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Dependence on Tetrahydrocannabinol in Rhesus Monkeys[1,2]

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ABSTRACT

The present studies examined whether dependence could be induced by continuous infusion of Δ-9-tetrahydrocannabinol (THC), as evidenced by behavioral disruptions during withdrawal of THC administration. Four rhesus monkeys lever pressed under fixed-ratio schedules for their daily food rations during four, daily, 0.5-hr sessions. Initially, the monkeys were tested before, during and after continuous i.v. infusions of THC. A dosage regimen of 0.05 mg/kg/hr for 10 days for three of the monkeys and a somewhat greater regimen in the fourth had little direct effect on rates of respondings for food during THC infusion, but marked decreases in response rates occurred in each monkey during withdrawal. These effects generally did not occur until the second day of withdrawal, and often lasted for over a week. These initial demonstrations were replicated subsequently with lower solvent concentrations. In the final study, THC was administered for a greater number of days and at higher dosages than during any of the previous studies. Response rate reductions again occurred within 2 to 3 days of withdrawal. When THC was readministered, reversal of the withdrawal effects occurred in that withdrawal-induced rate decreases were halted and rates increased subsequently. Administration of THC was discontinued after 2 to 3 days of its readministration. Response rates again decreased within the first 3 days after this withdrawal and then recovered slowly with time. Withdrawal of THC administration can disrupt operant behavior, and these disruptions can be reversed by resumption of THC administration, indicating that dependence upon this drug can be produced in rhesus monkeys.

Marihuana and other cannabis products remain among the most widely abused pharmacological substances (Richards, 1981). The constituent that is generally considered to possess the greatest behavioral activity in marihuana is THC. The results from previous studies involving discontinued THC administration have been inconsistent in demonstrating an abstinence syndrome. Many studies have been unable to produce a THC-related abstinence syndrome (Dewey *et al.*, 1972; Harris *et al.*, 1974; Leite and Carlini, 1974; McMillan *et al.*, 1970; Stefanis *et al.*, 1976), whereas others have obtained positive findings (Deneau and Kaymakcalan, 1971; Fredericks and Benowitz, 1980; Jones and Benowitz, 1976; Stadnicki *et al.*, 1974). A number of procedural differences among these studies may account for the inconsistent observations of THC-related withdrawal effects. One prominent difference is that different effects are often monitored from study to study for detection of dependence. For example, when observable signs characteristic of a typical abstinence syndrome such as that associated with the opiates or sedative-hypnotics are monitored during THC withdrawal, an abstinence syndrome is not observed usually (Dewey *et al.*, 1972). However, in other studies some evidence of dependence has been observed when effects not limited to those associated with narcotic or sedative-hypnotic dependence are examined. For example, Branch and co-workers (1980) found that rates of initiation of a complex operant task were reduced markedly in squirrel monkeys upon withdrawal of chronic THC administration.

Rates and patterns of schedule-controlled responding have been used frequently in the assessment of the effects of drug administration and tolerance to drug effects. Less frequently have schedule-controlled performances been used in the assessment of drug withdrawal (Balster, 1985). When schedule-controlled performances have been used to monitor the effects of drug withdrawal they have been shown to be sensitive indicators of dependence (*e.g.*, Thompson and Schuster, 1964; Holtzman and Villarreal, 1973), often effective after drug conditions insufficient to induce observable syndromes indicative of physical dependence (*e.g.*, Slifer *et al.*, 1984). Not only do schedule-controlled performances offer sensitivity in assessing drug-withdrawal effects, but because response rate and pattern are used as the main dependent measures, a greater degree of objectivity is obtained than in more traditional procedures that rely on observational techniques. Additionally, the use of response rate as the principal datum permits graded expressions of the degree of withdrawal effects because by nature it is a

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[3] Postdoctoral trainee supported by National Institute on Drug Abuse Grant DA-07027.

ABBREVIATIONS: THC, Δ-9-tetrahydrocannabinol; FR, fixed-ratio; EL-620, emulphor-620.

continuous variable, unlike nominal variables most often used in traditional approaches.

In order to determine whether or not THC could induce dependence we used schedule-controlled response base lines for monitoring withdrawal effects, thereby attempting to capitalize on the attributes of operant methodologies described above. We investigated the dependence-producing potential of THC by applying the general procedure described by Slifer *et al.* (1984) in which the disruption of food-maintained operant behavior in rhesus monkeys was used for characterizing the effects of phencyclidine withdrawal. We additionally examined the effects of solvent concentration and whether withdrawal effects could be reversed by readministration of THC.

Methods

Subjects. Four adult male rhesus monkeys (*Macaca mulatta*) served as subjects throughout all experiments. The monkeys had been used in previous experiments in which drugs other than THC were adminstered and had been drug-free a minimum of 3 months before the start of the present experiment. The monkeys' weights at the beginning of the study ranged from 6.7 kg (monkey M320) to 12.7 kg (monkey 7623).

Apparatus. The monkeys were housed individually and continuously in enclosed fiber glass cubicles (0.8 × 0.8 × 1.0 m) equipped with filtered ventilation systems. Within the cubicle a food hopper separated two response levers mounted on the clear Plexiglas door. When scheduled, 1 g of banana-flavored pellets (Noyes Formula L) were delivered by an externally mounted pellet feeder into the food hopper. Three 28-V jewelled lamps were mounted above each lever. An audio speaker, through which a white-noise alarm could be sounded, was mounted behind the left lever. Events within the cubicles were controlled and recorded by programming equipment located in adjacent rooms.

Catheterization. The monkeys were prepared surgically with chronically indwelling catheters under phencyclidine-pentobarbital anesthesia. The silicone rubber catheter (0.8-mm lumen) was inserted into either a femoral, internal jugular, or external jugular vein. The distal end of the catheter was passed s.c. to an exit point on the animals' backs. Each monkey wore a lightweight stainless steel tubular harness with an attached spring arm (H & M Engineering, Chicago, IL). The spring arm was connected to the rear of the cubicle. The catheter was threaded through the protective spring arm and out the rear of the cubicle, where it was connected to a syringe pump (Sage Instrument Co., model 355, White Plains, NY). The harness and spring arm allowed free movement within the cubicle but prevented the monkeys from leaving the cubicle. Some of the monkeys also wore a nylon vest (Alice King Chatham Medical Arts, Los Angeles, CA) to prevent them from removing the catheter. During all experiments, drug or vehicle solutions were administered at a rate of 1.0 ml/hr, continuously, throughout the day.

