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Morphine-induced conditioned place preference in rhesus monkeys: Resistance to inactivation of insula and extinction

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ABSTRACT

Drug addicts experience strong craving episodes in response to drug-associated cues. Attenuating these responses using pharmacological or behavioral approaches could aid recovery from addiction. Cue-induced drug seeking can be modeled using the conditioned place preference procedure (CPP). Our previous work showed that conditioned place preference (CPP) can be induced by administration of increasing doses of morphine in rhesus monkeys. Here, we investigated whether expression of morphine-induced CPP can be attenuated by inhibiting activity of insular cortex or by repeated unreinforced exposures to the CPP test. The insula has been demonstrated to be involved in addiction to several drugs of abuse. To test its role in morphine CPP, bilateral cannulae were implanted into the insula in seven adult monkeys. The CPP was established using a biased apparatus by intramuscular injections of morphine at increasing doses (1.5, 3.0 and 4.5 mg/kg) for each monkey. After the monkeys established morphine CPP, their insulae were reversibly inactivated by bilateral microinjection with 5% lidocaine (40 μl) prior to the post-conditioning test (expression) of CPP using a within-subject design. The microinjections of lidocaine failed to affect CPP expression when compared to saline injections. We subsequently investigated morphine-associated memory during six episodes of CPP tests performed in these monkeys over the following 75.0 ± 0.2 months. While the preference score showed a declining trend with repeated testing, morphine-induced CPP was maintained even on the last test performed at 75 months post-conditioning. This observation indicated strong resistance of morphine-induced memories to extinction in rhesus monkeys. Although these data do not confirm involvement of insula in morphine-induced CPP, our observation that drug-associated memories can be maintained over six drug-free years following initial experience with morphine has important implications for treatment of drug addiction using extinction therapy.

1. Introduction

Drug addiction is a chronic, relapsing disease characterized by compulsive drug seeking and bouts of intense drug craving (De Vries & Shippenberg, 2002; Le Moal & Koob, 2007). Drug relapse can persist long after the cessation of drug use in humans (Hunt, 1971; Pickens et al., 2011; Robbins, Ersche, & Everitt, 2008). The high rate of relapse after detoxification is a primary clinical problem and constitutes a major challenge in the treatment of drug abuse (Aguilar, Rodriguez-Arias, & Minarro, 2009).

Conditioned place preference (CPP) is a widely used experimental protocol to assess the conditioned reinforcing properties of cues associated with drugs of abuse in laboratory animals (Borges, Duarte, Nogueira, & Barros, 2015; Portillo & Paredes, 2009; Vindenes, Handal, Ripel, Boix, & Morland, 2006; Wang et al., 2012). CPP is based on classical Pavlovian conditioning and is used to evaluate the rewarding effects of objects or experiences (Bardo

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& Bevins, 2000; Stephens et al., 2010). CPP can be established across different species including humans (Astur, Carew, & Deaton, 2014). Our previous work has shown that CPP can be induced by morphine treatment using a biased place conditioning paradigm in rhesus monkeys and lasts at least 15.3 ± 1.7 months (Wang et al., 2012). Pharmacological and behavioral approaches are being explored to treat drug addiction. Both approaches can be tested using CPP. In the present study we have assessed the ability of intracranial manipulations of insula as well as repeated exposure to drug-associated cues to decrease morphine-induced CPP.

The insula has been of recent particular interest in the study of drug addiction (Contreras, Ceric, & Torrealba, 2007; Garavan, 2010; Naqvi, Rudrauf, Damasio, & Bechara, 2007). The insula is a highly interconnected structure embedded deep in the brain that is subdivided into the anterior insula (AI) and posterior insula (PI) by the central insular sulcus in humans and rhesus monkeys (Paxinos, Huang, & Toga, 1999; Singer, Critchley, & Preuschoff, 2009). The insula is the central nervous system's hub for receiving, processing and integrating signals relevant for the body with external stimuli to affect ongoing motivated behavior (Cechetto & Saper, 1987; Craig, 2003; Naqvi & Bechara, 2010; Paulus & Stewart, 2014).

Studies using lesion approaches have shown that the insula has a crucial role in acquisition, consolidation, maintenance, and extinction of conditioned taste aversion (CTA) in rodents (Roman, Lin, & Reilly, 2009; Roman & Reilly, 2007; Slouzkey, Rosenblum, & Maroun, 2013; Stehberg & Simon, 2011). In addition, the insula can be involved in the processing and expression of drug-induced CPP. Damage to the human insula can lead to an abrupt and profound disruption of addiction to cigarette smoking (Naqvi et al., 2007). Electrical stimulation of the insula region attenuates nicotine-taking and nicotine-seeking behaviors in naïve male Long-Evans rats (Pushparaj et al., 2013). Bilateral injection of lidocaine, a reversible inhibitor of neuronal activity, into the rat insula, reverses amphetamine-induced CPP (Contreras et al., 2007). Intra-insular administration of muscarinic acetylcholine receptor antagonists and nitric oxide inhibitors attenuates morphine-induced CPP in rats (Ma et al., 2014; Wu et al., 2014).

