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A Method for Exposing Primates to Marihuana Smoke That Simulates the Method Used by Human Marihuana Smokers

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PRYOR, G. T. AND C. S. REBERT. *A method for exposing primates to marihuana smoke that simulates the method used by human marihuana smokers*. PHARMACOL BIOCHEM BEHAV **34**(3) 521–525, 1989.—A method was developed for exposing rhesus monkeys to marihuana smoke under conditions simulating those used by human marihuana smokers. An ADL II smoking machine was used to generate puffs of smoke of constant volume from marihuana or placebo cigarettes at a constant rate. The outlet of the smoking machine was connected by Tygon tubing to an airtight face mask covering the monkey's nose and mouth. The monkey was seated in an airtight Plexiglas chamber with only its head protruding. When a puff of smoke was generated, a vacuum was imposed on the chamber causing the monkey to inhale deeply and hold its breath. After 6 seconds, the vacuum was removed and the monkey was allowed to exhale and breathe fresh air freely until the next puff. Delivery of smoke to the monkey by this method was more efficient than allowing the monkey to voluntarily inhale the smoke. The method was used in acute dose-effect experiments and, subsequently, in long-term experiments. It may be useful with other animal species and for efficiently delivering other airborne substances of abuse.

Rhesus monkeys Marihuana Forced inhalation of marihuana smoke
Blood Δ^9-THC and COHb saturation from marihuana smoke

IN order to study possible persisting behavioral, electrophysiologic, and neuropathologic consequences of chronic marihuana smoking in rhesus monkey, we sought a method for delivering the smoke that would be both efficient and reliable. Heath *et al.* (1) described such a method whereby a monkey was placed in an Isolette® infant respirator with its head protruding. A small mask that held the cigarette was connected to tubes and valves such that when the respirator was activated the monkey was forced to inhale the smoke deeply and hold its breath for a predetermined time. Between forced inhalations, the monkey was allowed to breathe normally. This method, although involuntary for the monkey, is essentially the same method used voluntarily by human smokers to achieve efficient delivery of the major psychoactive ingredient in marihuana (Δ^9-tetrahydrocannabinol; Δ^9-THC) with each puff.

Using the general concept described by Heath *et al.* (1), we designed and developed a similar system that is described in this report. Unlike the system of Heath *et al.* (1) in which the cigarette was positioned in the mask such that the monkey's inspiration drew in the smoke, we used an ADL-II smoking machine to generate a puff of smoke of constant volume. Higher blood levels of Δ^9-THC observed after forced inhalation, compared to the levels obtained when the monkeys were allowed to voluntarily breathe the artificially generated puffs of smoke, clearly demonstrated the increased efficiency of delivery of THC using the forced inhalation method.

METHOD

Subjects

Preliminary experiments were carried out with two juvenile male rhesus monkeys (8 kg, Hazelton Research Animals, Inc., Alice, TX) to determine the general feasibility of the method. The main experiments involved 31 male rhesus monkeys of similar age (3 to 4 years old). They were obtained in two cohorts (15 from Key Lois, Inc., Summerland, FL, Division of Charles River and 15 from Hazelton Research Animals, Inc., Alice, TX). They were housed individually in $81 \times 81 \times 91$ cm stainless steel primate cages in a room with temperature controlled at $24 \pm 1°C$ and relative humidity of 40 to 60%. Lights in the room were on from 0700 to 1900 hr. The monkeys were fed 6 to 8 Purina pellets twice daily, supplemented with fruit once weekly; water was available at all times during the experiments described here.

Exposure System

Figure 1 shows a diagram of the exposure system. An ADL II smoking machine (Arthur D. Little, Inc., Cambridge, MA) was fitted with a special cigarette holder to accommodate the placebo or marihuana cigarettes and special cams to generate puffs at the rate of 1, 2, or 3 per min. The ADL II smoking machine operates by drawing a puff of smoke into a 35-ml cylinder and then, by a valve-switching arrangement, forcing it into a Tygon stand-tube (1.3 cm i.d. by 96.2 cm length) attached to the monkey's face mask (see below). The airtight face mask was fitted with directional valves so the monkey could inhale only through the stand-tube. Except during the period when the puff of smoke was forced into the stand-tube (about 0.3 sec), fresh air was available through the inlet to the stand-tube. The valves were switched when the monkey exhaled to allow the air to exit into a tube that terminated in an exhaust hood.