Daily conditions. The monkeys had previous experience pressing the right lever for banana pellet deliveries according to either FR 100 or 150 schedules (FR 100: monkeys M321 and 6189; FR 150: monkeys M320 and 7623). Under these schedules every 100 (FR 100) or 150 (FR 150) presses of the right lever resulted in a 1-g banana pellet delivery. Presses at the left lever did not have programmed consequences. Banana pellets were available throughout all experiments during four, daily, 30-min sessions that began at 4:00 P.M., 10:00 P.M., 4:00 A.M. and 10:00 A.M. During each session the lights above the right lever were illuminated. In addition, a white-noise alarm was sounded at the start of each session and was terminated by the first press of the right lever. Routine housekeeping (cubicle cleaning and animal inspections) was performed between 7:00 A.M. and 8:30 A.M. daily.

Initial inductions of dependence and retest. After catheterization, saline was infused continuously for a minimum of three days. During this experiment, days of THC administration were preceded and followed by days of vehicle administration. The first experiment consisted of three phases during which vehicle then THC and then vehicle again were infused continuously. During vehicle conditions a solution of EL-620, 95% ethanol and 0.9% saline (1:1:8, respectively) was infused. This solution concentration was chosen inasmuch as it is able to serve as a vehicle for up to 20 mg/ml of THC (Carney *et al.*, 1977) and was capable of accommodating any THC dose we anticipated testing before the start of the experiment. Lower vehicle concentrations were sufficient to solubilize the tested THC solutions.

Initially, vehicle solutions were infused until 5 consecutive days of stable food-reinforced responding occurred. Vehicle solutions were then replaced by 0.05 mg/kg/hr of THC. After 10 days of THC infusion, vehicle was again administered until behavior recovered. This procedure involving predrug vehicle for at least 5 days, 0.05 mg/kg/hr of THC for 10 days, followed by postdrug vehicle, was repeated for monkeys 7623, M321 and 6189 (referred to as the Second Determination). Monkey M320 was tested after the First Determination under a 10-day drug regimen of 0.05, 0.075, 0.113 and 0.169 mg/kg/hr of THC for 2, 2, 2 and 4 days, respectively. Because of a lack of a withdrawal effect under both the first and second 10-day regimens, monkey M320 was tested subsequently under a 16-day drug regimen of 0.05, 0.075, 0.113 and 0.169 mg/kg/hr of THC for 2, 2, 2 and 10 days, respectively. Naloxone was administered to each monkey to test whether a dependence of the opiate type was being induced during THC administration. Naloxone challenges (0.3 mg/kg i.m.) were given 5 min before the start of the last THC session during the second 10-day regimen.

The daily rate of responding for food, averaged across the four 30-min sessions, was the major dependent variable used to assess drug and abstinence effects. In addition, each morning the monkeys were inspected closely and any unusual behavior was recorded. Observations were also made 1 to 2 hr before the start of the 4:00 P.M. session during the first 3 days of withdrawal and signs of withdrawal were recorded on standardized checklists used previously in this laboratory (Balster and Woolverton, 1980).

Blood samples (up to 5.0 ml) were drawn from the saphenous veins of monkey M320 as close to 2:00 P.M. as possible on selected days before, during and after THC infusion during his 16-day regimen. Attempts to draw blood from other monkeys were discontinued because the phlebotomy procedure occasionally caused marked changes in their operant behavior. The blood samples of monkey M320 were centrifuged, the plasma extracted, frozen and analyzed subsequently by gas-chromatography/mass spectrometry for plasma levels of THC (analysis by Dr. Rodger L. Foltz of the Center for Human Toxicology, Salt Lake City, UT).

Manipulation of solvent concentration. As mentioned above, the solvent concentration used during vehicle conditions during Determinations 1 and 2 was chosen because it was able to serve as a vehicle for up to 20 mg/ml of THC and was capable of accommodating the high THC doses we anticipated needing to administer in order to induce dependence. We were surprised to observe marked withdrawal effects after the modest dosage regimens used during Determinations 1 and 2 and did not need to use large THC doses requiring concentrated solvent solutions. In order to demonstrate that the concentrations of emulphor and ethanol were not essential determinants of the withdrawal effects observed during these earlier demonstrations, the conditions of Determination 2 were replicated in these same animals except that predrug and postdrug vehicle concentrations were identical to that present in the solvent for THC during its administration. For monkeys 7623, M321 and 6189 predrug and postdrug vehicles were identical in concentration to that needed to solubilize an amount of THC necessary to obtain 0.05 mg/kg/ml (monkey 7623: 0.615%; monkey M321: 0.415%; monkey 6189: 0.35% concentrations by volume of El-620:ethanol in 0.9% saline). For monkey M320 the vehicle concentration was 1.07% EL-620:ethanol, which was equal to the concentration needed to solubilize 0.169 mg/kg/ml of THC, the highest concentration of THC administered to this monkey. All expressions of vehicle concentration represent the combined percentage of EL-620 and 95% ethanol, by volume, in 0.9% saline; for example, every milliliter of 1.07% EL-620:ethanol contains 0.00535 ml of EL-620, 0.00535 ml of 95% ethanol and 0.9893 ml of 0.9% saline.

After 5 consecutive predrug vehicle days of stable response rates during which there were no obvious increasing or decreasing trends in responding, THC was administered continuously for 10 days at a rate of 0.05 mg/kg/hr for monkeys 7623, M321 and 6189. Monkey M320 was again administered THC at a rate of 0.05, 0.075, 0.113 and 0.169 mg/kg/hr for 2, 2, 2 and 10 days, respectively. After the last day of THC administration, vehicle was again administered for a minimum of 14 days.

