PHARMACOLOGY AND TOXICOLOGY

Changed Morphine Sensitivity of Morphine-Dependent Rats after Laser Exposure of the Cerebral Prefrontal Cortex

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We studied the effect of bilateral laser exposure of the cerebral prefrontal cortex in morphine-dependent rats on their sensitivity to positive reinforcing and toxic effect of morphine. Laser exposure led to partial deafferentation of the cingulum. Exposure of the anterior and central areas of the prefrontal cortex stimulated self-administration of low morphine doses and had no effect on self-administration of high concentrations. Exposure of the posterior area of the prefrontal cortex did not appreciably change self-administration of medium and low morphine doses, but self-administration of high doses significantly increased. Exposure of the anterior area of the prefrontal cortex significantly decreased the sensitivity to the positive reinforcing effect of morphine. Exposure of the posterior prefrontal cortex decreased the sensitivity to the toxic effect of morphine.

Key Words: *morphine; intravenous self-administration; prefrontal cortex; cingulate gyrus*

Two structural and functional systems of the brain are involved in the formation of opioid dependence. One of them includes the paragiant-cell nucleus, solitary tract nucleus, and locus coeruleus [4], the other, mesocorticolimbic dopamine system, consists of neurons of the ventral tegmentum and brain areas, where terminals of these neurons are located, including nucleus accumbens and prefrontal cortex [3,8]. The prefrontal cortex is a heterogeneous structure including limbic structures of the cortex, in particular the cingulate gyrus.

Brain tomography in heroin addicts showed decreased metabolism of neurons in the cingulate gyrus. On the other hand, their activity increases after acute injection of opiates [2]. Cingulotomy in patients with opium narcomania suppresses the use of narcotic [5,7,9]. This fact and the decrease of pathological craving after cingulotomy, presumably due to suppression of the obsessive-compulsive component of this craving, suppression of memory traces of narcotic use and reduction of emotional reaction to stimuli associated with narcotics, substantiate neurosurgical treatment of opiate narcomania adopted in some Russian clinics. This treatment consists in cryo- or laser destruction of the anterior cingulate gyrus. However, there are no data on the effect of cingulotomy on opiate sensitivity in opiate addicts.

We studied the effect of bilateral laser exposure of the cerebrocortical prefrontal zones of morphine-dependent rats on their sensitivity to positive support and toxic action of morphine.

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MATERIALS AND METHODS

Experiments were carried out on 40 male Wistar rats weighing 180-200 g at the beginning of the experiment. The rats (8-10 animals per cage) were kept at artificial (12 h) illumination with free access to water and standard combined fodder.

During stage I of the study physical dependence on morphine was formed in rats. To this end, they were injected with morphine hydrochloride in doses from 10 to 35 mg/kg for 5 days twice a day at 12-h intervals, after which the rats received daily injections of 35 mg/kg until the start of self-administration procedure. On day 6, two-component synthetic catheters were implanted (under ketamine narcosis, 100 mg/kg) through the jugular vein into the vena cava superior. The intravenous part of the catheter was a silicone tube (Dow Corning Corp.) with external diameter of 1.2 mm, 25 mm long. The rest of the catheter was a vinyl tube (Dural Plastic and Engineering) with an outer diameter of 1.0 mm, 55 mm long, connected to the intravenous part with a special adapter (Small Parts Inc.) at one end and fixed to the skin on the back of the neck on the other.

