SCIENCE CHINA
Life Sciences

• RESEARCH PAPERS •

November 2010 Vol.53 No.11: 1356–1360
doi: 10.1007/s11427-010-4077-2

Chronic morphine treatment decreases acoustic startle response and prepulse inhibition in rats

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Received November 14, 2009; accepted February 3, 2010

The reward-related effects of addictive drugs primarily act via the dopamine system, which also plays an important role in sensorimotor gating. The mesolimbic dopamine system is the common pathway of drug addiction and sensorimotor gating. However, the way in which addictive drugs affect sensorimotor gating is currently unclear. In previous studies, we examined the effects of morphine treatment on sensory gating in the hippocampus. The present study investigated the effects of morphine on sensorimotor gating in rats during chronic morphine treatment and withdrawal. Rats were examined during treatment with morphine for 10 successive days, followed by a withdrawal period. Acoustic startle responses to a single startle stimulus (115 dB SPL) and prepulse inhibition responses were recorded. The results showed that acoustic startle responses were attenuated during morphine treatment, but not during withdrawal. PPI was impaired in the last 2 morphine treatment days, but returned to a normal level during withdrawal.

morphine treatment, sensorimotor gating, startle response, prepulse inhibition

Citation: Meng Z Q, Zhou D M, Wang J H, *et al*. Chronic morphine treatment decreases acoustic startle response and prepulse inhibition in rats. Sci China Life Sci, 2010, 53: 1356−1360, doi: 10.1007/s11427-010-4077-2

Repeated treatment with morphine is associated with changes in brain structure and function. Studies of the neural basis of addiction have shown that the mesolimbic dopamine (DA) system projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) and the medial prefrontal cortex (mPFC) is involved in the neural mechanisms of drug reward and reinforcement [1–3]. In addition to these dopamine-related brain areas, addictive drugs also affect dopamine neurons in other areas, including the striatum, amygdala, and ventral pallidum [2].

The startle reflex is a reflexive response to an abrupt or intense stimulus for a defensive purpose. This behavior exists across a wide range of species, from rodents to humans. The acoustic startle response (ASR) is a type of startle reflex that is exhibited in response to intense audio stimuli. ASR is typically suppressed when an intense startling stimulus is preceded by a weak stimulus (a prestimulus). This effect is called prepulse inhibition (PPI) [4–6]. PPI reflects the function of sensorimotor gating. Some psychiatric disorders such as schizophrenia show impaired sensorimotor gating function, which is expressed as a reduced PPI [7–9]. Previous studies in rats revealed that PPI is regulated by DA neural circuits between the limbic cortex (including the temporal and medial prefrontal cortex), the ventral striatum, ventral pallidum and the pontine tegmentum [10]. Thus, the neural substrates regulating PPI appear to overlap substantially with the brain circuitry involved in drug addiction. This neural overlap involved is of interest to researchers studying the neural interaction between drug addiction and sensorimotor gating. One study reported that acute

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nicotine administration increased sensory gating in rats [11]. However, chronic cocaine administration was found to have no lasting effects on PPI in rats. This inconsistency may be related to differences in the molecular mechanisms underlying the action of different addictive drugs [1].

Our previous studies revealed that acute morphine treatment in rats significantly attenuated sensory gating ability, manifested as N40 suppression. Furthermore, gating function was found to gradually return to normal after several days of morphine withdrawal [14–16]. However, how chronic morphine treatment affects sensorimotor gating function is still unknown. The purpose of the present study was to investigate the possible modulating effects of chronic morphine treatment on sensorimotor gating during a lengthy period of morphine administration and withdrawal.

1 Materials and methods

1.1 Animals

16 adult male Sprague-Dawley rats weighing 280–320 g were obtained from a breeding center at Kunming Medical College (Kunming, China). Rats were housed in a room with an alternating 12-h light/12-h dark cycle, and a temperature of around 25°C. Food and water were available ad libitum. Rats were regularly handled for one week before the experimental test to reduce the stress response. All experiments were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 86–23, revised 1985).