Reversal of withdrawal effects with readministration of THC. To further characterize the THC dependence phenomenon in these animals, THC was readministered after brief (3 days or less) withdrawal periods in order to test whether this would reverse withdrawal-induced response-rate reductions. During the drug condition of this experiment, THC was administered at higher doses and for a greater number of days than had been tested previously. These tests involved five conditions: the predrug vehicle, the drug, the postdrug vehicle, the reversal test and the postreversal vehicle conditions. Vehicle solutions for each subject administered during the predrug vehicle, reversal test and postdrug vehicle conditions were identical in the concentration of EL-620, 95% ethanol and saline as those used during the immediately preceding experiment. The duration of the drug condition and the doses of THC administered were different for each monkey (monkey 7623: 2, 4, 4 and 9 days of 0.05, 0.075, 0.113 and 0.169 mg/kg/hr, respectively; monkey M320: 2, 2, 2, 2, 4 and 10 days of 0.05, 0.075, 0.113, 0.169, 0.253 and 0.38 mg/kg/hr, respectively; monkey M321: 2, 4, 4 and 29 days of 0.05, 0.075, 0.113 and 0.05 mg/kg/hr, respectively; monkey 6189: 4, 4, 4 and 4 days of 0.05, 0.075, 0.113 and 0.169 mg/kg/hr, respectively). The doses of THC and the number of days each dose was administered were selected with the objective of maximizing the likelihood of a marked dependence phenomena while minimizing the occurrence of response-rate disruption during the infusion regimen. After 3 days of administering 0.113 mg/kg/hr of THC to monkey M321, response rates fell to zero levels. An attempt to restore responding in monkey M321 by lowering the dose to 0.05 mg/kg/hr initially was without effect; only after 10 days of THC administration at this lower dose did response rates begin to increase. While response rates were at near-zero levels (from the 9th through the 18th day of THC administration) supplementary feedings of banana pellets (from 30–60 pellets/day) were given this monkey in order to maintain his health.

During the reversal test condition the highest dose predicted to be well tolerated by the monkey was administered by continuous infusion (monkey 7623: 0.169 mg/kg/hr; monkey M320: 0.38 mg/kg/hr; monkey M321: 0.05 mg/kg/hr; monkey 6189: 0.05 mg/kg/hr).

Drugs. THC was obtained from the National Institute on Drug Abuse (Rockville, MD). Stock solutions of THC (100 mg/ml) were prepared using a solvent composed of EL-620 (GAF Corp., New York, NY) and 95% ethanol in a 1:1 concentration. Working drug solutions were prepared using appropriate amounts of stock solution dissolved in 0.9% saline (Carney *et al.*, 1977). Naloxone hydrochloride was obtained from Endo Laboratories, Inc. (Wilmington, DE) and its dosage is expressed as the salt.

Results

Substituting vehicle for saline did not have effects on operant behavior. Group mean daily response rates for the last 3 days of saline and for the first 3 days of vehicle were both 2.4 responses/sec. Additionally, the general behavior of the monkeys during morning inspections was not observed to be different during the two conditions.

Response rates during the last 5 days of the predrug vehicle condition ranged from 1.8 responses/sec for monkey M321 to 2.9 responses/sec for monkey 7623 during Determination 1 (fig. 1). THC administration did not have consistent disruptive effects on behavior. Generally, the response rates for food during THC administration were within the range occurring during predrug vehicle administration. Only monkey 6189 showed any evidence of a downward trend in response rates during THC administration, whereas the rates of monkeys M320 (during both 10-day regimens, not shown in fig. 1, and the 16-day regimen, fig. 1) and 7623 were relatively unchanged. Response rates of monkey M321 during THC infusion were consistently higher than vehicle rates. Additionally, clear overt changes in the monkeys' general behavior were not observed during THC administration, although the animals' handlers noted that they were less aggressive and excitable.

Although responding was little affected during THC administration, marked reductions in response rates occurred during withdrawal of THC. As shown in figure 1, reductions in rates of responding occurred within 48 hr of THC withdrawal during Determination 1 and 2 for three of the four monkeys. Monkey M320 did not show reductions in response rates comparable to the other monkeys after either 10-day THC regimen (not shown in fig 1); however, response-rate reductions to near-zero levels occurred within 72 hr after the 16-day drug regimen (lower left panel in fig. 1).

Figure 2 shows cumulative records for monkey M321 for all four 30-min sessions on the final day of vehicle control administration, the final day of THC administration and for the first 2 days of withdrawal during Determination 2. Overall response rates were higher on the first day of withdrawal relative to the final day of THC administration, which in turn were higher than the final day of vehicle control. On the second day of withdrawal, overall response rates were very low and the monkey earned only 10 food pellets on this day. When responding did occur on the second day of withdrawal, running response rates were within the range of running rates of previous conditions. Except for a few instances of pausing after responding had started before completion of a FR, local topographies of responding on the second day of withdrawal were not markedly different from nonwithdrawal conditions for this monkey. The patterns of responding shown in figure 2 for monkey M321 were also typical of response patterns of the other monkeys, both during THC administration and withdrawal.

The monkeys did not show dramatic signs of abstinence upon gross visual inspection and usually looked indistinguishable from the drug or predrug condition. Absent were signs characteristic of an opioid or depressant abstinence syndrome such as lacrimation, rhinorrhoea, emesis or behavior indicative of cramping. However, some changes in overt behavior were noted on the observational checklists including hyperreactivity, yawning, aggressiveness and bruxism. Only one monkey ever showed signs of convulsive activity. Monkey M321 convulsed on the 7th day during the withdrawal period during Determination 1, for which he had to be treated. Treatment consisted of i.v. and i.m. administrations of diazepam. Within 48 hr of convulsing, monkey M321's response rates dropped to zero and he ceased eating. Renewed eating was stimulated with hand-offered food, flavored with vitamin syrup. The amount of hand-offered food was eliminated progressively until monkey M321 once again began to lever press for all his daily food by the 18th day of withdrawal. Within the subsequent 3 days monkey M321's response rates returned to predrug levels.

