently been used to reactivate drug-context association[21,27]. However, some

Table 1 | Group assignment, timeline and treatment for experiment

	Treatment					
	Day 1–7	Day 10–11	Day 14	Day 15	Day 18–19	Day 22
Group [a]	Conditioning	Reactivation1(R1)	Conditioned LA test	Sensitization test1	Reactivation2(R2)	Sensitization test2
Sal (n = 11)	CS(20 min) + Sal(2 hr)	Sal(20 min)	CS (20 min) + Sal(1 hr)	3Mor(2 hr)	Sal (30 min) + Sal	3Mor(2 hr)
Mor-R-Sal (n = 11)	CS(20 min) + 5Mor(2 hr)	Sal(20 min)	CS (20 min) + Sal(1 hr)	3Mor(2 hr)	2Mor(30 min) + Sal	3Mor(2 hr)
Mor-R-Pro (n = 11)	CS(20 min) + 5Mor(2 hr)	10Pro(20 min)	CS(20 min) + Sal(1 hr)	3Mor(2 hr)	2Mor(30 min) + 10Pro	3Mor(2 hr)
Mor-NR-Pro (n = 8)	CS(20 min) + 5Mor(2 hr)	No R + 10Pro	CS(20 min) + Sal(1 hr)	3Mor(2 hr)	No R + 10Pro	3Mor(2 hr)

[a]Abbreviations: Sal, saline; Mor, Morphine hydrochloride; Pro, Propranolol; R, Reactivation; NR, no Reactivation; CS, Conditioned Stimulus.

researchers suggest that reactivation requires the re-experience of a conditioning session and it does not occur after contextual or drug exposure alone[22,24,33,34]. It is important to efficiently and effectively reactivate drug related memory in order to fully assess the reconsolidation-interfering effect of propranolol treatment. Therefore, in the current study morphine treatment-related memory was reactivated in two consecutive days in which propranolol was administered immediately after CS- or US- primed reinstatement sessions. The goal of this study was to test the feasibility of propranolol's disrupting effect on the reconsolidation of conditioned hyperactivity and locomotor sensitization induced by morphine. We also tested the effect of different retrieval types in reactivating memory reconsolidation underlining locomotor sensitization.

Results

The group assignment, timeline and treatment for the experiments were shown in Table 1. Briefly, the experimental procedure consists of three sessions, the acquisition of conditioned hyperactivity and locomotor sensitization, reactivation and intervention, conditioned hyperactivity and locomotor sensitization test.

The acquisition of conditioned hyperactivity and locomotor sensitization. During the conditioning sessions, conditioned hyperactivity is defined as the locomotion during the 20 min prior to drug administration. As shown in Fig. 1A, repeated two-way ANOVA showed a significant main effect of days ($F_{6,\ 222} = 6.82$; $p < 0.001$), but the interaction of group × days ($F_{18,222} = 0.853$; $p = 0.67$) and the effect of groups ($F_{3,\ 37} = 1.33$; $p = 0.28$) were not significant. Rats received morphine (Mor-R-Sal, Mor-R-Pro and Mor-NR-Pro groups) showed higher locomotion than rats received saline (Sal group) during the conditioning sessions. However, there were no statistically differences among the groups that received morphine ($p > 0.05$). The locomotor sensitization to morphine is defined as the progressive increase in locomotion observed after intermittent administrations of morphine. As shown in Fig. 1B, during the daily 120-min locomotor recording, statistical analysis revealed a significant effect of days ($F_{6,222} = 27.22$; $p < 0.001$), an interaction of group × days ($F_{18,222} = 6.748$; $p < 0.001$) and an effect of groups ($F_{3,\ 37} = 17.129$; $p < 0.001$). Rats given morphine (Mor-R-Sal, Mor-R-Pro and Mor-NR-Pro groups) during the training sessions showed significantly higher locomotion level than those received saline ($p < 0.05$ for every session). Moreover, although there were fluctuations during the training session, their locomotion level on Day 7 was significantly higher than that on Day 1 ($p = 0.003$, $p = 0.001$ and $p = 0.04$ for group Mor-R-Sal, Mor-R-Pro and Mor-NR-Pro), which demonstrated the progressive development of morphine locomotor sensitization. However, the locomotion level in rats receiving saline did not show systematic changes across days ($F_{6,\ 60} = 1.57$, $p = 0.17$).