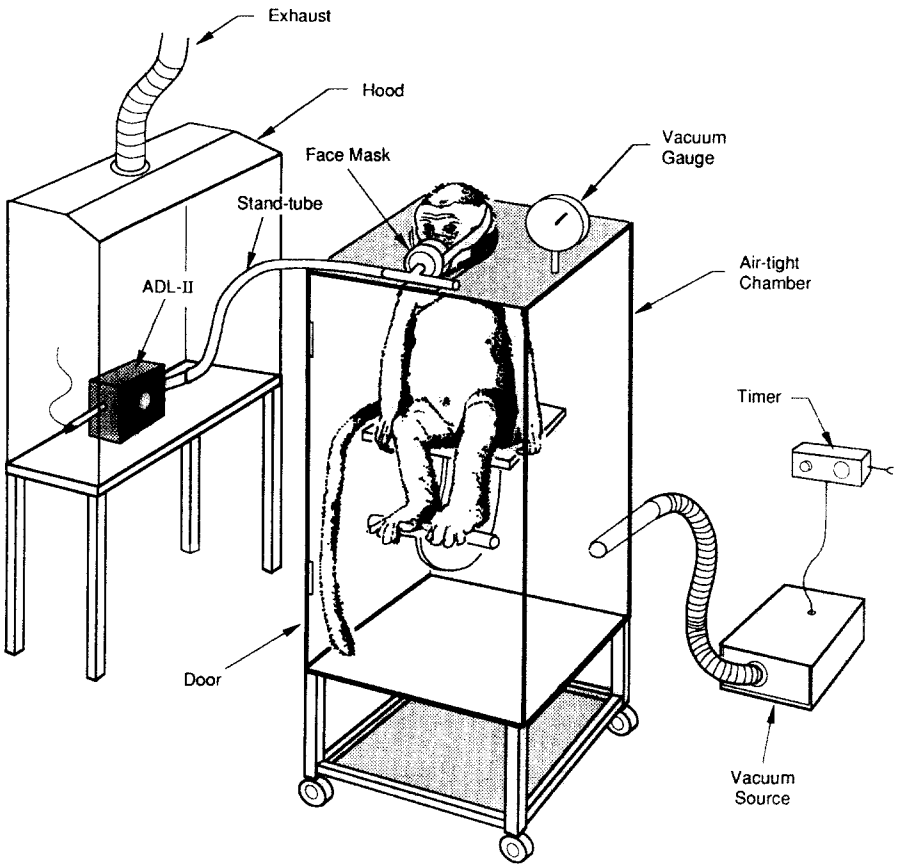

FIG. 1. System for exposing monkeys to the smoke from marihuana and placebo cigarettes. The ADL-II smoking machine generates a 35-ml puff of smoke into the stand-tube at which time a vacuum is imposed on the chamber, forcing the monkey to inhale deeply and hold its breath for 6 sec. The vacuum is then released, and the monkey is allowed to breathe fresh air normally between puffs.

During exposures the monkey was seated in a sealed Plexiglas chamber ($36 \times 46 \times 66$ cm) with its head protruding from the top. The chamber had a door on the front, and there was a slot in the top large enough to accommodate the monkey's neck; the monkey was put in the chamber by sliding its neck in the slot. Once the monkey was seated, a grooved insert was slid into the slot. The insert was held in place and the top was sealed when the front door of the chamber was closed. A latex dam was placed around the monkey's neck to further seal the top. An industrial vacuum cleaner (Model VNB-7, Metropolitan Vacuum Cleaner Co. Inc., Suffern, NY), attached by a hose to the chamber and located in a sound attenuating plywood cabinet, was used to impose a vacuum on the chamber. The operation of the vacuum cleaner was controlled by an electronic timer. A gauge located in the top of the chamber was used to measure the vacuum imposed. The vacuum created by this arrangement was 63.5 to 76.2 cm of water, which was sufficient to cause the monkey to inhale deeply (as judged visually by an expanded chest cage) and hold its breath for the timed duration of the vacuum. This arrangement was also usually sufficient to cause the monkey to clear all of the visible smoke from the stand-tube with each inspiration. Without the vacuum, 2 to 4 inspirations were required to clear the stand-tube of smoke, depending on the monkey's depth of breathing. After initial adaptation to the system, no discomfort to the monkeys was apparent. Upon release of the vacuum, normal breathing was restored immediately.