1.2 Morphine administration and startle response measures

Startle measures were performed with a four-unit automated acoustic startle response-testing instrument. Each unit contained a small plexiglass cylinder (10 cm in diameter and 28 cm in length) fixed on a platform under which a sensitive sensor was attached. Stimulus presentation and startle response signal-sampling were conducted using a PC in another room. During experiments, rats were restricted within the cylinders, which were placed into a cabinet with 65 dB SPL white background noise. Acoustic stimuli were delivered through a speaker above the cylinders. Startle measures were conducted between 9∶00 AM and 12∶00 AM on each experimental recording day.

In the test session, rats were first placed into the testing cylinder for a 5 min acclimation period, during which rats received no stimuli but background noise. The test session then began, with 10 trials of single 20 ms 115 dB SPL startle stimuli followed by 50 training trials, consisting of 10 trials of 115 dB SPL pulse stimuli, 10 trials of blank stimuli (NOSTIM), and 30 trials of prepulse startle stimuli. Prepulse stimuli included a 20 ms 115 dB SPL pulse, preceded by three types of 20 ms non-startling stimuli (PP70, PP75 and PP80). The interval between prepulse stimuli and startle stimuli was 100 ms, and all stimuli were randomly delivered within a group of test trials. Inter-trial intervals (ITI) were randomly assigned to a time between 27 and 32 s. Percentage of PPI was calculated as (startle response to the 115 dB SPL startle stimuli–response to pulses with the prepulse)/startle response to the 115 dB SPL startle stimuli×100.

Rats were tested with the startle response and prepulse inhibition protocol for 2 successive days before morphine administration. Thus, they were adapted to the testing procedures, and these results were used as baseline. All rats were randomly assigned into the morphine treatment group and the saline treatment group based on the baseline. From day 3 to day 12, the morphine group (n=8) were injected with morphine (10 mg kg^{-1}, i.p.) twice daily. The interval between the two morphine injections was at least 6 h. 30 min after each morphine injection, the startle response and PPI were tested. From day 13 to day 19, there was no morphine administration, and spontaneous withdrawal was experienced for 7 d. The startle response was tested once daily during the withdrawal period. The control group (n=8) was treated in an identical way except they were injected with saline instead of morphine.

1.3 Statistical analysis

Acoustic startle response data are presented as mean±SE of the mean. Data were analyzed with a two-way repeated analysis of variance (ANOVA) in SPSS 13.0. Treatment difference was used as a between-subjects factor, and test day as a within-subjects factor. Differences were considered significant at P<0.05 and extremely significant at P<0.01.

2 Results

2.1 Baseline startle response

No differences were found either in the responses to a 115 dB SPL startle stimulus or in the responses to all three types of prepulse (PP70, PP75 and PP80) startle stimuli during the baseline test. Startle responses and prepulse inhibition are shown in Figure 1 (A1 and A2 indicate baseline adaptation days 1 and 2).

2.2 Startle response during morphine treatment

In the morphine treatment group, responses to the startle pulse tended to be attenuated compared with the saline treatment group during 10 morphine treatment days. However, the reduction in startle response of the morphine treatment group was only significant on the 3rd day, compared with the saline treatment group (F(1, 14)=4.81, P=0.046< 0.05). Startle responses and prepulse inhibition are shown in