Conditioned hyperactivity during reactivation sessions. During reactivation and intervention sessions, no data were recorded for rats that received propranolol in their home cages. Fig. 2 presented the locomotor activity data for Mor-R-Pro, Mor-R-Sal and Sal

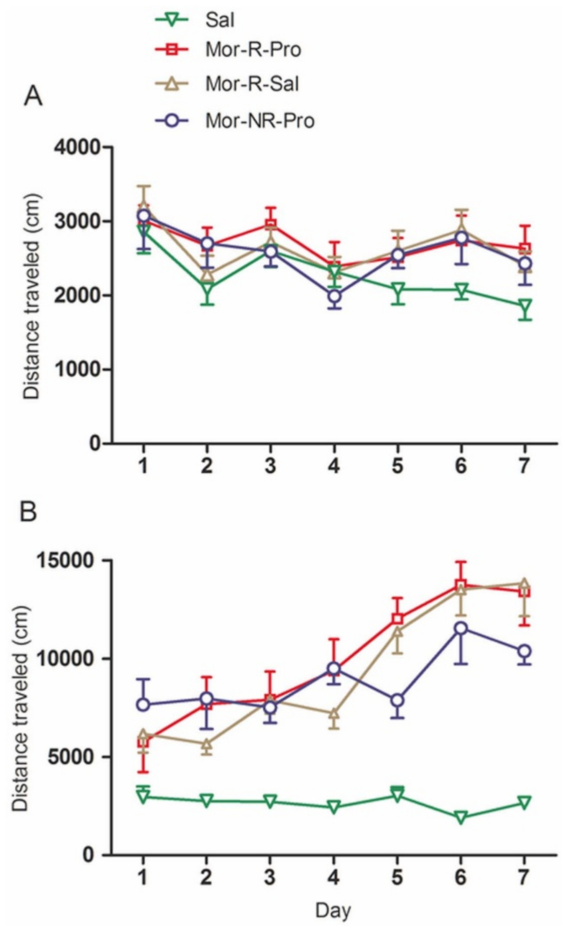

Figure 1 | Induction of conditioned hyperactivity and locomotor sensitization. Rats were put into locomotion chambers for 20 min (paired with CS) with an injection of 5 mg/kg morphine (Mor group) or saline (Sal group) afterwards. The locomotor activity was recorded for another 120 min. This session was conducted during seven consecutive days (days 1–7). (A) Locomotor activity collected during the 20-min period prior to drug injection; (B) Locomotor activity collected during the 120-min period after morphine injection. Note that the statistical significance was not shown on Fig. 1B for the group comparisons because Mor-R-Sal, Mor-R-Pro and Mor-NR-Pro groups were all significantly higher than Sal group throughout the seven days.