After recovery period (7 days), during which the rats were kept in individual boxes and were daily injected with morphine (35 mg/kg), they were placed (50 min) into experimental boxes (Lafayette Instruments Inc.). Free end of the implanted catheter was connected through a liquid rotating contact to a precise dose pump (Harvard Apparatus). After the rat pressed the lever in the box, the pump was activated via computer software and 1 mg morphine dissolved in 50 μl isotonic NaCl was infused into the vena cava superior through the catheter. The injection was followed by a 17-sec latent period, during which lever pressing did not lead to new injections. The latent period was paralleled by light switching off in the experimental box. During the first 3-5 days the rats received morphine injection after one lever pressing, during the next 2 days after three, and during the next 3 days after 5 lever pressings. Training was over after about 10 days, when all rats formed a stable habit of intravenous self-administration of morphine. On days 10, 11, and 12 the rats in experimental box received 0.5, 1.0, or 1.5 mg morphine, respectively, after pressing the lever 5 times. The procedure was repeated during the next 3 days. On day 16 the animals were narcotized with ketamine, the skull was scalped, a hole was drilled, and a rigid optical fiberglass lightguide (20 μ) was stereotactically inserted into the anterior cingulate gyrus in accordance with the coordinates given in the Atlas [6]: A=1.0, L=0.5, H=2.5 (8 rats); P=1.0, L=0.5 (8 rats); H=2.5 or AP=0, L=0.5, H=2.5 (6 rats) first on one and then on the other side. The lightguide was connected to the ALTO diode laser and 0.3 W radiation was conducted for 10 sec. On days 17, 18, and 19 stability of morphine self-administration (1 mg/injection) was tested. On days 20, 21, and 22 the rats received 0.5, 1.0, or 1.5 mg morphine, respectively, after pressing the lever 5 times. On days 23, 24, and 25 the last procedure was repeated. The rats were then decapitated, the brain was removed for morphological study of the consequences of laser exposure. The morphology of the destroyed region was studied on serial 20-μ sections.

The results of self-administration of different morphine doses before and after laser exposure of the prefrontal cortical structures were compared. The differences were considered significant at $p<0.05$, the t test for unpaired variables was used.

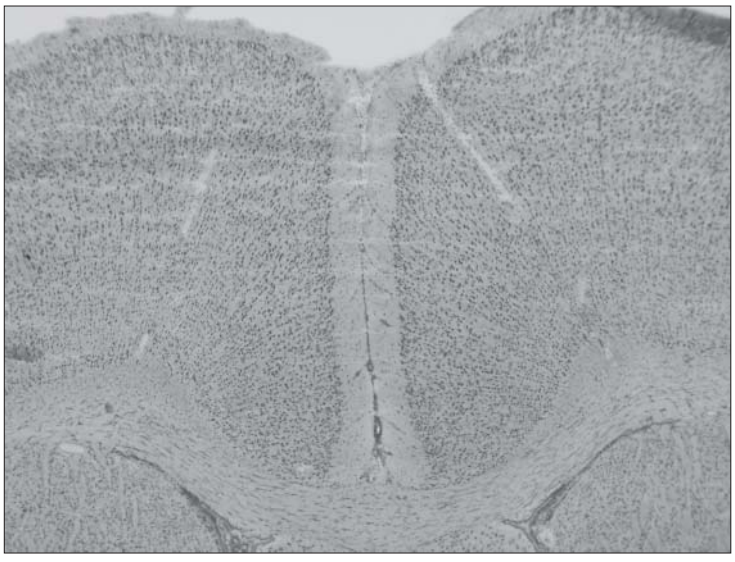

Fig. 1. Morphological changes in rat prefrontal cortex after laser exposure.

RESULTS

Laser exposure of the brain (on average of 2 mm downward and laterally, along the cingulate gyrus) led to tissue destruction, death of neurons, and disruption of neuronal connections. The cingulate gyrus looked intact (Fig. 1).

Laser exposure changed the number of intravenous self-administrations of morphine. Exposure of the anterior and central areas of the prefrontal cortex increased the number of morphine self-administrations. Before exposure the animals made 3.9±0.3 self-infusions of morphine per session, after exposure 6.0±0.5 self-infusions. The dose/number of infusions curve became more abrupt, that is, the number of self-administrations of low doses increased significantly, while the number of self-administrations of high concentrations of morphine remained unchanged (Figs. 2, 3). Laser exposure of the posterior areas of the prefrontal cortex did not lead to appreciable changes in the number of self-administrations of medium and low doses of morphine, while the number of self-administrations of high doses increased significantly. Injecting 2 mg morphine per infusion, the rats self-injected up to 70 mg/kg per session, which more than 2-fold surpassed the control values and led to the state of deep narcotic intoxication (Figs. 2, 3).

