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Lesion of olfactory epithelium attenuates expression of morphine-induced behavioral sensitization and reinstatement of drug-primed conditioned place preference in mice

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ABSTRACT

Previous studies have shown that olfactory impairment by disrupting the olfactory epithelium prior to morphine administration attenuated the development addiction-related behaviors. However, it is unclear whether olfactory impairment will affect the expression of already established addiction-related behaviors. To address this issue, mice were conditioned with morphine to induce behavioral sensitization and condition placed preference (CPP). After an abstinence period, the animals were subjected to either an intranasal $ZnSO_4$ effusion (ZnE) or sham treatment with saline. Behavioral sensitization and CPP reinstatement were evaluated 24 h later, as well as the expression of c-Fos protein, a marker of activated neural sites, in brain regions of interest. It was found that ZnE treatment attenuated morphine-induced behavioral sensitization and reinstatement of CPP. Compared to the saline-treated ones, the ZnE-treated animals showed reduced c-Fos expression in the nucleus accumbens (NAc) associated with behavioral sensitization, and in the NAc, cingulate cortex, dentate gyrus, amygdala, lateral hypothalamus and ventral tegmental area associated with CPP reinstatement. Together, these results demonstrated that acute olfactory impairment could attenuate already established addiction-related behaviors and expression of c-Fos in drug addiction related brain regions, perhaps by affecting the coordination between reward and motivational systems in the brain.

1. Introduction

Drug addiction is primarily characterized by uncontrollable drug-seeking behaviors and chronic drug administration (Steketee and Kalivas, 2011). Drug addiction is also known to be associated with dysfunction of many brain systems, including the memory, control and motivational systems (Gladwin et al., 2011; Hyman, 2005). Brain dysfunction may contribute to the high rates of relapse in addicted individuals, even after long periods of abstinence are achieved (Dijkstra et al., 2007).

Behavioral sensitization and conditioned place preference (CPP) are commonly used paradigms to study addiction/relapse behaviors. Behavioral sensitization measures increase in the activating effects of a drug following repeated administration (Cadoni et al., 2001); and CPP measures the ability to pair a drug with environmental cues (Aguilar et al., 2009). Both behavioral sensitization and CPP can be reinstated after the extinction of drug related training with the presentation of addiction-related stimuli, such as a physical drug injection and/or drug-related cues that include paraphernalia, drug-associated odors and sounds, drug availability, or drug-using partners (Biala et al., 2009; Epstein et al., 2006).

Olfaction is known to play an important role in many cognitive processes, such as learning and memory, social interactions, development of kinship between individuals, and/or nourishment seeking (Sanchez-Andrade and Kendrick, 2009). Importantly, olfaction has also been implicated in addiction. For example, our previous studies found that lesion of the olfactory epithelium prior to morphine treatment attenuated the development of drug-related behavioral sensitization and CPP (Niu et al., 2012). Bulbectomy was shown to disrupt the expression of cocaine-induced place preference (Calcagnetti et al., 1996). Olfactory priming following an extinction procedure has been shown to reactivate the place preference in a food-induced CPP paradigm (La Mela et al., 2010).

In the present study, we aimed to investigate whether acute olfactory deprivation following the development of addiction-related behaviors has an effect on the increased motivational reactivity of the animals to drugs and/or drug-associated environmental cues that are associated

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with relapse into drug abuse following abstinence. Mice were subjected to repeated morphine injections, and developed addiction-related behaviors (i.e., as assessed by the CPP paradigm). After a period of abstinence to extinct the addiction-related behaviors, the animals were subjected to intranasal effusion of either $ZnSO_4$ or saline. The ZnE-treated animals showed impaired olfaction, as demonstrated by an olfactory discrimination test. Reinstatement of morphine-induced behavioral sensitization and CPP was measured 24 h later. C-Fos immunohistochemistry was used to investigate the possible neural mechanisms underlying the modulatory effects of olfaction on the reinstatement of morphine-induced behavioral sensitization and CPP.

2. Materials and methods

2.1. Animals

Male Institute of Cancer Research (ICR) mice, weighing 20–25 g and 8 weeks of age at the beginning of the experiment, were purchased from Animal Center, Kunming Medical College, Kunming, China, and housed in plastic cages with 8 mice per cage and food and water available ad libitum. Room temperature was maintained at 25 °C under a 12-hour light/dark cycle with white lights on from 08:00 to 20:00 h. Mice were gently handled twice a day (at 09:00 and 15:00) during the first week after arrival.

2.2. Drugs

Zinc sulfate was purchased from Zhejiang Xianju Pharmaceuticals Company (Zhejiang, China), and morphine hydrochloride from the Shenyang No.1 Pharmaceutical Company Limited (Shenyang, China).

