Neural Correlates of the Reinstatement of Heroin-Seeking Behavior in Rats by fMRI

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Introduction. Relapse to compulsive drug-seeking behavior long after detoxification is a major problem in the treatment of drug addiction. In human addicts, re-exposure to an addictive drug, a stressful event or drug-associated environmental cues often induce or intensify drug craving and precipitate relapse even after prolonged period of abstinence. Similarly, an extinguished drug-seeking behavior can be triggered in laboratory animals by a priming injection of an addictive drug, acute foot-shock stress or by exposure to drug-associated cues. This reinstatement of drug-seeking behavior in experimental animals has been widely accepted as a relapse model for study of the neural mechanisms underlying drug craving and relapse in humans (1). It is generally believed that brain reward systems are involved in both drug reward and relapse. However, there are two opposing views on the role of brain reward systems in mediating drug craving and relapse. One hypothesis suggests that relapse is triggered by drug-like action in brain reward systems, which may become sensitized to addictive drugs (2). Another contrasting hypothesis was proposed, that relapse is evoked by paradoxical processes such as dysphoria or physical withdrawal responses (3). The present fMRI study was designed to test the hypothesis that reinstatement of heroin-seeking behavior in laboratory animals has neural correlates that are enduring functional alterations that are precipitated during repeated heroin self-administration (SA). Such knowledge could be important for us to understand the neural mechanism underlying drug craving and relapse.

Materials and Methods. *Animal Preparation:* Thirty male Sprague-Dawley rats weighing between 300-350 g at the start of the experiment were housed and maintained individually on a 12/12h light/dark cycle (lights on at 8:00 p.m.) with free access to food and water. All behavioral procedures were performed during the dark phase. *Surgery:* Under sodium pentobarbital anesthesia (60 mg/kg ip.), the rats were implanted with a chronic silicone rubber jugular catheter that passed subcutaneously to terminate on a head assembly. Seven days were allowed for recovery from surgery before SA training. The catheter in the jugular vein was maintained by daily injections of saline. *Heroin-SA procedure and behavior test:* *Apparatus:* The self-administration chambers had two levers located 5 cm above the floor, but only one lever (an active, retractable lever) activated the infusion pump. Presses on the other lever were also recorded. The modified 22-gauge cannulae were connected to liquid swivels with PE-50 tubing that was protected by metal springs. The swivels were connected to syringes attached to the infusion pumps. The SA boxes were controlled by a Med Associates system. *Procedures:* The experiment included SA training, withdrawal and reinstatement testing phases. During the SA training phase, rats were trained for three hours per day to intravenously self-administer saline (0.1 ml/injection) or heroin (0.1 mg/kg/injection) under a fixed ratio-2 (FR2) schedule of reinforcement. After regular SA was achieved, all of the rats underwent the withdrawal phase in the home cages of the colony room for 8-9 days. Then, each group of rats was divided into two subgroups for reinstatement testing and fMRI scanning, respectively. During the reinstatement testing phase, rats were given an initial extinction session for three hours, during which rats were re-exposed to the same SA chambers, and the drug-associated contextual cues-induced reinstatement of drug-seeking behavior (lever presses) was recorded. When the extinction criterion (≤10% of responses on the active lever per hour) was reached during the last 1-2 hours of the extinction session, each rat in both the saline and heroin withdrawal groups received a priming injection of heroin (0.1 mg/kg, iv) and the subsequent reinstating response of lever presses was recorded during another three-hour session. *fMRI experiments:* fMRI experiments were performed on a Bruker Medspec 3T/60cm scanner using a custom-built, 2-inch long, 1.5-inch-diameter RF birdcage volume coil, inserted into an in-house-made cylindrical local gradient coil. A single-shot, gradient echo EPI sequence was used for functional imaging with FOV=3.5 cm, slice thickness=2 mm, image matrix=64 x 64, giving an in-plane image resolution of 550 x 550 µm, TR=1 sec, TE=27.2 ms, and bandwidth=±62.5 kHz. Each rat was scanned for 30 min. A heroin (0.1 mg/kg) challenge was administered intravenously 10 min into the 30 min scan. *Data analysis:* BOLD-weighted voxel time courses were fitted with a DiffExp model using the AFNI. Voxels were considered significantly activated with a threshold F test ≥ 6.8 ($P < 0.05$ after Bonferroni correction). Nine regions of interest were defined from the four slices (interaural 11.2 mm – 5.2 mm): prefrontal cortex, cingulate gyrus, nucleus accumbens, olfactory tubercle, caudate putamen, parietal cortex, hypothalamus, thalamus and hippocampus. A student's T test was employed for statistical analysis of behavioral tests and fMRI results. To compare the differences in fMRI results between the heroin-SA group and saline control group, two parameters were employed – the number of activated voxels and the percentage change in area under the curve (AUC%) of those activated voxels.