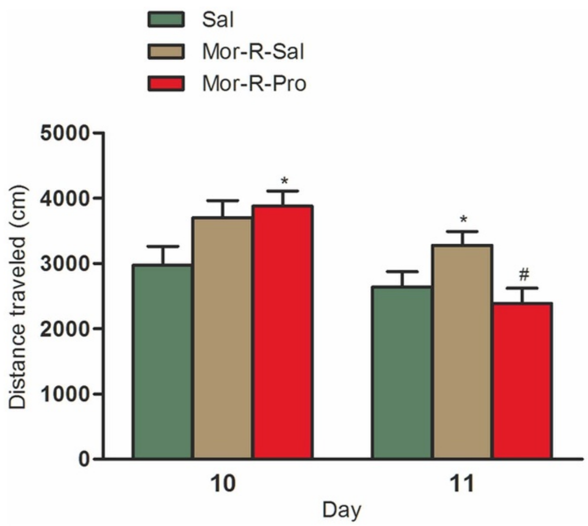

Figure 2 | The conditioned hyperactivity during the CS reactivation sessions. Two days after the conditioning sessions, rats were transported into the locomotion chambers for a 20-min re-exposure trial on day 10. Immediately after re-exposure, rats were administered 10 mg/kg propranolol (Mor-R-Pro) or saline (Mor-R-Sal) and placed back into home cages. The non-reactivation control group (Mor-NR-Pro) received propranolol in home cages. The same intervention was repeated on day 11. * $p < 0.05$ compared with Sal group (day 10 and day 11); $^{\#}$ $p < 0.01$ compared with Mor-R-Sal group (day 11).

groups. Repeated two-way ANOVA showed an effect of days ($F_{1, 30} = 28.09$; $p < 0.001$), an interaction of group × days ($F_{2, 30} = 6.98$; $p = 0.003$) but no effect of groups ($F_{2, 30} = 2.6$; $p = 0.091$). On Day 10, one-way ANOVA revealed a significant group effect ($F_{2, 30} = 3.35$, $p = 0.049$). Post-hoc test showed that the locomotor activity of Mor-R-Pro group was significantly higher than Sal group ($p = 0.021$), while there was no significant difference between Mor-R-Pro group and Mor-R-Sal group ($p = 0.634$). Mor-R-Sal group exhibited higher locomotion than Sal group ($p = 0.06$). These results indicated that Mor-R-Pro and Mor-R-Sal groups expressed similar conditioned locomotor activity. On Day 11, one-way ANOVA for groups reached significant level ($F_{2, 30} = 4.17$, $p = 0.025$). Post-hoc test showed that the locomotor activity of Mor-R-Pro group was significantly lower than Mor-R-Sal group ($p = 0.009$) but there was no significant difference as compared to Sal group ($p = 0.429$). However, the locomotor activity of Mor-R-Sal group was significantly higher than Sal group ($p = 0.05$). These results suggest that propranolol administration after reactivation can disrupt the conditioned hyperactivity 24 h later.

Conditioned hyperactivity test. On Day 14, all groups were tested for conditioned locomotor activity. As shown in Fig. 3A, one-way ANOVA for the 20-min locomotor activity revealed a significant group effect ($F_{3, 37} = 4.29$, $p = 0.011$). The locomotor activity level of Mor-R-Sal and Mor-NR-Pro groups were significantly higher than Sal group ($p = 0.05$ and $p = 0.003$, respectively); however, the locomotor activity level between Mor-R-Pro group and Sal group was not significantly different ($p = 0.215$). As shown in Fig. 3B, one-way ANOVA for the 60-min locomotor activity after saline injection also revealed significant group effect ($F_{3, 37} = 5.55$, $p = 0.003$). The locomotor activity of Sal and Mor-R-Pro groups were significantly lower than Mor-R-Sal ($p = 0.011$, $p = 0.033$) and Mor-NR-Pro ($p = 0.001$, $p = 0.005$) groups. These results indicated that propranolol administration following CS reactivation is enough to disrupt the reconsolidation for conditioned hyperactivity, and this effect could not be attributed to