Plasma levels of THC were determined in monkey M320 on selected days before, during and after THC administration during his 16-day regimen. These plasma levels of THC (nanogram per milliliter) in monkey M320 are shown connected to their respective days with arrows in the lower left frame of

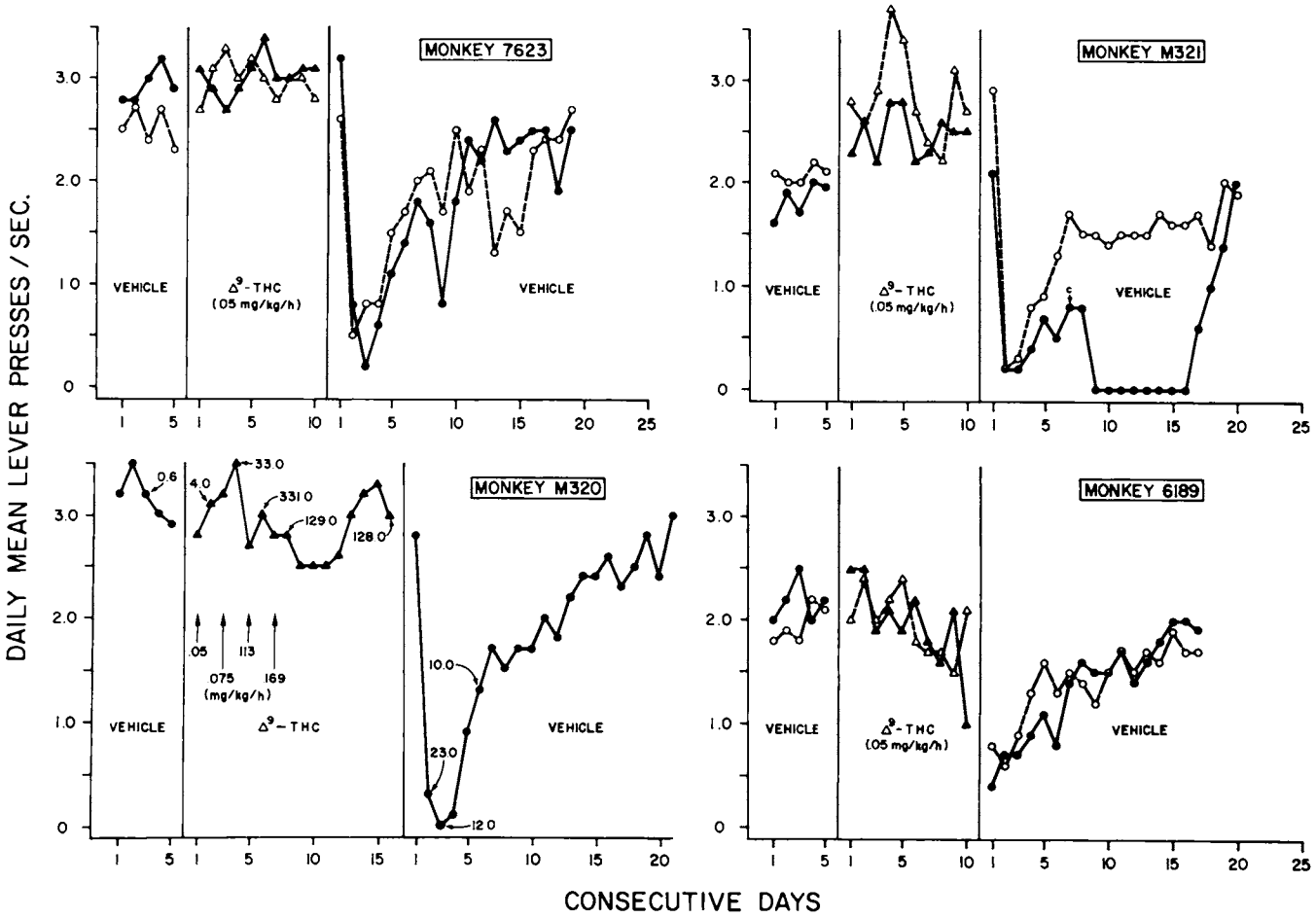

Fig. 1. Effects of continuous i.v. infusion of THC and of its withdrawal on operant behavior of four rhesus monkeys. Daily average response rates are shown for each monkey during the predrug vehicle, THC and postdrug vehicle conditions. Each symbol is the daily mean of four, 0.5-hr sessions during which the monkeys could obtain banana pellets according to FR reinforcement schedules. For monkeys 7623, M321 and 6189 the filled symbols indicate the First Determination and the unfilled symbols indicate the Second Determination. For monkey M320 the filled symbols indicate the results from the 16-day regimen. THC infusion doses in milligrams per kilograms per hour are indicated for each monkey. The numbers at the top of the frame for monkey M320 indicate levels of THC (nanograms per milliliters) in plasma samples drawn on the days indicated. The "C" above the seventh day of withdrawal during the First Determination for monkey M321 indicates that this monkey had convulsed on that day (see text).

figure 1. During the period of THC infusion, plasma levels of THC ranged from 4.0 to 331.0 ng/ml. Initial decreases in plasma THC levels in monkey M320 from 128.0 ng/ml on the last day of drug administration to 12.0 ng/ml on the third day of withdrawal were accompanied by sharp reductions in response rates. Responding began to recover during withdrawal when accompanied by further reductions in plasma THC levels below 12.0 ng/ml.

Administration of 0.3 mg/kg naloxone i.m. 5 min before the 10:00 A.M. session on the last day of THC administration during the second 10-day regimen did not precipitate withdrawal or alter the monkey's behavior in any observable way. The group mean response rate was 2.58 responses/sec during this session, preceded by the naloxone challenge, which was similar to the group mean rate of 2.50 responses/sec obtained during the 10:00 A.M. session of the preceding day.

When the conditions of Determination 2 were replicated systematically using lower solvent concentrations during vehicle conditions, withdrawal-induced reductions of responding were seen again (fig. 3). Maximum response-rate decreases occurred usually between the third and fifth days of withdrawal. Response-rate decreases during withdrawal occurred usually later than, and were usually not as great as, the decreases occurring during the withdrawal episodes of Determinations 1 and 2. As in the first experiment, the THC infusion regimens used in this study generally had little effect on response rates. The only clear exceptions were monkeys M320 and 6189. In monkey M320, the final increase in the daily dosage to 0.169 mg/kg/hr resulted in a temporary disruption in responding. Monkey 6189 showed progressively lower response rates over the 10 days of THC infusion, very similar to what occurred with this subject in the first experiment. Monkey M321's infusion pump malfunctioned after the sixth day of drug delivery and he did not receive any drug for 24 hr. During the day in which drug was not delivered, monkey 321's response rates decreased by 50%, relative to his response rates on the immediately preceding drug day.

