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Electroacupuncture Facilitates Recovery of Male Sexual Behavior in Morphine Withdrawal Rats

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The effect of electroacupuncture (EA) on the sexual behavior of male rats undergoing morphine withdrawal was studied by measuring various parameters of sexual behavior. In addition, the total serum testosterone (TST) concentrations in male rats at different times of morphine administration and abstinence were measured. Acute and chronic administration of morphine severely inhibited the sexual behavior of the rats and lowered their TST concentrations. TST concentrations recovered to normal within 24 h after the last morphine injection, while sexual behavior remained suppressed for at least 7 days. Electroacupuncture (2/100 Hz alternately) administered once daily for 7 days during morphine withdrawal facilitated the recovery of male sexual behavior and increased TST concentrations to above normal. The effect of EA on sexual behavior may involve both neuronal and hormonal pathways.

KEY WORDS: Morphine dependent/withdrawal; sexual behavior; total serum testosterone; electroacupuncture.

INTRODUCTION

Long-term opiate abuse has long been known to adversely affect male sexual behavior, even after abstinence (1–3). Sexual inability can cause feelings of inadequacy and depression and may be a social stressor that elicits relapse to drug abuse (4). Sexual activity does not appear to provoke reinstatement of drug-abusing behavior (5), suggesting that it is a beneficial rather than a harmful factor for rehabilitation from opioid addiction. Our recent clinical observations showed that former heroin addicts treated with 2/100-Hz transcutaneous electrical stimulation had a better recovery of sexual function, accompanied by a significant increase in serum luteinizing hormone (LH) and total serum testosterone (TST) concentrations (6). The purpose of the present study was to confirm our clinical observation in rats serving as an appropriate animal model to assist in unveiling the possible underlying mechanisms.

EXPERIMENTAL PROCEDURE

Care of the Animals. All experiments were performed on male Sprague-Dawley rats (obtained from the Institute of Animal Research, Chinese Academy of Science, Beijing, China), weighing 230–250 g at the beginning of the experiment. They were housed six per cage, with the room temperature maintained at 21 ± 3°C, relative humidity at 50%, under a natural light/dark cycle. The Committee on Animal Care and Use of the Peking University approved the experimental procedures.

Induction of Experimental Morphine-Dependent Rats. Rats were given morphine hydrochloride (obtained from Qinghai Pharmaceutical Factory, China) dissolved in normal saline to a final concentration of 20 mg/ml. It was administered intraperitoneally twice a day

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(10:00–10:30 AM and 10:00–10:30 PM) for 4 weeks, according to the following schedule (7): 12.5 mg/kg per day on days 1 and 2, 25 mg/kg per day on days 3 and 4, 50 mg/kg per day on days 5 and 6, and maintaining 100 mg/kg per day to the end. Control rats received the same volume of normal saline.

Preparative and Test Procedures for Blood Samples. Blood (0.5 ml) was taken from the caudal vein of each rat. Serum was extracted and tested by enzyme immunoassay (EIA). The EIA kit for TST (DSL-10-4000) was purchased from Diagnostic Systems Laboratories (San Antonio, TX) and had a sensitivity of 0.04 ng/ml. The cross reaction with corticosterone was less than 0.1%.

Evaluating Procedures for Sexual Behavior in the Rat. Male rats were prescreened before the experiment, and only those that performed ejaculation within 30 min were used in subsequent experiments. Screening and testing were conducted under dim red light during the dark portion (11:00 PM–2:00 AM) of the light/dark cycle. Copulatory behavior was recorded by video camera and then viewed on a monitor by experienced observers blind to the experimental arrangement. For both screening and testing, individual male rats were placed in a carton box (60 × 50 × 40 cm) with pine wood shaving bedding and allowed a 5-min adaptation period. A receptive female rat was then introduced. The following measurements were taken: mount latency (ML), intromission latency (IL), and the ejaculation latency (EL). The test ended immediately after the rat completed its first ejaculation within 30 min, or when one of the following standards was met: (i) the rat did not perform the first mount in 5 min; (ii) the rat did not perform its first intromission in 15 min; (iii) the rat did not complete its first ejaculation in 30 min. Receptive female rats were induced via subcutaneous injections of 10 μg of estradiol benzoate 48 h and 500 μg progesterone 5 h (both dissolved in olive oil), per rat, before testing. Only those females demonstrating a high level of receptive behavior were used.