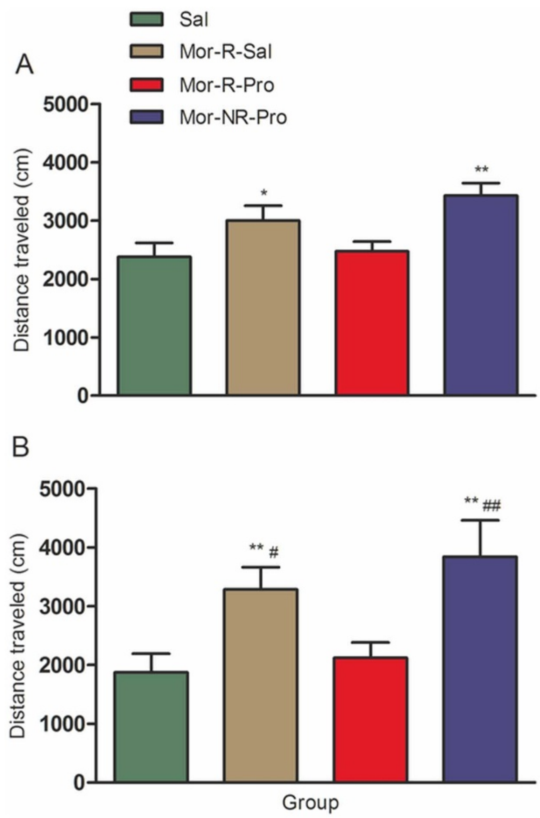

Figure 3 | The conditioned hyperactivity challenge test after CS reactivation and propranolol intervention. Two days after reactivation sessions, rats were first tested for 20 min in locomotion chambers, their locomotion was recorded for another 60-min in a drug-free state after an injection of saline. (A) Locomotor activity collected during the 20-min period prior to saline injection. (B) Locomotor activity collected during the 60-min period after saline injection. * $p < 0.05$, ** $p < 0.01$ compared with Sal group; $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$ compared with Mor-R-Pro group.

propranolol administration per se, for the conditioned hyperactivity of Mor-NR-Pro group remained higher conditioned hyperactivity.

The locomotor sensitization test. On Day 15, a morphine (3 mg/kg) challenge was used to test locomotor sensitization. As shown in Fig. 4A, one-way ANOVA for the locomotor activity revealed significant group effects ($F_{3, 37} = 10.57$, $p < 0.001$). The locomotor activity of Mor-R-Pro, Mor-R-Sal and Mor-NR-Pro groups was significantly higher than that of Sal group (all $p < 0.001$), whereas no significant differences were found among Mor-R-Pro, Mor-R-Sal and Mor-NR-Pro groups ($p > 0.05$). These results suggest that the locomotor sensitization remained unaltered in spite of the fact that the conditioned hyperactivity was disrupted by propranolol treatment after CS reactivation. On Day 22, after a small dose of morphine (2 mg/kg) reactivation and propranolol treatment, another challenge dose of morphine (3 mg/kg) was used to test the locomotor sensitization. As shown in Fig. 4B, one-way ANOVA revealed significant group effects ($F_{3, 37} = 17.8$, $p < 0.001$). The locomotor activity of Mor-R-Pro, Mor-R-Sal and Mor-NR-Pro groups was significantly higher than that of Sal group ($p < 0.001$ for Mor-R-Pro and Mor-R-Sal, $p = 0.047$ for Mor-NR-Pro). Unexpectedly, the locomotor activity of Mor-R-Pro group was significantly higher than Mor-R-Sal and Mor-NR-Pro group ($p = 0.01$ and $p < 0.001$). These results suggest that a drug-primed reactivation followed by propranolol treatment could not disrupt the expression of locomotor sensitization. In contrast, propranolol enhanced the magnitude of locomotor sensitization to morphine.