During the final series of THC infusions before the reversal tests, all monkeys, except monkey M321, either were not affected greatly by, or became tolerant to, THC administration as high as 0.169 to 0.38 mg/kg/hr (fig. 4). At these dosages their response rates by the end of the infusion regimen overlapped the range of response rates occurring during the predrug vehicle condition. As explained under "Methods," monkey M321's response rates declined to near-zero levels after receiving 0.113 mg/kg/hr and he had to be returned to 0.05 mg/kg/

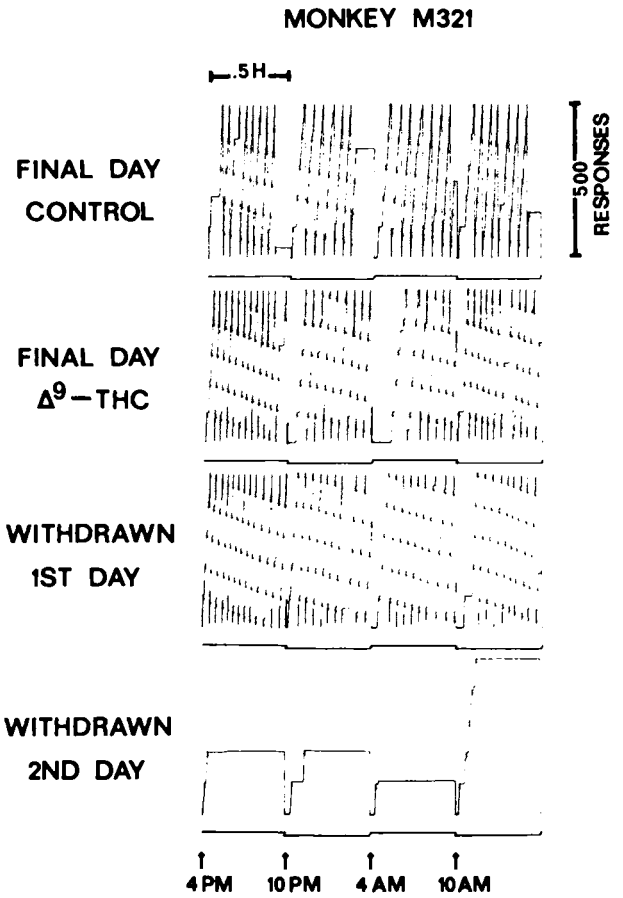

Fig. 2. Cumulative records of monkey M321 for each of the 0.5-hr food sessions obtained on the final vehicle control day, the final day of THC administration and the first two days of withdrawal during Determination 2. The upper pen stepped vertically for each press of the lever. Each time 100 presses of the lever occurred a banana pellet was delivered and was registered by a downward deflection of the upper pen. Downward or upward deflections of the lower pen's tracing indicated the end of a 0.5-hr session.

hr in an attempt to reinstate responding. Monkey M320 was administered the highest daily THC dosage (*i.e.*, 0.38 mg/kg/hr) of all the monkeys without incurring marked response-rate reductions.

During the postdrug vehicle condition in this experiment, response rates again declined during the 2 to 3 days of withdrawal of THC (fig. 4). Upon readministration of THC during the reversal-test condition, the downward trends in response rates for all monkeys were reversed and rates increased to levels exceeding those during withdrawal and to within the range of rates occurring during the drug condition. Signs of sickness were exhibited by monkey M321 during the postdrug vehicle condition (*e.g.*, retching and lassitude), which were attenuated during the reversal test condition.

When vehicle was again readministered during the postreversal vehicle condition, response rates again decreased but generally not to the lowest levels occurring during the initial postdrug vehicle condition (fig. 4). Except for monkey M321 (whose postreversal vehicle condition was terminated after only 8 days due to catheter problems), response rates during the postreversal vehicle condition increased subsequently to within the ranges occurring within the drug conditions.

Control rates of responding during the predrug vehicle conditions which preceded all four THC regimens were very similar across the four, 0.5-hr sessions for monkeys 7623, M320 and M321 and did not show systematic, diurnal variations. Occasionally, during the predrug vehicle conditions, near-zero rates occurred during the 4:00 A.M. session for monkey 6189; at other times during the predrug vehicle conditions his rates were always above 1.5 responses/sec. Generally, during THC administration, responding occurred usually at similar rates throughout the day.

During the THC withdrawal conditions of Determinations 1 and 2 for monkeys 7623, M321 and 6189, and after the 16-day drug regimen for monkey M320, response rates became markedly reduced, relative to previous conditions, by the second or third day. On these days of withdrawal, response rates during all four, 0.5-hr sessions were reduced relative to rates occurring on the last day of drug administration and during the predrug vehicle control days. Usually, the earliest evidence of a withdrawal-related response-rate suppressive effect did not appear before the fourth session on the first day of withdrawal (*i.e.*, at the 10:00 A.M. session, 18 hr after withdrawal). The exception to this generality was monkey 6189 who showed reductions in responding during earlier sessions on the first day of withdrawal. It should be noted that an attempt was made at approximately 2:00 P.M. on the first day of withdrawal during Determination 1 to draw blood from monkey 6189 for eventual THC plasma level determination. The phlebotomy procedure greatly disturbed monkey 6189 as evidenced by unusually aggressive and hyperexcitable overt behavior he displayed after these attempts to obtain a blood sample. During the session after the phlebotomy attempts with monkey 6189, very low rates of responding occurred. No attempts were ever made to draw blood from monkey 6189 again.

During the lower solvent replication tests, the withdrawal-induced response-rate reductions occurred usually later than, and did not become as great as, those occurring during earlier regimens. However, during the two or three days of initial THC withdrawal during the reversal study response-rate reductions occurred as early as the end of the first day of withdrawal, similar to the time of onset during withdrawals in Determinations 1 and 2. When THC was readministered during the reversal study, increases in response rates occurred as early as the first (monkeys 7623 and M320), second (monkey 6189) and fourth (monkey M321) sessions on the first day of readministration.