Face Mask

For efficient operation a relatively airtight face mask was found to be essential, both to assure that all of the smoke was inhaled by the monkey and to prevent the smoke from leaking out and irritating the monkey's eyes. A number of masks were designed and used with success. However, the mask described here is the easiest to fabricate with readily available materials.

Figure 2 shows a diagram of the mask and the attached valving arrangement. A toddler-size nonconductive human face mask (Vital Signs, Inc., Totowa, NJ) was used as the basic unit and found appropriate to fit the monkeys we used (other sizes are available). The inner cushion was carefully cut off with a scalpel leaving only the hard plastic foundation. A medium-size black rubber glove (obtainable at hardware stores) was cut off just below the base of the thumb. The narrow part of the wrist portion of the glove was stretched to fit over the face mask and secured and sealed there with rubber cement. A 3.8-cm elastic band (obtainable from fabric stores) was stapled around the glove near the face mask to add stability and ensure a tight fit. A 3.8-cm Velcro strip was then stapled to each side of the mask (one male and one female) to provide flaps that were drawn tightly around the monkey's head and secured there.

A T-adaptor was attached to the inlet of the face mask. One side of the T was connected to the stand tube. A one-way valve (Model 001671, Inspiron Corp., Cucamunga, CA) was attached to the other side of the T, which allowed air to exit, but not to enter, the mask. Thus, only inspiration through the stand-tube was possible, with exhalation through the other side of the T. The exhaled smoke was exhausted by tubing to a nearby hood.

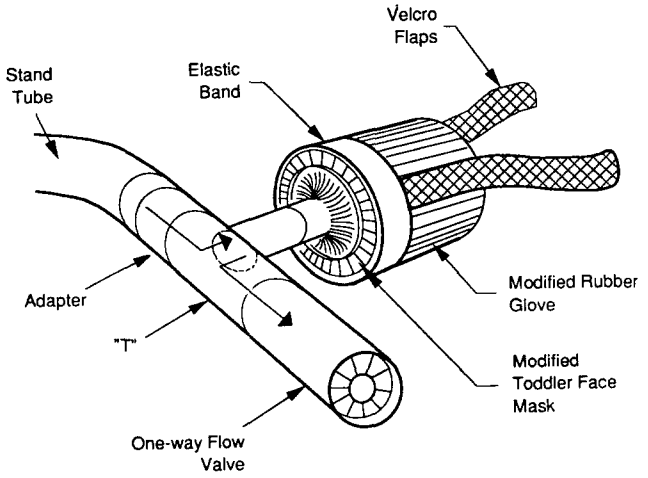

FIG. 2. Face mask used to expose monkeys to the smoke from marihuana and placebo cigarettes. See text for a description of fabrication.

Measurement of Δ^9-THC and Carboxyhemoglobin (COHb)

Blood (3 ml) was taken from the saphenous vein using a sterile 1-cc tuberculin syringe and 21-g needle. The sample was transferred to a 4-ml vacutainer tube. For COHb determinations, a 200-μl aliquot was drawn into a Allied Instrumentation Laboratory Co-oximeter (Model IL282, Lexington, MA) and analyzed immediately. The rest of the sample was stored on ice until all samples were collected for that day. The samples were then centrifuged and stored at 4°C until analyzed for Δ^9-THC. Radioimmunoassay kits obtained from Research Triangle Institute (RTI; Research Triangle Park, NC) were used to measure THC. ^{125}I-Δ^8-THC was the radioligand. Δ^9-THC diluted in naive monkey serum was used as standard.