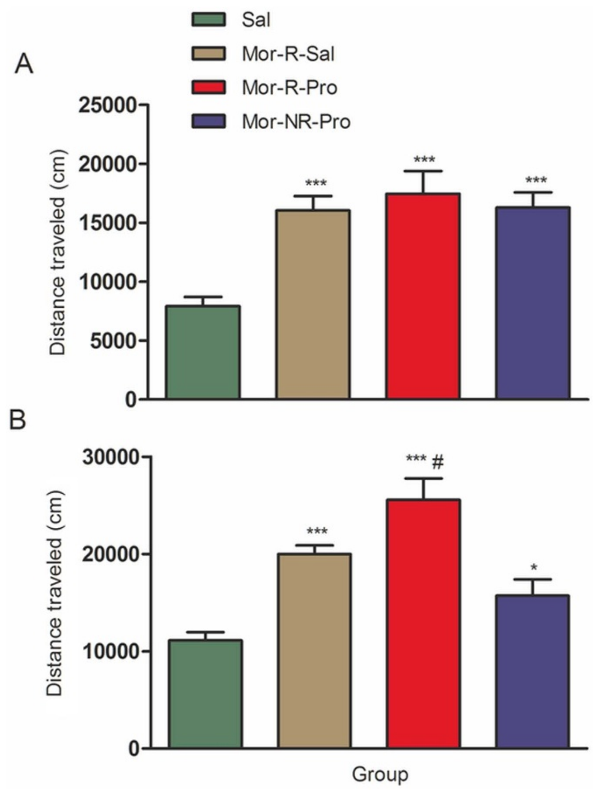

Figure 4 | The locomotor sensitization test. (A) The locomotor sensitization challenge test after CS reactivation and propranolol intervention. All rats were challenged with 3 mg/kg morphine on day 15. *** $p < 0.001$, compared with Sal group. (B) The locomotor sensitization challenge test after CS + US reactivation and propranolol intervention. All rats were challenged with 3 mg/kg morphine on day 22. * $p < 0.05$, *** $p < 0.001$, compared with Sal group; # $p < 0.01$, compared with group Mor-R-Sal and Mor-NR-Pro groups.

Discussion

Two methods were used in the current study to measure the conditioned hyperactivity: one was the 20-min recording in the locomotion chambers without any treatment, and the other was an additional 60-min locomotion recording, which has been used by other researchers[35,36]. The results of both methods showed that propranolol treatment following reactivation inhibited the development of conditioned hyperactivity, which is consistent with previous studies which have demonstrated post-retrieval impairment effect of drug-mediated behaviors[27,28,31]. The disrupting effects of propranolol could not be attributed to the drug *per se*, because the conditioned hyperactivity remained unaltered if propranolol was given in the absence of reactivation.

Since the goal of reconsolidation studies is to retrieve the original memory trace, most studies accomplish this by using CS re-exposure, which is essentially a short extinction session. For example, animals are often put into the test chambers without the presence of drug, or they perform an instrumental behavior for a drug-associated cue, or they are presented with the cue non-contingently[33]. According to trace dominance theory[37], the extinction and reconsolidation processes compete with each other and the amnestic agents will block the dominance process. The prevailing concept in reconsolidation studies is that reconsolidation sessions need to be short to avoid extinction. The time period of exposure for reactivation varies markedly depending on the type of experiment. For example, in fear conditioning studies, reactivation is often only 30 s but in drug abuse studies the re-exposure session can be as much as 30 min because enough time is typically allowed for the animal to perform the behavior[33]. The reconsolidation process was also influenced by the strength of memories. For instance, strong memories were found to be more resistant to reconsolidation, but could be rendered labile again only if the reminder session was prolonged[13,18]. In the current study, 20 min re-exposure to training chamber were chosen, which is based on our pilot study that the current time period is enough to develop conditioned hyperactivity and to avoid extinction process. To effectively disrupt the reconsolidation process, the reactivation and amnestic intervention were carried out twice based on previous studies[20,26,38].

Noradrenaline neurotransmission plays a critical role in learning and memory processes. Specifically, β-adrenoceptors are involved in long-term potentiation[39] and consolidation of memory[40]. The β-adrenergic receptor activation is also important for post-retrieval stabilization of memories, as systemic injections with the β-adrenoceptor antagonist propranolol impairs the expression of aversive memories in rats that received reactivation[41]. Using self-administration paradigm, propranolol persistently disrupts reconsolidation of Pavlovian associations between environmental conditioned stimuli and appetitive reinforcers when administered immediately after memory reactivation[25]. In addition, reactivation of drug-related memories and concomitant propranolol administration disrupt subsequent expression of cocaine-[27] and morphine-[29,31] induced CPP. In this study, the conditioned hyperactivity induced by Pavlovian pairing of context and morphine could also be disrupted by administration of propranolol after the retrieval trial, which is consistent with previous reports. In the earlier studies, many researchers used protein synthesis inhibitors to disrupt the reconsolidation process. Although protein synthesis inhibitors can effectively attenuate *de novo* protein synthesis, their non-specific toxicity precludes their clinical use. The β-adrenoceptor antagonists are already available for human use, and there is evidence for use of propranolol as an amnestic to treat posttraumatic stress disorder (PTSD)[42,43]. In this sense, propranolol which interferes with memory reconsolidation processes might open the door to novel treatment for drug addiction and other psychiatric disorders.