2.3. Olfactory deprivation

Mice were subjected to intranasal $ZnSO_4$ (ZnE) or saline effusion (SalE) in an abdomen-up position under ketamine anesthesia (80 mg/kg). Each nostril of the animal was injected with 20 μl of sterile 10% $ZnSO_4$ solution or physiological saline (0.9%, w/v) with a 20-μl microsyringe fitted with a polished blunt end.

2.4. Recording of locomotor activity

For each session, locomotor behaviors of the animal were recorded continuously for 15 min in a wooden open-field test apparatus (35 cm × 35 cm × 30 cm) by an automatic tracking system equipped with a computer-monitored infrared motion detector (The Shenzhen Jiameikang Technology Co. Ltd., Shenzhen, China). The total distance traveled within the 15-min recording period was calculated and used as a measure for locomotor activity (Chefer and Shippenberg, 2009; Cordonnier et al., 2007).

2.5. CPP test

The CPP apparatus consisted of two testing wooden chambers of equal size (30.7 cm × 31.5 cm × 34.5 cm) that were separated by a corridor chamber (7 cm × 12 cm × 34.5 cm) having a guillotine door on each side. The two testing chambers had different visual features (horizontal or vertical black and white/black stripes at equal intervals) and floor textures that were smooth or rough. During the expression test, the animal was placed in the corridor chamber with the guillotine doors on both sides open, and allowed to explore the entire apparatus for 15 min (Popik et al., 2006). A digital camera was suspended from the center of the ceiling to track and record the activities of the mouse in the apparatus. The time of the animal spending in the three chambers was analyzed manually offline. The preference score was calculated using the following formula: time spent in the drug-paired chamber/(time spent in the drug-paired chamber + time spent in the non-drug-paired chamber). To minimize cross-animal contamination, 75% ethanol was used to fully clean the apparatus between subsequent tests with different animals.

2.6. Behavioral experiments

All behavioral tests were conducted from 09:00 to 15:00, and in accordance with the National Care and Use of Animals guidelines approved by the Chinese National Animal Research Authority and conformed to international guidelines for the ethical use of animals. Each behavioral experiment used an independent set of animals.

2.6.1. Experiment 1: olfactory discrimination test

An odor discrimination test was performed to verify the olfaction-depriving effects of ZnE (Luo et al., 2002; Malkesman et al., 2010). For each animal, the test was performed 24 h after the ZnE (n = 9) or SalE (n = 9) treatment. The mice were habituated to a cotton-tipped applicator infused with mineral oil inserting into their home cage 1 h before the test, and then transferred to a dimly-lit room for the discrimination test. Each animal was placed in a clean 24 cm × 14 cm × 13 cm plastic cage with a fresh layer of corn-cob bedding covered by a removable Plexiglass cover, on which a 1.25-cm-diameter hole was drilled for delivering odorants. The test consisted of three phases: 1) baseline: one exposure (3 min) to a cotton tip dipped in mineral oil (Shanghai Chemical Reagent, Shanghai, China), during which the time spent by the animal on sniffing was recorded; 2) habituation: an interval of 45 min during which no cotton tip was presented to the animal; 3) testing: exposure (3 min) to a cotton-tipped applicator infused with mineral oil solution of 15% phenethyl acetate (Aladdin, ID: 103-45-7, Shanghai, China). The chemical has a rose-like order. The time spent by the animal on sniffing during the 3-min exposure period was recorded. The entire test was recorded on a CCD camera, and the sniff duration was determined by two observers. The use of sniff duration to determine changes in olfactory function was validated by recent studies that showed sniffing was essential for acquisition of olfactory information (Kepecs et al., 2006).

2.6.2. Experiment 2: effects of ZnE on the expression of morphine-induced behavioral sensitization

A total of 64 mice were used for this experiment. The experiment to measure morphine-induced behavioral sensitization consisted of five phases: 1) adaptation, 2) conditioning, 3) abstinence, 4) nasal effusion and 5) expression. The timeline of the experiment is depicted in Fig. 2A.

2.6.2.1. Adaptation. Each mouse was allowed to explore the locomotor chamber freely for 60 min each day for 3 consecutive days. The locomotor activity of each animal measured 4 h after the last adaptation exploration was defined as the baseline (day 0).

2.6.2.2. Conditioning (days 1–4). After the adaptation period, the mice were randomly assigned into two groups (n = 32 for each group), which received a daily intraperitoneal injection of morphine (40 mg/kg, 0.1 ml) or the same amount of 0.9% saline, respectively, for four consecutive days (Cordonnier et al., 2007). On each day, the animal was placed in the open-field apparatus immediately after the injection, with its locomotor activities measured as described in Section 2.4.

2.6.2.3. Abstinence (days 5–27). All mice were subjected to a 23-day abstinence period, during which each animal was placed in the open-field apparatus for 15 min on each day, with no any other treatment given.

2.6.2.4. Nasal effusion (day 28). On day 28, half of the morphine-conditioned animals received ZnE treatment (n = 16), and the other