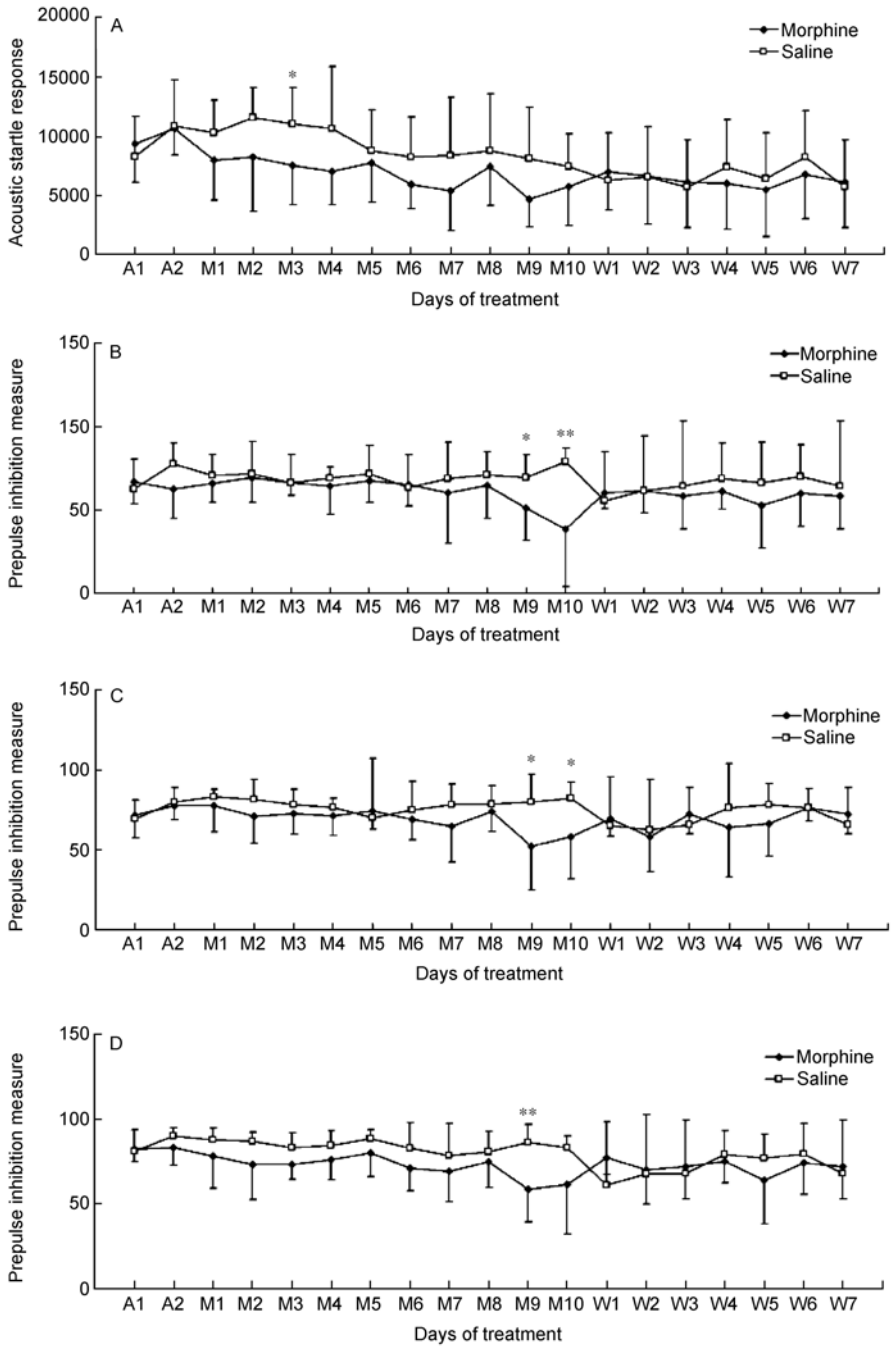

Figure 1 The effects of morphine administration on acoustic startle response (ASR) and prepulse inhibition (PPI). A, Startle response to the sole startle stimulus in morphine (10 mg kg^{-1}) and saline treatment rats (—◆—, morphine treatment group, n=8; —□—, saline treatment group, n=8). B, Response to the prepulse stimulus of 70 dB SPL plus startle stimulus (PPI) in morphine (10 mg kg^{-1}) and saline treatment rats. C, Response to the prepulse stimulus of 75 dB SPL plus startle stimulus (PPI) in morphine (10 mg kg^{-1}) and saline treatment rats. D, Response to the prepulse stimulus of 80 dB SPL plus startle stimulus (PPI) in morphine (10 mg kg^{-1}) and saline treatment rats. Data are presented as mean±SE and were recorded at 30 min after injections. A1 and A2 represent startles in 2 successive days of baseline adaptation tests. M1–M10 represent startles in tests of 30 min after morphine administration days 1–10. W1–W7 represent startles during withdrawal days 1–7. *, P<0.05; **, P<0.01 by two-way ANOVA.

Figure 1 (M1–M10 indicate morphine treatment on days 1–10).