Marihuana and Placebo Cigarettes

Marihuana and placebo cigarettes were obtained from the RTI

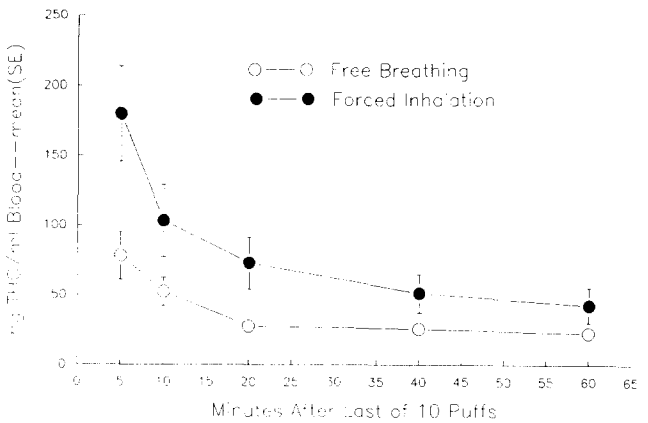

FIG. 3. Disappearance of Δ^9-THC from blood after exposure to marihuana smoke by free breathing compared with forced inhalation. Two naive monkeys (not used in the main experiments) were exposed to marihuana smoke by two methods. In the first method, the monkeys were allowed to breathe the smoke voluntarily. In the second method, the monkeys were forced to inhale each puff of marihuana smoke and hold it for 6 sec by imposing a vacuum on the chamber in which they were seated. Smoke was delivered at the rate of two puffs per min (each puff was 35 ml). Each monkey received 10 puffs. Blood was sampled as indicated and Δ^9-THC was measured by RIA. The experiment was repeated three times for each monkey under each exposure method.

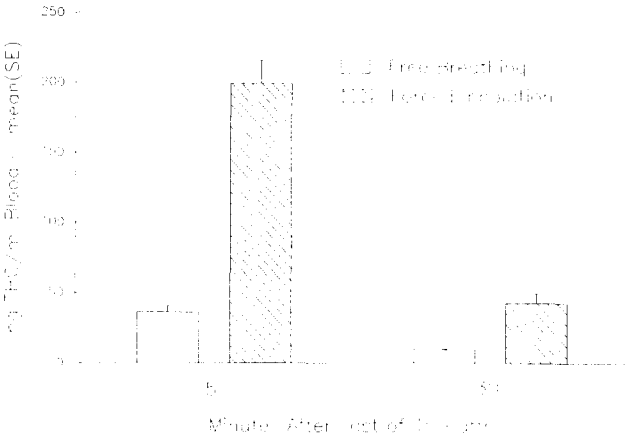

FIG. 4. Comparison of blood levels of Δ^9-THC 5 and 30 min after exposure to ten puffs of marihuana smoke by voluntary or forced inhalation. Two monkeys were exposed to ten puffs of marihuana smoke delivered at the rate of three puffs per min. They were allowed to voluntarily breathe the smoke, or they were forced to inhale and hold each puff for 6 sec. The experiment was repeated four times for each monkey under each experimental condition.

through contract with the NIDA. The marihuana cigarettes weighed 813 mg (average) and contained 2.62% Δ^9-THC. Isopropanol-extracted marihuana cigarettes, also supplied by the RTI, contained 0.004% Δ^9-THC. The cigarettes were stored frozen in sealed containers until thawed for use. All cigarettes were humidified (over NaCl-saturated water in a closed container) for at least 24 hr before use.

Procedure

A presmoke blood sample was taken while the monkey was in a standard primate chair (samples could not be obtained easily while the monkey was in the exposure chamber). The monkey was then transferred to the exposure chamber, the mask was fitted and secured, and the monkey was allowed to adapt for a few min. The smoking machine was turned on, and a cigarette was placed in the holder and lit. Puffs were generated at the rate of two or three per min. When a puff was forced into the stand-tube the technician activated a handswitch that initiated the vacuum for 6 sec. When the predetermined number of puffs had been delivered, the mask was removed from the monkey. The monkey was transferred back to the standard primate chair, and blood was sampled at predetermined times after the last puff. For experiments in which non-forced inhalation was being tested, the same procedures were followed except that the vacuum was never imposed.