Although conditioned hyperactivity could be disrupted by CS reactivation and propranolol administration, the morphine challenge results suggest that the expression of locomotor sensitization could not be disrupted by propranolol administration following CS reactivation. Considering drug-priming is a powerful reminder of the drug-associated memory, rats were given an injection of morphine (2 mg/kg) followed by 30 min freely activity in locomotion chamber, which served as a CS + US retrieval trial. A small dose of morphine (2 mg/kg) was chosen for the reason that we wish to reactivate the unconditioned drug effect in the premise not to enhance sensitization effect as much as possible. The reactivation duration (30 min) was chosen for the unconditioned morphine effect reach a higher level in 30 min. However, CS + US reactivation and propranolol intervention also could not disrupt locomotor sensitization. Both results suggest that the locomotor sensitization effect could not be erased by disrupting the reconsolidation process.

Several possibilities could attribute to the differential effects of propranolol on the reconsolidation of context-associated hyperactivity and morphine-induced sensitization.

First, in the current study, morphine-induced sensitization develops during conditioned training (Fig. 1), whereas context-associated hyperactivity requires the longer periods and perhaps a period of abstinence. These results are similar to the study conducted by the group of Li[6], although there are differences in training session and abstinence period. Also, Kosowski et al[4] have shown that the conditioned response to nicotine could not be demonstrated until the sensitized locomotor activity response reached a plateau phase, which suggests that a maximal level in the expression of behavioural sensitization to nicotine precedes the onset of conditioned increase of locomotor activity. Therefore, we can infer that the memory associative strength of context-associated hyperactivity is smaller than that of morphine-induced sensitization. Previous studies have shown that stronger memory is less readily reactivated[18].

Second, Anagnostaras and colleagues[7,44] have demonstrated that there are three memory processes underling locomotor sensitization: drug exposure causes non-associative cellular changes which are essential for locomotor sensitization; drug exposure initiates an inhibitory associative process which attenuates the expression of locomotor sensitization in the unpaired environmental context; drug exposure initiates an excitatory associative process which facilitates the expression of locomotor sensitization in the paired environmental context. In their experiments, rats received repeated injections of amphetamine or saline in group-specific environments. Following these treatments some groups were given an electroconvulsive shock when memories of the drug experience were reactivated (and therefore vulnerable to disruption) in order to produce retrograde amnesia. Their results have shown that the electroconvulsive shock affects the expression of sensitization in unpaired animals but not in paired animals. The experiment conducted by the group of Anagnostaras[7] is also considered a reconsolidation study, which showed that electroconvulsive shock after reactivation could disrupt the inhibitory associative effect, whereas it has no effect on locomotor sensitization caused by non-associative effect. However, their results have also indicated that the electroconvulsive shock after reactivation could not disrupt the excitatory associative effect, which is in contrast to our results. We have confirmed that the association between drug and context can be disrupted by blocking the reconsolidation process, whereas the non-association of neuroplasticity induced by intermittent drug treatment is not simultaneously disrupted by the same intervention. These results imply a dissociated memory mechanism between conditioned hyperactivity and locomotor sensitization.