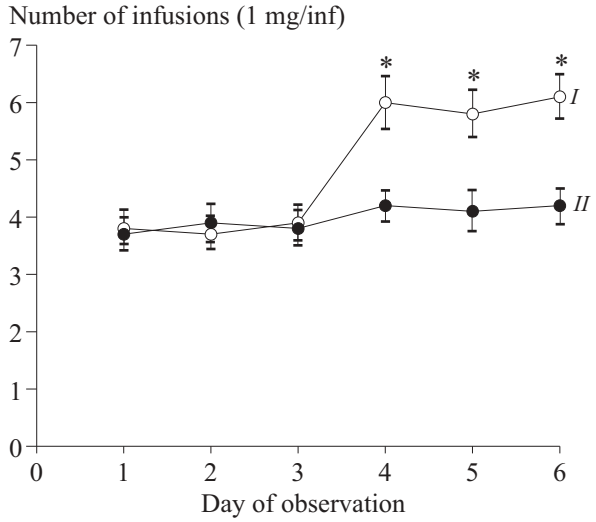

Fig. 2. Daily self-administrations of morphine before (1, 2, 3) and after (4, 5, 6) exposure of the anterior and central (*I*) and posterior (*II*) rat prefrontal cortex. Ordinate: number of infusions. *$p<0.05$ compared to this parameter before exposure.

Hence, laser exposure of the anterior and central areas of the prefrontal cortex seems to lead to partial deafferentation of the cingulate gyrus and caused a decrease in the sensitivity to the positive reinforcing effect of morphine. Exposure of the posterior prefrontal cortex led to a decrease in the sensitivity to the toxic effect of morphine. These data indicate an important role of the prefrontal cortex in the formation and development of pathological craving for opiates, underlying the formation of opiate dependence. Destruction of the anterior cingulate gyrus really led to suppression of formation of physical dependence from morphine [1].

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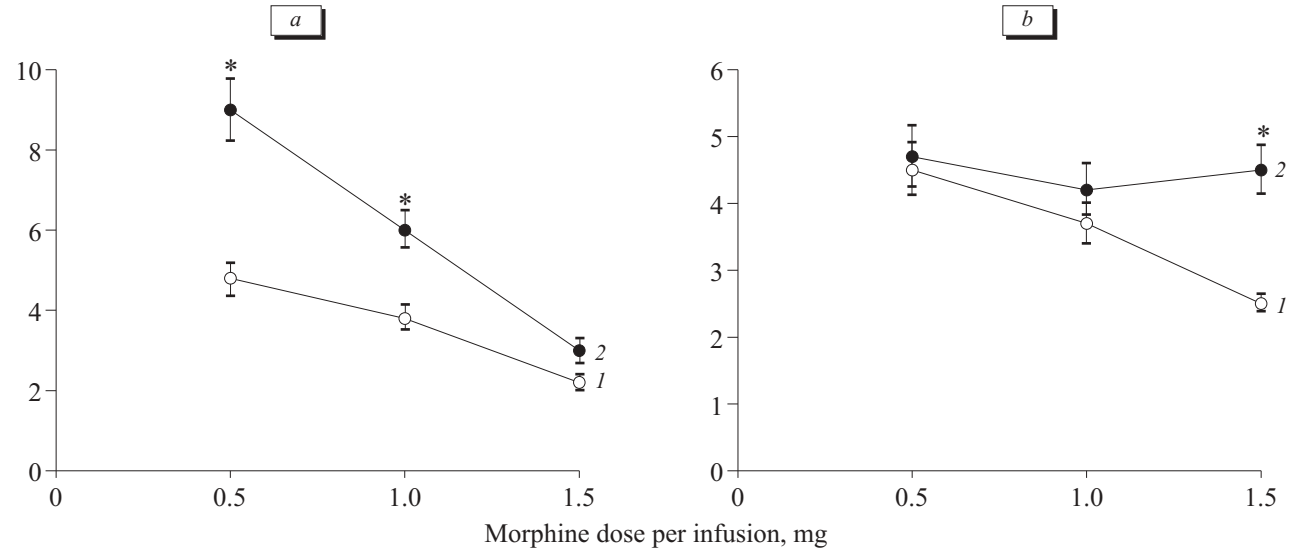

Fig. 3. Number of morphine infusions self-injected by rats before (*1*) and after (*2*) exposure of the anterior and central (*a*) and posterior (*b*) frontal cortex. *$p<0.05$.

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