Results. When the rats were re-exposed in the same SA chambers after 8-9 days of heroin withdrawal, the rats with a heroin-SA history displayed an immediate, burst-like non-reinforced drug-seeking (lever-presses) behavior within the initial 30 min, which was gradually extinguished. Following this initial extinction phase, an acute heroin (0.1 mg/kg, iv) priming injection also triggered remarkable reinstatement of the extinguished heroin-seeking behavior (Fig. 1). In contrast, no such a drug-seeking behavior was observed in the saline-SA rats. The absolute intensities (AUC%) of these positive and negative BOLD signals in heroin-SA rats were significantly lower than those in the saline control rats on the four coronal slices of the rat brain (Fig. 2a). The numbers of activated voxels in the prefrontal cortex, parietal cortex, and nucleus accumbens regions were about 80% less in the heroin-SA rats compared with controls (Fig. 2b).

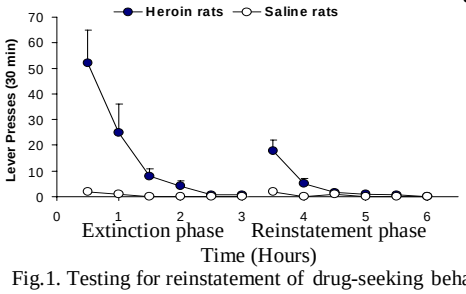
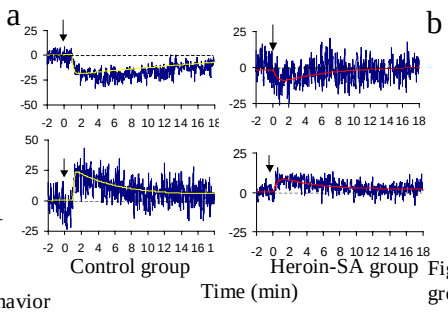


Fig.1. Testing for reinstatement of drug-seeking behavior



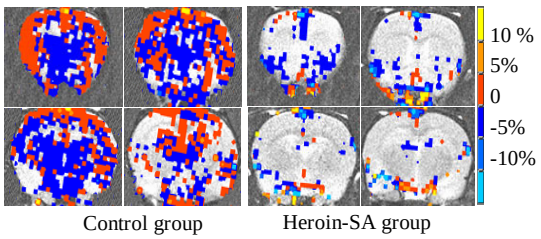


Fig.2. a: DiffExp fitting on time course of control and heroin-SA group, respectively. b: Priming-induced activation map of control and heroin-SA group, respectively.

Discussion. The major finding of this study is that contextual, environmental cues-induced or heroin priming-induced reinstatement of drug-seeking behavior 8-9 days after heroin withdrawal coincides with long-term desensitization in heroin-induced functional activation or inhibition in distinct brain regions. This suggests that the relapse of drug-seeking behavior observed in heroin-dependent subjects may be associated with this persistent reduction in opiate actions, as shown in this rat brain model. In general, opiate addicts usually require escalating doses of the drug to produce the same levels of the subjective effects. This progressive increases in drug intake, or decrease in drug effect, for the same dose of the drug is called tolerance or desensitization. Termination of high-dose frequent use usually causes severe opiate withdrawal symptoms, which may lead to drug craving and relapse. The mechanism underlying the dysphoria during opiate withdrawal has been thought to be related to a hypofunctional status of brain reward systems (4). The present fMRI study provides direct evidence in support of this hypothesis by demonstrating a remarkable reduction in heroin-induced brain activity in specific brain regions after prolonged heroin withdrawal. Our observation is also consistent with findings that cocaine-dependent human subjects showed less DA response to methylphenidate challenge than that of in drug naive subjects detected by PET (5). This study suggests that chronic opiate SA produces enduring neuroadaptation or desensitization in opiate receptor signaling. As such, reinstatement of drug-seeking behavior may represent a compensatory response or motivated effort to achieve the same subjective experienced previous by increasing drug intake.

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