Responses to PP70, PP75 and PP80 prepulse startle stimuli were not significantly different between the morphine treatment and saline treatment group from M1 to M8. However, on M9 and M10, morphine treatment significantly attenuated prepulse relative to saline treatment. For PP70 prepulse inhibition on day 9, F(1, 14)=4.88, P<0.05;

on day 10, $F(1, 14)=10.12$, $P<0.01$. For PP75 prepulse inhibition on day 9, $F(1, 14)=5.73$, $P<0.05$; on day 10, $F(1, 14)=5.95$, $P<0.05$. For PP80 prepulse inhibition on day 9, $F(1, 14)=12.65$, $P<0.01$. No significant difference was found on day 10, $F(1, 14)=4.22$, $P=0.059$.

2.3 Startle response during morphine withdrawal

No significant differences were found either in the responses to startle stimuli or in responses to any of the three types of prepulse (PP70, PP75 and PP80) startle stimuli during the withdrawal period. Data are shown in Figure 1 (W1–W7 indicate morphine withdrawal days 1–7).

3 Discussion

Morphine treatment had an acute effect on the startle response to a single startle stimulus after 30 min of morphine injection. Specifically, morphine had a tendency to attenuate the startle response over 10 d of morphine treatment. On the 3rd day, the startle response was significantly attenuated compared with the saline treatment group. During the withdrawal period, the startle response in the morphine group was reinstalled to the normal level.

Morphine did not have an acute effect on PPI, at any of the three levels of prepulse stimuli (PP70, PP75 and PP80) during the first 8 morphine treatment days. Some previous studies reported that morphine had little influence on PPI [12]. However, we found a tendency toward attenuated ASR and PPI related to morphine administration, demonstrating that morphine had acute effects. On the last 2 morphine treatment days, PPI was impaired by morphine treatment and quickly reinstalled on the 1st day of the withdrawal period. This result indicates that morphine had an accumulating and chronic modulatory effect on PPI. This finding supports the notion that the DA system is changed by chronic morphine treatment. In addition, repeated morphine administration may cause sensitization, enhancing the effects of the drug [13].

We speculated that the dose of morphine we used (10 mg kg^{-1}, twice daily) was high enough to affect PPI neural circuitry. Although one of our previous studies used a dose of 30 mg kg^{-1} twice daily, another used a dose of 10 mg kg^{-1} once daily. In these earlier studies, sensory gating of N40 suppression in rats was found to be reduced on the 1st day of morphine treatment, an effect that lasted several days during morphine withdrawal [14–16]. It is possible that this finding is related to sensory gating of the N40 being more sensitive to morphine exposure compared with sensorimotor gating.

The current results indicate that there is substantial overlap between neural circuitry involved in regulating reinforcement and withdrawal symptoms, and neural mechanisms that modulate PPI. Previous evidence indicates that several brain areas are involved in both processes, including the prefrontal cortex, portions of the extended amygdala, central amygdala, and the bed nucleus of the stria terminalis and ventral pallidum [17–20]. Thus, one potential explanation for our results is that morphine-induced drug dependence and behavioral traits related to craving have a delayed effect because of neural sensitization. The sensorimotor gating deficits we observed in the present study may have been caused by increased DA levels. Morphine administration can enhance the DA level in related brain areas, and some previous research demonstrated that apomorphine disrupted PPI and ASR through the D1, D2 and D4 receptor [21–22]. Considering the almost instant modulating effect on N40 suppression, it is difficult for morphine administration to affect sensorimotor gating. More research is necessary to investigate the dynamic changes in sensory and sensorimotor gating with simultaneous recordings of auditory evoked potential paradigms and PPI paradigms during morphine treatment and withdrawal phases.

4 Conclusion

Overall, our results revealed that the startle response and prepulse inhibition in rats were attenuated in the late phase during a lengthy period of morphine treatment, but returned to the normal level during morphine withdrawal. Future studies are required to elucidate the underlying mechanisms of this attenuation, and to examine the effects of other addictive drugs on these neural processes.

This work was supported by the National Natural Science Foundation of China (*Grant Nos. 30470553, 30770700 and 30530270*), *National High-Tech Research and Development Program of China* (*Grant No. O7013810*), *Major State Basic Research Development Program of China* (*Grant Nos. 2005CB522803 and 2007CB947703*) *and Yunnan Science and Technology Program* (*Grant No. 2006PT08-2*).

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