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A new exposure model to evaluate smoked illicit drugs in rodents: A study of crack cocaine

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ABSTRACT

The use of smoked illicit drugs has spread dramatically, but few studies use proper devices to expose animals to inhalational abused drugs despite the availability of numerous smoking devices that mimic tobacco exposure in rodents. Therefore, the present study developed an inexpensive device to easily expose laboratory animals to smoked drugs. We used crack cocaine as the drug of abuse, and the cocaine plasma levels and the behaviors of animals intoxicated with the crack cocaine were evaluated to prove inhaled drug absorption and systemic activity. We developed an acrylic device with two chambers that were interconnected and separated by a hatch. Three doses of crack (100, 250, or 500 mg), which contained 63.7% cocaine, were burned in a pipe, and the rats were exposed to the smoke for 5 or 10 min (n = 5/amount/period). Exposure to the 250-mg dose for 10 min achieved cocaine plasma levels that were similar to those of users (170 ng/mL). Behavioral evaluations were also performed to validate the methodology. Rats (n = 10/group) for these evaluations were exposed to 250 mg of crack cocaine or air for 10 min, twice daily, for 28 consecutive days. Open-field evaluations were performed at three different periods throughout the experimental design. Exposed animals exhibited transient anorexia, increased motor activity, and shorter stays in central areas of the open field, which suggests reduced anxiety. Therefore, the developed model effectively exposed animals to crack cocaine, and this model may be useful for the investigation of other inhalational abused drugs.

1. Introduction

Many studies used animal models to evaluate the harmful effects of exposure to cigarette smoke and other related tobacco products, such as kretek cigars (Clark, 1990), narghiles, or water pipes (Miri-Moghaddam, Mirzaei, Arab, & Kaikha, 2014). These studies used different types of equipment, with greater or lesser degrees of sophistication and cost, to burn the tobacco and expose the animals to the smoke. However, the only factor these studies have in common is the wide availability of the raw material tobacco.

It is difficult to study the effects of animals exposed to illicit drugs through inhalation because of the amount of raw material that is required for these devices. Therefore, many laboratory animal studies of the effects of illicit drug exposure via the pulmonary route, such as marijuana, methamphetamine, and crack cocaine, are impaired for various reasons. Several reasons that hinder the performance of these studies include the legal difficulty in obtaining large amounts of illicit drugs, the ability to reproduce laboratory results worldwide, and the safety of the researchers when performing the experiment.

The use of illicit drugs, such as crack cocaine, methamphetamine, and new designer drugs (e.g., flakka), is a global public health concern. Several epidemiological studies focused on this issue, but most of these studies did not address multivariate or confounding factors that interfere with the purpose of the study, such as the concomitant use of other drugs (Bolla, Funderburk, & Cadet, 2000; Ferri, 1999), social stress (Mann & Bastos, 2015; Oteo Pérez, Benschop, Blanken, & Korf, 2015), and the health status of the user (Khalsa, Treisman, McCance-Katz, & Tedaldi, 2008). Therefore, the development of an easy and inexpensive laboratory animal exposure model for smoked drugs would provide a methodology to replicate laboratory conditions, increase our understanding of the toxic effects of inhaled drugs, and promote the development of therapeutic strategies for addiction.

Few studies addressed animal exposure to the smoke of burning drugs, despite robust results. The main problems of the existing apparatus include the complexity of drug burning and smoke exposure (Boni, Barr, & Martin, 1991; Burchfield, Abrams, Miller, & DeVane, 1991; He, Lidow, & Lidow, 2006) and the large amount of drug that is required to perform the experiment (Herculiani et al., 2009). Therefore, this

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study developed an inexpensive and easily assembled apparatus for smoke exposure of inhaled drugs that requires small drug amounts that are safe for the researcher. For this study, we used crack cocaine as the drug of abuse, which was legally obtained by judicial and police authorities. Cocaine plasma levels and the behavior of animals intoxicated with crack cocaine were evaluated to demonstrate drug absorption and systemic activity.

2. Materials and methods

2.1. Crack cocaine

Crack cocaine was obtained from drug trafficking products and was seized legally courtesy of the State Narcotic Division of São Paulo (DENARC-SP) pursuant to court order of the Hon. Judge Lucas Pereira Garciado from the Forum of Itapecerica da Serra, São Paulo (proc. Number: 007417-59.2012.8.26.0268). The crack rocks were tested for composition and contained 63.7% cocaine.

2.2. Ethics statement

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Committee on the Ethics of Animal Experiments of the School of Veterinary Medicine, University of São Paulo, Brazil approved the protocol (Permit Number: 2882/2013). All efforts were made to minimize animal suffering, reduce the number of animals used, and utilize alternatives to in vivo techniques when available. The experiments were performed in accordance with good laboratory practice protocols and quality assurance methods.