Discussion

The results of these studies indicate that dependence can be induced in rhesus monkeys by continuous i.v. infusion of THC. All four monkeys showed marked response-rate reductions for food during withdrawal from THC. Particularly noteworthy is the observation that these withdrawal effects occurred after modest THC regimens during which only small response-rate changes occurred and in which overt behavior was relatively unchanged. In fact, the dosage regimens used during the first three experiments were within the range of THC intake (on a milligram per kilogram basis) obtained by heavy cannabis users (LeDain *et al.*, 1972) and the plasma level obtained in monkey M320 on the final day of his 16-day THC regimen (128.0 ng/ml) is similar to peak plasma levels that have been determined in human plasma after the smoking of one, 1.64% THC marihuana cigarette (Lindgren *et al.*, 1981). On the other hand, 24 hr/day continuous i.v. infusion is not a naturalistic exposure

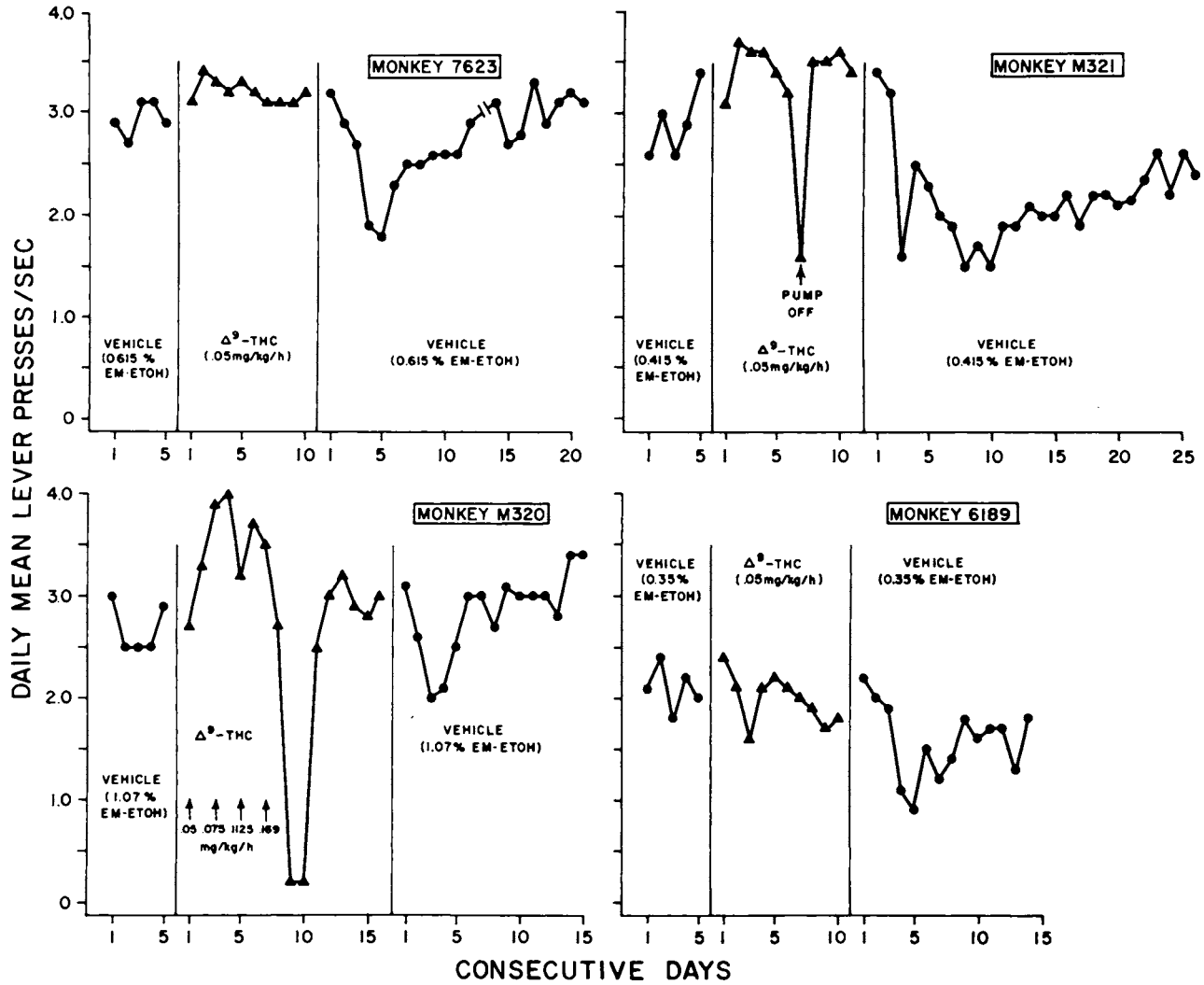

Fig. 3. Daily response rates during the predrug vehicle, drug and postdrug vehicle conditions when low solvent concentrations were used. Each symbol represents the mean of four, 0.5-hr sessions. Numbers in parentheses during vehicle administration represent the percentage of EL-620 and 95% ethanol (1:1) delivered in 0.9% saline. Numbers either in parentheses or associated with arrows during THC administration represent the amount of THC administered (milligrams per kilograms per hour). Due to a pump malfunction after the sixth day of drug administration for monkey M321, drug was not delivered, as indicated by the arrow.

regimen in that the normal route of THC administration in humans is by inhalation and exposure is not continuous. Whether similar dependence could be produced by more intermittent dosing is unknown and requires further study.

During the withdrawal-reversal experiment, resumption of THC infusion after 2 or 3 days of abstinence from THC halted the withdrawal-induced decrement in response rates and returned responding to within the range occurring during drug administrations. The ability of THC administration to reverse the effects on response rates seen during withdrawal provides evidence that the absence of THC was the critical factor mediating rate reductions during withdrawal. Further evidence that the disruption of responding after the discontinuation of THC infusion was due to THC dependence was supplied during the third 10-day THC regimen of monkey M321 when the pump malfunctioned (fig. 2). During the 24 hr that the pump was off and THC was not infused, response rates decreased by over 50% relative to rates occurring in the immediately preceding 6 days of drug infusion. When THC infusion was resumed, response rates of monkey M321 increased rapidly to levels preceding the pump malfunction. The results of this serendipitous reversal probe were similar to the results of the scheduled reversal tests occurring in the final experiment involving a longer THC regimen and higher THC doses. To our knowledge these are the first demonstrations of THC administration reversing behavioral disruptions occurring during THC withdrawal.