EA Stimulation. During EA, rats were kept in cylindrical plastic holders, with their hind legs and tails protruding. Two stainless steel needles 0.3 mm in diameter were inserted 5 mm into each hind leg, one in the acupoint ST36, 5 mm lateral to the anterior tubercle of the tibia, and the other in SP6, 3 mm proximal to the medial malleolus, at the posterior border of the tibia (Fig. 1). Constant current square wave electric stimulation produced by a HANS LH-800 programmed pulse generator (manufactured by Beijing University of Astronautics and Aeronautics, China) was given via the two needles for a total of 30 min. The frequency of stimulation used was 2/100 Hz (2 Hz alternating automatically with 100 Hz, each lasting for 3 s; with pulse width 0.6 ms at 2 Hz and 0.2 ms at 100 Hz). The intensity of the stimulation was increased stepwise from 1 mA to 2 mA and 3 mA, each lasting for 10 min [for details see (8)].

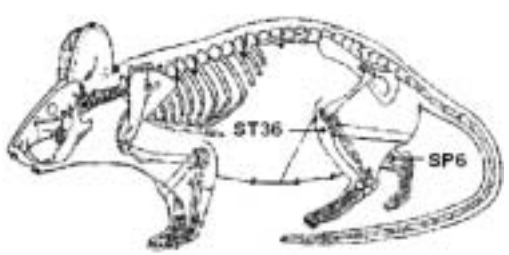

Fig. 1. Sketch map indicating the location of acupoints (ST36 and SP6) on the hind legs of the rat for the application of electroacupuncture (EA) stimulation.

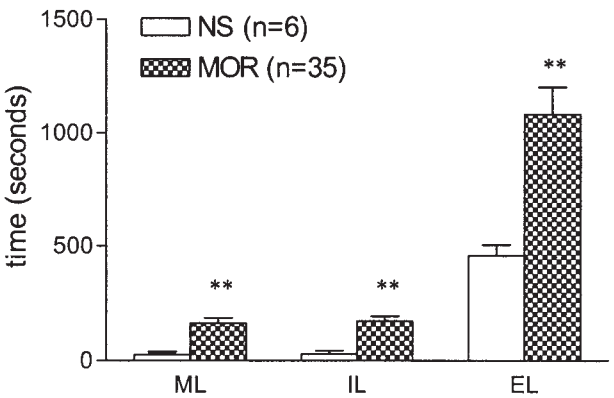

Fig. 2. Effect of chronic morphine administration on the sexual behavior of male rats. MOR, Chronic morphine administration group; NS, normal saline control group. **P < .01. ML, Mount latency; IL, intromission latency; EL, ejaculation latency. Numbers in parentheses indicate the number of animals in each group (N).

RESULTS

Forty-one prescreened sexually active male rats were randomly divided into two groups: chronic morphine-treated group (MOR, N = 35), and normal saline control group (NS, N = 6). Sexual behavior was tested 2 and 24 h after the last morphine/saline injection. As shown in Fig. 2, in the drugged state (2 h after the last injection), the ML, IL, and EL of morphine-dependent rats were significantly prolonged compared with those of the NS control rats (P < .01), indicating depressed sexual activity. During acute drug withdrawal (24 h after the last injection), sexual behavior of the morphine-dependent group was inhibited compared with that of the control group (P < .05, Fig. 3).

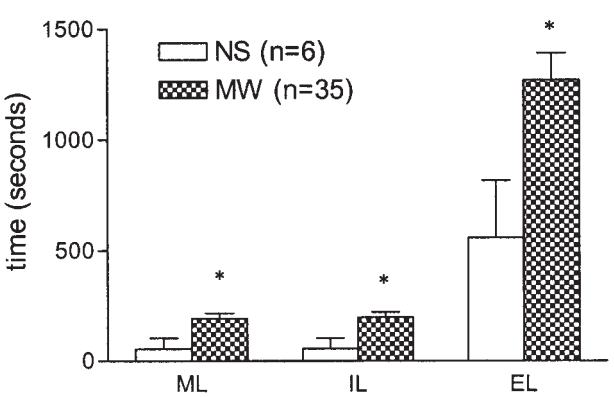

Fig. 3. Effect of acute morphine withdrawal on the sexual behavior of male rats. MW, Acute morphine withdrawal group; NS, normal saline control group. *P < .05. ML, Mount latency; IL, intromission latency; EL, ejaculation latency. Numbers in parentheses indicate the number of animals in each group (N).

There were 21 morphine withdrawal (MW) rats that did not complete ejaculation within 30 min. They were randomly divided into two groups. One group (EA group, N = 11) received EA 30 min per day for 7 days starting from the second day after the last morphine injection. The other group (mock-EA group, N = 10) was put in the holder for 30 min without EA. As shown in Figure 4, on the 7th day after the last morphine administration, the EL of rats in the mock-EA group remained at the same level without any recovery ($P < .05$), which was still significantly longer than that of the NS group ($P < .05$). In contrast, the EL of the EA-treated rats became shorter, showing no significant difference compared with that of the NS control group.