2.3. Animals

Male adult Wistar rats (10 weeks of age) were obtained from our own colony in the Department of Pathology, School of Veterinary Medicine and Animal Sciences, University of São Paulo. All animals were housed separately and received water and balanced rodent feed (Nuvilab CR1®) *ad libitum*. Cages were maintained under controlled conditions of temperature (22–24 °C), relative humidity (50–65%), and lighting (12 h/12 h light/dark cycle). All animals were checked daily until the end of the experimental period for clinical signs, soft feces, food waste, morbidity, and mortality. Food consumption and body weight gain were measured daily.

2.4. The model of exposure

The model comprises two chambers: the acrylic exposure chamber, where the smoke produced by burned crack cocaine outside of the chamber is aspirated under negative pressure (−70 mmHg) and confined; and an antechamber built with a polyvinyl chloride (PVC) tube, where the animals were placed before exposure. A connection lock between the chambers is attached to a door that is opened after the burning process to restore atmospheric pressure balance, and the animals move from the antechamber into the exposure chamber (Fig. 1).

The exposure chamber has the following internal dimensions, 34 × 21.5 × 22 cm (length × depth × height), and it was constructed of crystal acrylic walls (polymethyl methacrylate—12 mm thickness). All walls were glued using specific acrylic glue (methylmethacrylate based), and Phillips screws (25 × 3.5 mm) were used at strategic points of vulnerability where negative pressure was maximal. Two ½-inch ball valves were located at the top and side of the box, one valve for air outlet (connected to a vacuum pump) and the other valve to let in crack smoke (connected to the crack burner). A 4-in. round hole was created at one end of the exposure chamber to connect the antechamber.

The lock is very similar to a sliding door frame, which allows the door to run along its length and seal the display box bore. The acrylic plate port (12 mm) is 24 × 27 cm, and it is "sucked" against the outer wall of the exposure chamber when closed and subjected to a vacuum pressure to prevent leakage and the consequent loss of vacuum.

The antechamber was constructed of an opaque white PVC tube with a 4-in. diameter, which was coupled to the lock after the animals were placed inside. The crack smoke was confined to the exposure chamber after the completion of burning. The door is easily opened, and a displacement piston prods the animals (5 adult rats/period) to enter the exposure chamber. A transparent PET (polyethylene terephthalate) window (0.8 mm thickness) was placed on the tube side to provide better viewing of the animals before exposure.

2.5. The burner and burning

The burner was purchased from a store that specializes in the sale of products for smoking, water pipes, and other products. The burner is nothing more than a pipe adapted with two glass parts: one part is a

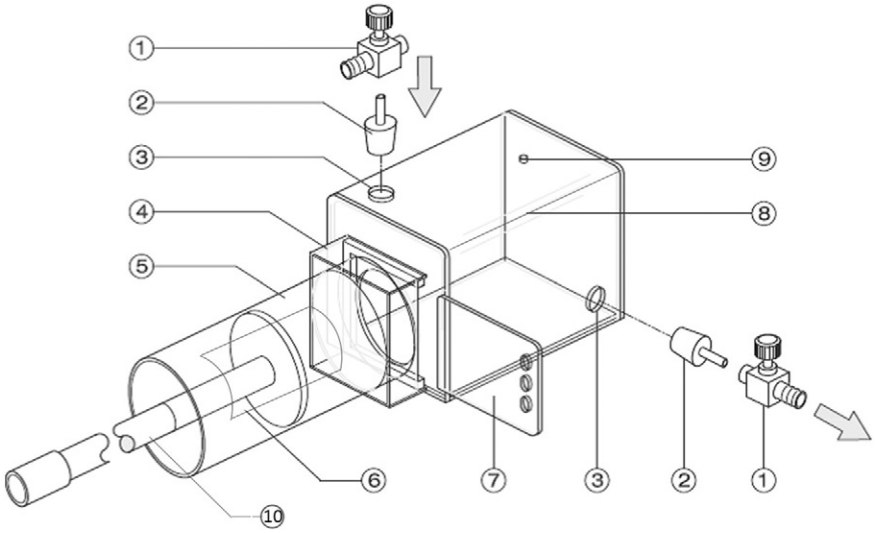

Fig. 1. The exposure model to evaluate smoked illicit drugs. An acrylic device with two chambers interconnected and separated by a door. 1) Half-inch ball valve; 2) valve adaptor; 3) opening for the adaptor; 4) sliding door frame; 5) antechamber where animals were placed before exposition; 6) polyethylene terephthalate (PET) window to see animals; 7) acrylic door 12 mm thickness; 8) exposure chamber of acrylic walls 12 mm thickness; 9) hole for use of probes; 10) piston to animal conduction.