In addition to the marked reductions on response rates during withdrawal, other changes in overt behavior were noted on the observational checklists. Occasionally aggressiveness, bruxism and hyperreactivity were observed during withdrawal. These changes in overt behavior during THC (or cannabis) withdrawal have been observed in part or in whole by others in rhesus monkeys (Deneau and Kaymakcalan, 1971; Fredericks and Benowitz, 1980; Stadnicki *et al.*, 1974), mice (Cutler *et al.*, 1975) and in humans (Jones and Benowitz, 1976; Mendelson *et al.*, 1984). Monkey M321 displayed the most pronounced effects during withdrawal in that he convulsed during Determination 1 and showed signs of illness including retching and lassitude during the postdrug vehicle conditions before the reversal tests which were, in part, relieved by THC readministration. However, it should be noted that monkey M321's

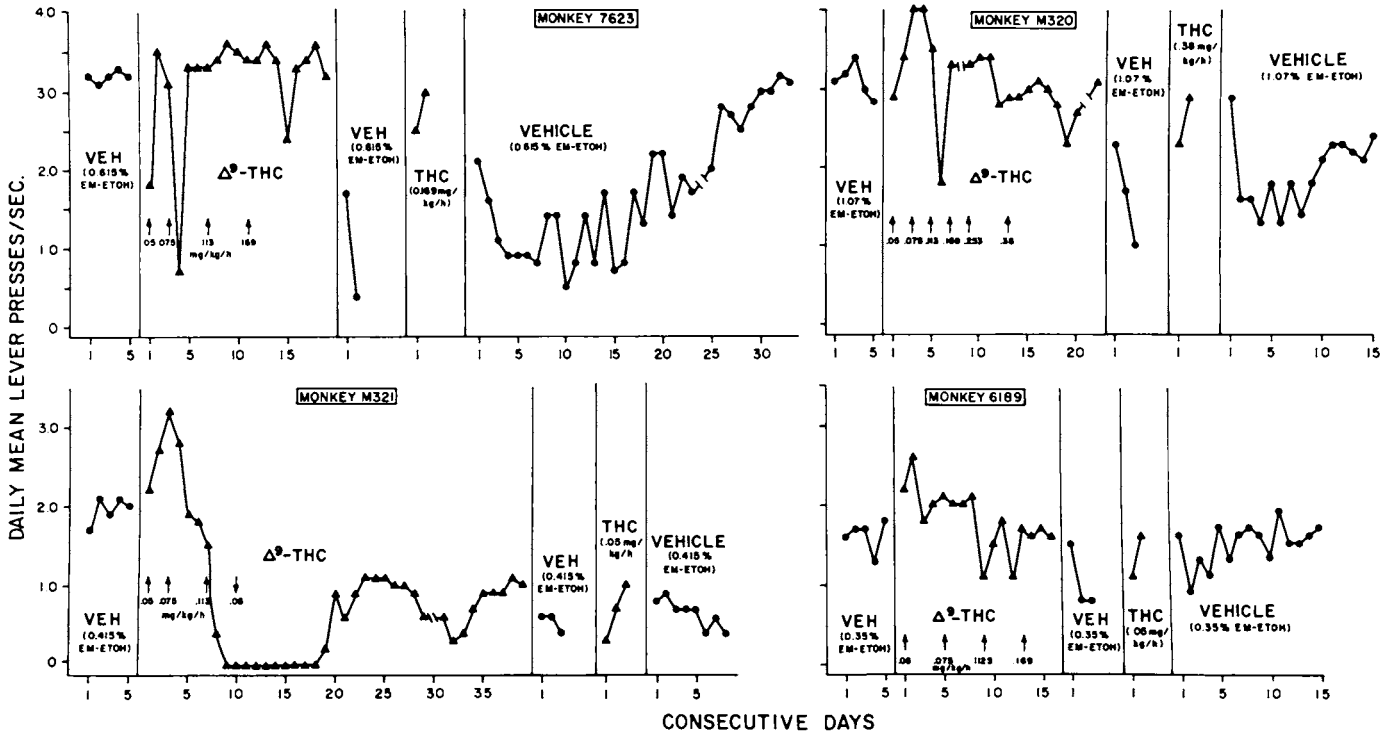

Fig. 4. Daily response rates during the predrug vehicle, the drug, postdrug vehicle, reversal test and postreversal vehicle conditions. Each symbol represents the mean of four, 0.5-hr sessions. Numbers in parentheses during vehicle administration represent the percent of EL-620 and 95% ethanol (1:1) in 0.9% saline. Numbers in parentheses or associated with arrows during THC administration represent the amount of THC administered (milligrams per kilograms per hour). Breaks in individual curves indicate that the specific solution was delivered but due to equipment problems response data were lost or not recorded.

convulsions occurred very late during withdrawal (on the 7th day) and did not recur during subsequent withdrawal episodes, including withdrawal from the THC dosage regimen which involved higher doses administered for more days during the reversal test study. Because of this delay in the emergence of convulsions and because of the lack of recurrence of convulsions during subsequent withdrawal episodes, it is uncertain whether this convulsive episode can be attributed solely to his THC history. No other monkeys convulsed or did they show any preconvulsive activity.

During all studies, peak abstinence-induced reductions of food responding occurred usually after at least 18 to 24 hr of THC withdrawal. This delay in the emergence of maximal abstinence effects is similar to that reported to occur in humans withdrawn from oral THC administration (Jones and Benowitz, 1976), in humans withdrawn from smoked marihuana (Mendelson *et al.*, 1984) and in rhesus monkeys withdrawn from crude marihuana extract (Stadnicki *et al.*, 1974). The tardy emergence of peak withdrawal effects may in part be due to the slow elimination of THC and its active metabolite.