Third, the behavioral activating effects of addictive drugs appear to be mediated by their actions on mesotelencephalic and related circuitry, especially dopaminergic projections to the striatum originating from the ventral tegmental area and substantia nigra, and glutamate projections originating from the neocortex[45–49]. Moreover, there are corresponding persistent presynaptic and postsynaptic changes in monoamine and glutamate neurotransmission in the striatum of sensitized animals[8,47,50], which may be related to persistent changes in the morphology of neurons in the nucleus accumbens and prefrontal cortex[51,52]. McDonald and White[53] postulated a triple memory system which included the hippocampal formation, the amygdala and the dorsal striatum. Within this account, the hippocampus is held to be responsible for the acquisition and retrieval of declarative memory and stimulus-stimulus associations. The amygdala system is believed to mediate Pavlovian associations between stimuli and contingencies, both reinforcing and aversive. The dorsal striatum mediates implicit, dopamine-modulated habit-based learning. Based on this theory, the neural substrate of conditioned hyperactivity is thought to be involved in the amygdala system, whereas locomotor sensitization appears to be mediated by mesotelencephalic dopamine system. The separate neural substrates may contribute to the differential effects of propranolol on conditioned hyperactivity and locomotor sensitization.

Bernardi et al[32] have noted that rats given anisomycin immediately after a reactivation session show decreased activity as compared to the saline group in response to a low-dose of cocaine challenge. Carrera et al[54] have also shown that a single post-conditioning trial treatment with a low dose of apomorphine could reverse apomorphine-induced locomotor sensitization in paired group, using a conventional paired/unpaired Pavlovian protocol. However, these reports are different from the current study in two important aspects: first, those studies did not distinguish the associative and non-associative effects in the challenge test, and thus it is not clear which component experiences memory reconsolidation; second, the conditioning session usually consists of multiple drug-context pairing in a typical locomotor sensitization paradigm and one-shot procedure induced sensitization used by previous studies might have different sensitization magnitude from the typical intermittent administration procedure. According to the current results, it is suggested that propranolol could not interact with the reconsolidation process of locomotor sensitization, which still needs to be confirmed.

It should be admitted that there were already differences in the response to the conditioned context in morphine-treated groups after previous propranolol/context exposure on days 10–11, so when they were re-exposed to the context on days 18–19, the precise associations reactivated by this context/morphine exposure might be different, which precluded drug associated memory to go into labile state. This may be another reason for the failure of propranolol to disrupt morphine sensitization. Unexpectedly, although a drug-priming reactivation and propranolol treatment did not disrupt the locomotor sensitization, a small dose of morphine injection and propranolol intervention enhanced the locomotor sensitization effect. It appears that a delayed interaction between propranolol and morphine enhanced the locomotor sensitization effect of morphine. Within the mesocorticolimbic dopamine system, several interactions between dopamine and noradrenaline transmission have been described that may underlie the effect of propranolol on the psychomotor effect of psychostimulant. Harris et al[55] reported that dopamine levels in the accumbens increased by an average of 700% over baseline levels in the presence of combined cocaine and propranolol. Vanderschuren et al[56] have found that propranolol enhanced the psychomotor stimulant effect of amphetamine and cocaine. However, the present study shows that propranolol is critical for the long term sensitizing effects of morphine. Because of the limited literature, it remains unclear by which mechanism propranolol interacts with morphine to enhance the sensitizing effects of morphine.

In conclusion, this study indicates that the conditioned hyperactivity caused by environmental cues associated with morphine treatment or by injection of saline can be erased by administration of propranolol after retrieval of related memory, which lasts for a much longer time. However, the morphine's sensitization effects induced by intermittent morphine administration cannot be blocked by disrupting the reconsolidation process. The results of the study also support propranolol as a useful pharmacological tool for blocking reconsolidation of drug-associated memories.

Methods

All experimental procedures were carried out in accordance with the 1996 National Institutes of Health Guide for the Care and Use of Laboratory Animals and the experimental procedures were approved by the Local Committee of Animal Use and Protection.

Animals. Adult male Wistar rats (200–220 g, Academy of Military Medical Science Animal Center, Beijing, China) were housed in standard lab Plexiglas cages (45 × 30 × 25 cm, length × width × height, 3 rats/cage) in a weather-controlled ventilated colony room on a 12-h-light/12-h-dark cycle (experiments were conducted during the light period) with free access to water and food in the home cages.

Drugs. Morphine hydrochloride (Shenyang First Pharmaceutical Factory, Shenyang, China) and propranolol (Sigma-Aldrich, St. Louis, MO) were dissolved with saline and injected intraperitoneally (i.p.) at a volume of 1 ml/kg.