RESULTS

Figure 3 shows the blood levels of Δ^9-THC at various times after the last of ten puffs of marihuana smoke delivered at the rate of two puffs per min in two naive monkeys. The levels were 78 (±17.2) ng/ml (mean ± SEM) 5 min after the last puff when the monkeys were allowed to breathe freely. When forced to inhale and hold the smoke the levels were 180 (±34.0) ng/ml 5 min after the last puff.

The experiment was repeated except that puffs were delivered at the rate of three per min (Fig. 4). Levels of Δ^9-THC after 5 min were slightly higher in this than in the first experiment under the forced-inhalation procedure. However, the levels were about half of those in the first experiment when the monkeys were allowed to

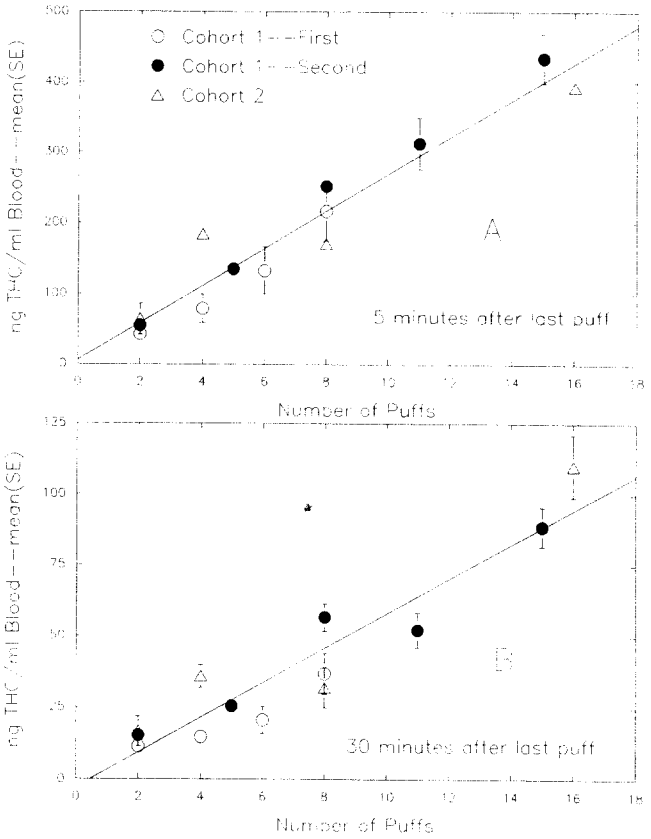

FIG. 5. Blood levels of Δ^9-THC as a function of the number of puffs of marihuana smoke by forced inhalation sampled 5 (A) or 30 (B) min after the last puff. Smoke was delivered at the rate of three puffs per min by the forced-inhalation method. Each point represents data from 9 or 10 monkeys in Cohort 1 and 4 to 6 monkeys in Cohort 2. Two determinations were made for the first cohort and one for the second cohort.

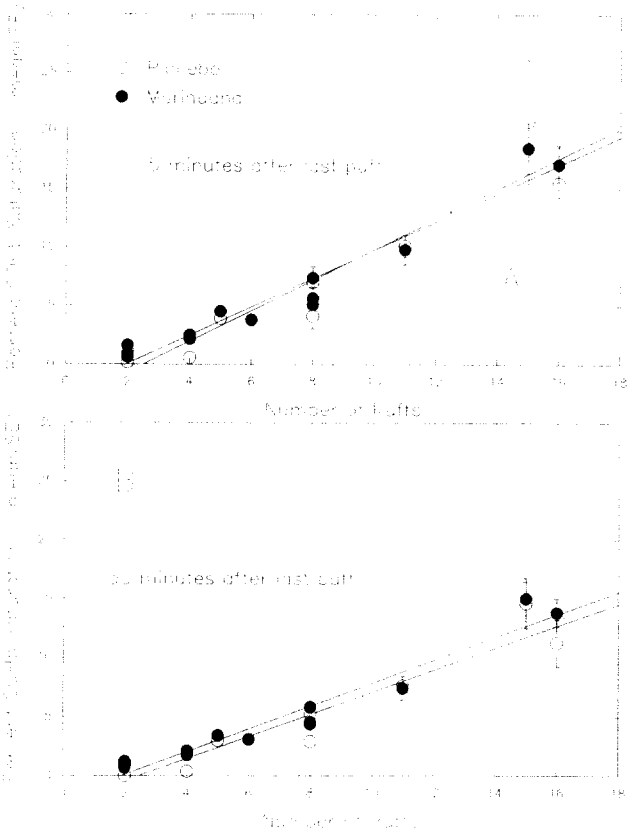

FIG. 6. COHb Saturation in blood of monkeys 5 (A) or 30 (B) min after exposure by forced inhalation to the smoke from placebo or marihuana cigarettes as a function of the number of puffs. Data are shown for both cohorts combined. Each point represents the data from 4 to 10 monkeys.