Another 30 male rats were randomly divided into two groups: chronic morphine administration group (MOR N = 20) and normal saline control group (NS, N = 10). They received MOR/NS injections as described in the previous experiment. Blood samples were collected (11:00–12:00 PM if not specified elsewhere) for TST measurement 2 h after the first injection (acute morphine administration), 2 h after the last injection (chronic morphine administration), and 24 h after the last injection (acute morphine withdrawal), respectively. Both acute and chronic morphine administration reduced TST to a significantly lower concentration (<0.10 ng/ml and 0.13 ± 0.014 ng/ml, respectively) compared with the control group (0.32 ± 0.076 ng/ml and 0.86 ± 0.21 ng/ml, respectively), $P < .01$ (Fig. 5A and 5B). Unlike the results of the behavior test, TST returned quickly to normal during acute morphine withdrawal (0.64 ± 0.16 ng/ml, Fig. 5C). From the second day of abstinence, the 20 morphine-treated rats were randomly divided into two groups. One group (EA) received EA while the other (mock-EA) did not, as described before. Seven days later, the TST level of the EA group (3.16 ± 0.51 ng/ml) was significantly higher than that of the mock-EA group ($P < .05$) and NS group ($P < .01$), respectively (Fig. 5D), suggesting a stimulating effect of EA on TST during morphine withdrawal.

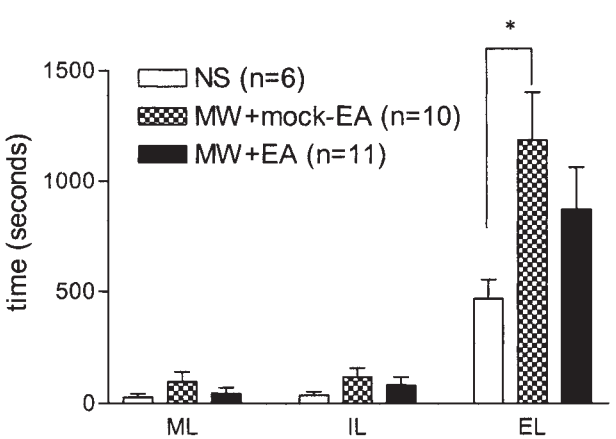

Fig. 4. Effect of EA on the sexual behavior of rats undergoing morphine withdrawal. NS, normal saline control group; MW+ mock-EA, rats undergoing morphine withdrawal restrained in the holder, without EA; MW+EA, rats undergoing morphine withdrawal receiving EA, *$P < .05$. ML, Mount latency; IL, intromission latency; EL, ejaculation latency. Numbers in parentheses indicate the number of animals in each group (N).

DISCUSSION

It has been well documented that chronic use of opioid drugs reduces male sexual behavior (9). The data reported here show that morphine administration for 4 weeks dramatically prolonged the ML, IL, and EL of male rats, which is consistent with early reports. Some studies on changes in sexual behavior under morphine withdrawal have produced conflicting results. Sexual behavior was either normal (7,10,11) or inhibited (3). The different outcomes of such morphine withdrawal experiments may be due to variations in the drug dosage, length of drug, abusing history, and individual sensitivity to opioid drugs. A rebound of sexual behavior should be expected when functional inhibition of the hypothalamus-pituitary-gonadal (HPG) axis is produced without visible histological changes. In cases in which severe organ atrophy occurs after long-term abuse of drugs, significant suppression of sexual behavior might be expected even after the drug is withdrawn. In our experiment, 21 out of 35 (60%) rats displayed diminished sexual behavior (no ejaculation within 30 min) 24 h after the last morphine injection. The sexual behavior of the remainder (14/35) was indistinguishable from that of normal saline controls (data not shown), and no rebound was observed.