Despite this evidence, no attempts have been made to test the role of the insula in the expression of morphine-induced CPP in non-human primates. In this study, we established CPP with increasing doses of morphine in seven rhesus monkeys. To investigate the role of insula in the expression of morphine-induced CPP in rhesus monkey, we reversibly inactivated insula by bilaterally injecting lidocaine and conducted CPP tests over 5–35 min after the injection. Previous work showed the effect of lidocaine as a reversible Na^+-channel blocker was greatest at 8 min after lidocaine infusion and may last for up to 30 min in the monkey cerebral cortex (Tehovnik & Sommer, 1997). Therefore, behavior in the 30 min following microinjection of lidocaine should reveal the effects of insula inactivation on the expression of morphine-induced CPP.

Following testing the role of insula, we assessed the sensitivity of morphine-induced CPP to repeated extinction sessions. Learning and memory systems are intimately involved in drug addiction (Robbins et al., 2008), and extinction therapy is a frequent approach to treat addicted individuals (Marlatt, 1990; Shalev, Grimm, & Shaham, 2002). The time scale of the extinction of drug-associated memory is important for drug addiction treatment. Previous experiments have shown that morphine-induced CPP is persistent over time and can be maintained for up to 12 weeks when rats are tested every 2 weeks or 6 weeks (Mueller, Perdikaris, & Stewart, 2002). Drug related memories are even stronger and longer in people, and the rate of drug relapse in humans is high (Fuchs, Lasseter, Ramirez, & Xie, 2008). A previous study from our group demonstrated that morphine-induced CPP can last 15 months. However, effects of repeated extinction sessions on morphine-induced CPP have not been previously assessed. To bridge this gap we conducted six sets of CPP tests over 75.0 ± 0.2 months following our initial experiment. Our results demonstrated a profound resistance of morphine-induced CPP to extinction.

2. Materials and methods

2.1. Animal

Adult rhesus monkeys (*Macaca mulatta*) weighing 6–10 kg from breeding colonies at the Kunming Institute of Zoology (KIZ) were used in these experiments. Seven monkeys underwent surgeries and were used to establish morphine-induced CPP (pre-CPP, and post-CPP at 0 months), six of these monkeys were involved in intra-insula injection experiment using a within-subject design, and five to six of these monkeys were progressed into the extinction experiment (post-CPP at 15.8 ± 0.8 months in six monkeys, 35.4 ± 0.9, 50.6 ± 2.4, 60.8 ± 2.0, and 75.0 ± 0.2 months in five monkeys).

Monkeys were housed singly in a temperature-controlled (21 ± 2 °C) animal facility and were maintained on a 12 h light/dark cycle (lights on from 7:00 to 19:00 h). Monkeys had free access to tap water and were punctually supplied food three times a day. Experiments were performed between 8:00 and 17:00 h. Monkeys selected were healthy at the beginning of the experiment and remained healthy through 6 years.

All experimental procedures involving animals were performed in accordance with the guidelines for the National Care and Use of Animals approved by National Animal Research Authority (NARA). All efforts were made to minimize animal suffering and to reduce the number of animals required.

2.2. Drugs

The morphine hydrochloride ($C_{17}H_{19}NO_3{\cdot}HCl{\cdot}3H_2O$) was obtained from Sheng Yang 1st Medical Company as a 10 mg/1 ml per ampule solution. Hydrochloric acidulated ketamine, lidocaine and atropine were sourced from the He Nan Run Hong Pharmaceutical Company. Sodium pentobarbital was from Shang Hai Westang BIO-TECH Company.

2.3. Surgery and stereotaxic localization

All surgical procedures were conducted on monkeys anesthetized with hydrochloric acidulated ketamine (10 mg/kg, i.m.) and maintained with sodium pentobarbital (20 mg/kg, i.m.). Atropine (0.05 mg/kg, i.m.) was used to reduce salivation and other secretions. Body temperature was maintained at normal levels using a heating pad during surgery. The monkey's head was fixed on the stereotaxic apparatus after anesthesia.

2.3.1. MRI scanning

After disinfection, cleaning and exposure of skull, four rigid glass tubes filled with vitamin AD were anchored to the monkey's skull as previously described (Jing et al., 2010). These four glass tubes functioned as a reference point in MR imaging and the skull to determine the three-dimensional coordinates of the AI and PI (Fig. 1) according to the monkey brain atlas (Paxinos et al., 1999). Applying this method, the error for implantation of microelectrodes into the brain of rhesus monkeys was reported to be less than 1 mm (Jing et al., 2010). After each monkey underwent MRI of the brain (GE, Signa Excite Twinspeed 1.5 T), all four glass tubes were removed and holes on the skull that anchored the tubes but did not go through the skull were used as markers of the next