breathe freely, perhaps reflecting a reduction of rate and/or depth of inhalation adopted by the monkeys to avoid the irritating properties of the smoke.

The main experiments were done using two cohorts of monkeys obtained about one year apart. Before starting the chronic smoking phase of the experiments, the monkeys were acutely exposed to various amounts (i.e., number of puffs) of marihuana or placebo smoke using the forced inhalation method, and levels of Δ^9-THC and COHb were measured. Figure 5A shows that levels of Δ^9-THC increased approximately linearly from 2 puffs (about 50 ng/ml) to 16 puffs (about 400 ng/ml) when measured 5 min after the last puff. When measured 30 min after the last puff, the levels ranged from about 15 to 100 ng/ml (Fig. 5B).

Figure 6 shows the COHb saturation levels accompanying the inhalation of smoke from placebo and marihuana cigarettes. Reflecting the amount of carbon monoxide inhaled by the monkeys, COHb increased from about 2% after 2 puffs to about 20% after 16 puffs when measured 5 min after the last puff. COHb saturation declined only slightly after 30 min reflecting the slow dissociation of CO from Hb at these low levels. There were no differences between placebo and marihuana cigarettes in the levels of COHb produced.

DISCUSSION

The method described above provides an efficient way of delivering marihuana smoke to rhesus monkeys under relatively well controlled conditions. Doses of Δ^9-THC (and presumably the other cannabinoids in marihuana, which were not measured) can be varied systematically by varying the number of puffs delivered. The efficiency of the method was demonstrated by the markedly higher blood levels of Δ^9-THC achieved with forced inhalation compared with those measured when the monkeys were allowed to regulate their breathing voluntarily.

Interestingly, the levels achieved under the free-breathing condition were much lower in the second than in the first experiment. It is possible that this difference reflected the different puff rates between the two experiments (two versus three puffs per min, respectively). However, this explanation is unlikely because no such decrease was seen under the forced-inhalation procedure (indeed, there was a slight increase). It is more likely that the monkeys learned to reduce the rate and/or depth of their breathing from the first to the second experiment. This source of variability is inherent to the free-breathing mode of exposure, but is minimized by the forced inhalation method, making it the procedure of choice, especially for chronic experiments.

The smoke from both placebo and marihuana cigarettes was clearly aversive to the monkeys, and some had to have their heads restrained to prevent them from disturbing the mask and causing leaks. After repeated exposures, most of the monkeys adapted to the situation and ceased to struggle while having the mask put on and breathing the smoke. Nevertheless, some monkeys continued to try to remove the mask by moving their heads back and forth (throughout these and the subsequent chronic experiments), which undoubtedly created variability in the doses of Δ^9-THC they received. We also observed that some monkeys tried to resist the

vacuum imposed, and their inhalations did not appear to be as deep or as consistent as those of monkeys who did not so resist. However, measures of respiratory parameters were not made in these experiments to evaluate this possible source of variability.

In spite of the shortcomings just mentioned (some of which could be minimized by further modifications of the system and/or the operating procedures), the method described appears satisfactory and is relatively easy to implement. It was used successfully in chronic experiments (results to be reported elsewhere) of 6 and 12 months duration in which smoking was carried out 1 to 3 times daily, five days per week. It has clear advantages over methods where the monkey is allowed to regulate its own respiration [e.g., (2)]. By appropriate modification of the source of the inhalant, the method could also be adapted for studies of other volatile substances such as "crack" and various solvents, where the mode of inhalation in humans is similar to that of marihuana. Similarly, the method could be adapted to other animal species such as cats, dogs, and some rodents.

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