It is interesting that our results also indicated a facilitating effect of EA on sexual behavior in rats undergoing morphine withdrawal. The EL of the EA-treated rats was shortened, showing no significant difference compared with that of normal rats, whereas in the rats without EA (mock-EA group), the EL remained elevated 7 days after the last morphine injection. To study the mechanism involved in this facilitating effect, we measured TST, because it is one of the major terminal products of the HPG axis and thus can be used as an index to evaluate the activity of the whole axis. In addition, gonadal hormones have been shown to regulate the ability of copulation in rodents and influence sexual motivation in

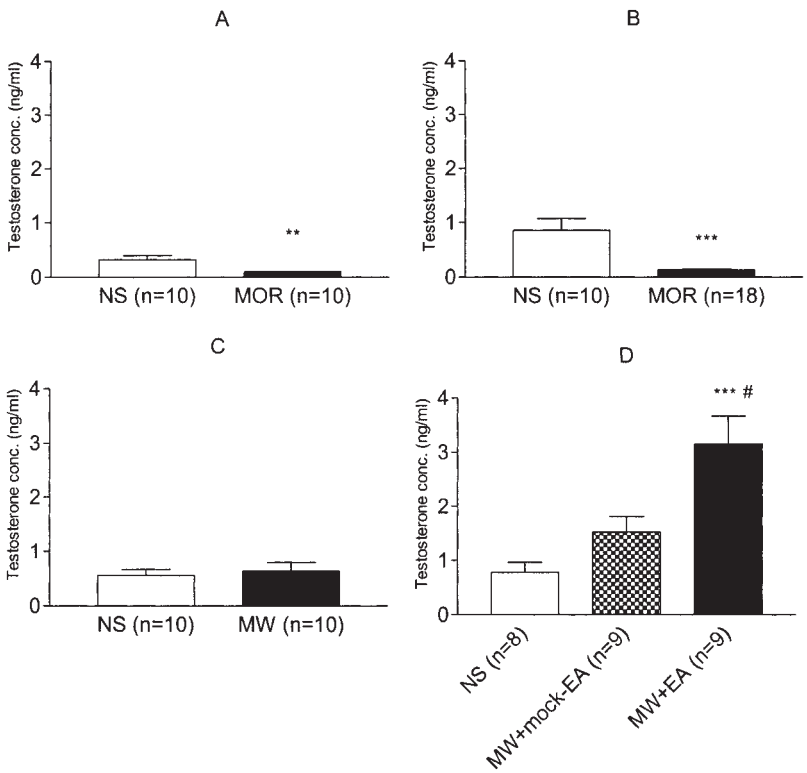

Fig. 5. Total serum testosterone concentrations during morphine administration, during morphine abstinence, and after EA. A, Acute morphine administration. B, Chronic morphine administration. C, acute morphine withdrawal. D, Effect of EA. ***$P < .001$ compared with NS. $^{\#}P < .05$, compared to mock-EA. NS, Normal saline control group; MW+mock-EA, rats undergoing morphine withdrawal restrained in the holder, without EA; MW+EA, rats undergoing morphine withdrawal receiving EA. *$P < .05$. ML, Mount latency; IL, intromission latency; EL, ejaculation latency. Numbers in parentheses indicate the number of animals in each group (N).

primates (12). Our data indicate that in addition to the impaired sexual behavior, the TST concentration was significantly lowered by both acute and chronic morphine administration. An unexpected finding was the rapid recovery of TST within 24 h of the last morphine injection in contrast to the long-term inhibition of sexual behavior, which appears to have weakened the correlation between testosterone concentrations and sexual behavior. However, this discrepancy may be resolved if we also consider the impairment induced by morphine on the target organs of testosterone. Thus our results suggest that after long-term use of opioid drugs, the target organs of testosterone might also suffer some degree of impairment resulting in their poor response to testosterone. In such circumstances, normalizing TST alone was not sufficient to increase sexual behavior. In contrast, EA, by increasing the TST to a higher than normal concentration, may partially compensate the lowered responsiveness of those target organs and lead to a recovery of sexual behavior.

However, increasing the TST alone cannot explain the effect of EA. It is known that EA can stimulate sexual behavior in castrated rats (13–15). Therefore it is likely that other pathways in the central nervous system (CNS) are involved. Early studies on EA have demonstrated that it is able to modulate various neural pathways in the CNS (16). For example, it was reported that EA induces GnRH release from the ventromedial hypothalamus in female rats (17). So it is possible that the facilitatory effect of EA might be mediated by a similar pathway. The medical preoptic area (MPOA) of hypothalamus has been shown to play a key role in controlling sexual behavior (18–20). Testosterone was also suggested to promote copulation by upregulating nitric oxide synthase in the MPOA, which in turn enhances dopamine release (21). All of these could serve as possible target sites for EA.

CONCLUSION

Based on the data we present in this paper and results of similar earlier work, we speculate that EA accelerated the recovery of sexual behavior of rats

undergoing morphine withdrawal by both increasing the peripheral sexual hormone concentration and modulating central neural pathways. EA has recently been shown to be effective in the treatment of heroin addiction (16,22). This study provides evidence that EA also facilitates the recovery of depressed sexual behavior, which in turn may help rehabilitation of former opioid addicts to resume a normal life.

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