Apparatus. Locomotor activity was measured by an automated video tracking system with four customer-made activity chambers as described previously (Li et al., 2010). The chambers were made of black Perspex plastics (40 × 40 × 50 cm, length × width × height). A video camera was mounted at the top of the chamber, which was connected to a PC to record the locomotion of rats. The video documents (stored in the computer) were analyzed by the LA analysis software (Institute of Psychology, Chinese Academy of Sciences, Beijing, China). The locomotor activity was expressed as the total distance traveled for a predetermined period of time.

To increase the salience of the CS, as described in Li et al. (2010), 1.5 ml of 50% acetic acid dropped on absorbent cotton served as the CS and was replaced daily immediately before the session started. The cotton was held in a porous metal container and put in the top corner of the chamber out of the reach of rats.

Behavioral experiments. The acquisition of conditioned hyperactivity and behavioral sensitization sessions has been described in detail previously[6] with modifications. Briefly, 41 rats were randomly assigned into one of four groups (see Table 1). These sessions were conducted during seven consecutive days (Day 1–7). On each conditioning day, all rats were put into the locomotion chambers for 20 min (paired with CS) with an injection of morphine (5 mg/kg) or saline afterwards. The locomotor activity was measured for the following 120 min. All groups were maintained in their home cages without any drug treatment during Day 8–9. The dose of morphine used in this study (5 mg/kg) was chosen based upon a previous report[6] and our pilot studies (data not shown) which revealed that this dose of morphine produced the most robust hyperactivity and locomotor sensitization.

Two days after the conditioning sessions, rats were transported into the locomotion chambers for a 20-min re-exposure trial on Day 10. This re-exposure manipulation served as a CS retrieval trial intended to reactivate the association between morphine and CS in rats given morphine during training sessions. Immediately after re-exposure, rats were administered with propranolol (10 mg/kg) or saline and placed back into their home cages. The non-reactivation control group received propranolol in home cages. The dose of propranolol used in this study (10 mg/kg) was chosen based upon previous reports[25,31]. In order to effectively disrupt the reconsolidation process, the CS re-exposure trial and amnestic intervention were repeated on Day 11. All groups were remained undisturbed in their home cages during Day 12–13.

On Day 14, the conditioned hyperactivity of rats was first tested for 20 min in locomotion chambers. To fully model the CS, rats were then given an injection of saline and their locomotion was recorded for another 60-min in a drug-free state[35,36]. On Day 15, all rats received an injection of morphine (3 mg/kg) and the locomotor activity was measured for 120 min to serve as locomotor sensitization test.

Two days after locomotor sensitization test, on Day 18, rats were transported into the locomotor cages for 30 min following an injection of morphine (2 mg/kg, i.p.), which served as a CS + US retrieval trial. Immediately after re-exposure, rats were administered propranolol (10 mg/kg) or saline and placed back into home cages. The CS + US re-exposure trial and amnestic intervention were repeated on Day 19. The non-reactivation control group received propranolol in home cages. All groups were remained undisturbed in their home cages during Days 20–21. On Day 22, all rats were given a second morphine (3 mg/kg) challenge test.

Data analyses. Two-way mixed factorial ANOVAs were performed on the data with the between-subjects factors of group and within-subjects factors of day for the conditioning and reactivation/intervention sessions. When a significant effect of group versus day interaction was recorded, one-way ANOVA was used to analyze the differences in the conditioned hyperactivity and locomotor sensitization among different groups. Post hoc analyses (bonferroni test) were performed for assessing specific group comparison wherever indicated by ANOVA results (with $p < 0.05$). The behavioral data obtained from the conditioning test and sensitization test were analyzed using a one-way ANOVA. Wherever indicated by the ANOVA results (with $p < 0.05$), possible differences among groups were analyzed by bonferroni test. The data were expressed as means ± SEM. The levels of statistical significance were set at $p < 0.05$.

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