There are multiple possible mechanisms mediating the reductions in response rates during THC withdrawal. One important question relevant to the withdrawal-induced response-rate reductions is whether during withdrawal the appetite for food was decreased (anorexia) and subsequently lever pressing was reduced or whether lever pressing was affected directly and food consumption necessarily was reduced. The latter alternative could be tested by determining whether lever pressing maintained by other reinforcers would also be similarly affected during withdrawal. In any case, anorexia remains a possible determinant of the withdrawal effects on response rates and has been cited as a sign of the THC abstinence syndrome by other researchers (*e.g.*, Deneau and Kaymakcalan, 1971; Jones and Benowitz, 1976).

Naloxone challenges had no observable effects on behavior, indicating that the THC regimens did not induce dependence of the morphine type. The naloxone dose administered (0.3 mg/kg i.m.) is considerably more than is required to precipitate an abstinence syndrome in a morphine-dependent rhesus monkey (Aceto, 1984). Naloxone has been reported to precipitate narcotic-like withdrawal signs in rats that had been administered THC i.p. (Hirschhorn and Rosecrans, 1974). There are numerous procedural differences between the present study and the previous study by Hirschhorn and Rosecrans (1974) including differences in species of animal tested, THC regimen, route of drug administration and dose of naloxone administered which could account for these observed differences in naloxone's effect.

During repeated withdrawal episodes from continuous phencyclidine infusion, rhesus monkeys showed decreased sensitivity to withdrawal-disruption of operant behavior (Slifer *et al.*, 1984). Some tolerance to the withdrawal effects of THC seemed evident in the present studies also. Generally, less reduction of response rates occurred during withdrawal from THC during the third, 10-day THC regimen (or the second, 16-day regimen for monkey M320) than during earlier withdrawal episodes from identical regimens (figs. 1 and 3). The vehicle concentration of emulphor and ethanol during withdrawal was lower in these latter tests than in the initial tests which might, by itself, have influenced the magnitude of the withdrawal-induced response-rate reductions. However, it is unlikely that the concentration of the vehicle during withdrawal was an essential deter-

minant of behavioral disruptions because during the 2 or 3 days of withdrawal during the reversal experiment a similar magnitude of response-rate reduction occurred as during these initial studies despite the use of vehicles identical to that used during regimens producing more modest withdrawal effects. The ability to recapture a marked withdrawal effect with THC regimens involving higher doses administered for a greater duration (during the reversal experiment) when the effect had been diminishing during earlier, repeated exposures to THC withdrawal also suggests that the monkeys became tolerant to the withdrawal effects of THC. Branch and co-workers (Branch *et al.*, 1980) also observed "tolerance" to repeated withdrawals of chronic THC administration. In their study all squirrel monkeys tested showed marked decreases in the rates of initiation of multiple response-operant tasks when THC was withdrawn for the first time, indicative of an abstinence effect. However, when daily THC administration was terminated for the second time the depressive effect on rate was not observed. It is interesting to speculate what the determinants of the development of tolerance to the withdrawal effects of a drug may be. Is there a parallelism between the determinants of the development of tolerance to the effects of a drug's withdrawal and its direct effects? An examination of the determinants of the development of tolerance to the withdrawal effects on operant behavior of THC and other drugs is an objective for future research.

The principal datum used to detect and track the dependence effects of THC in this study was rate of schedule-controlled behavior. Just as the rate of behavior has been shown to have advantages when used in the analysis of the effects of drug presence, so too can these advantages be applied to studying the effects of drug absence, such as in the analysis of drug dependence (Balster, 1985; Schuster and Thompson, 1969). Response rate is an objective measure that is quantifiable and highly sensitive. Response rate is also a sensitive measure of drug abstinence, often indicating a withdrawal effect in animals that appear outwardly normal. For instance, in the present study reductions in response rates for food indicative of drug dependence often occurred in the absence of observable changes in gross behavior. A similar sensitivity was shown by Slifer and her colleagues (Slifer *et al.*, 1984) when they demonstrated reductions of rates of operant behavior during withdrawal from phencyclidine in the absence of dramatic changes in gross behavior. Another indication of the sensitivity of response rate for detecting drug dependence is seen by the longer duration of recovery of response rate relative to the duration of recovery of normal gross behavior. In the present study it often took over 2 weeks for response rates to return to control levels after withdrawal of THC infusion. Slifer *et al.* (1984), studying recovery from withdrawal of phencyclidine, and Holtzman and Villarreal (1973), studying recovery from withdrawal of morphine, also demonstrated that response rates of operant behavior remained disrupted much longer than the disruption of gross behavior. There have been other demonstrations of the usefulness of operant behavior in the analysis of drug dependence besides those mentioned above; for example, in the study of morphine dependence in cats (Djahanguiri *et al.*, 1966), in rats (Ford and Balster, 1976; Steinfels and Young, 1981; Tye and Iversen, 1975) and in rhesus monkeys (Thompson and Schuster, 1964), of ethanol dependence in rats (Ahlenius and Engle, 1974) and of THC dependence in squirrel monkeys (Branch *et al.*, 1980).

In an earlier study (Slifer *et al.*, 1984), disruptions in food-maintained operant behavior during withdrawal from continuous phencyclidine infusions in rhesus monkeys were observed which were similar to those observed during withdrawal from THC. These changes in operant behavior after phencyclidine were also unaccompanied generally by overt signs of abstinence. Although neither THC nor phencyclidine abusers typically show prominent signs and symptoms of withdrawal requiring medical treatment, as do abusers of opiates and depressants, it would appear that more subtle withdrawal effects on behavior could occur. Perturbations of behavior caused by drug withdrawal indicate behavioral dependence (Schuster and Thompson, 1969; Balster, 1985). Procedures, such as were described in this report, may prove very useful in studying the behavioral dependence-producing properties of drugs such as THC.

The clinical significance of our findings invite speculation. The dosage levels of THC we infused were modest and exposure was as short as 10 days. The fact that reliable behavioral effects were observed during withdrawal suggests that more attention should be focused on possible behavioral sequelae of discontinued cannabis use in human users. Clear withdrawal signs and symptoms have been observed in humans receiving high doses of THC (Jones and Benowitz, 1976) or smoked marihuana (Mendelson *et al.*, 1984); however, the more subtle behavioral effects which we suggest may be produced by low doses may contribute to the maintenance of cannabis use, and warrant further investigation.

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