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Cross-Tolerance Between Inhaled Cannabis and Intraperitoneal Injections of Δ^9-THC[1]

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FRIED, P. A. *Cross-tolerance between inhaled Cannabis and intraperitoneal injections of Δ^9 THC* PHARMAC BIOCHEM. BEHAV. 4(6) 635–638, 1976. – Male and female rats were exposed to Cannabis smoke or placebo once every second day for 32 days. Following these 16 trials all animals were injected once intraperitoneally with 4 mg/kg THC. After every third inhalation trial and after the injection the rats were placed on a movement sensor for 3 min Cannabis smoke significantly reduced activity, relative to baseline scores, during the first 10 inhalation trials but by the thirteenth exposure, tolerance was evident. When the animals were injected with THC, the male rats who had been exposed to Cannabis smoke significantly increased their activity whereas the females did not alter their activity relative to the last inhalation trial. In contrast rats of both sexes that had been exposed to placebo smoke significantly decreased their activity following the injection. This intermodal cross-tolerance is discussed in terms of the role of conditioning in the development of tolerance

Marihuana (Cannabis sativa) Δ^9-tetrahydrocannabinol Inhalation Injection Activity cross-tolerance

EXPERIMENTS examining the neurological, pharmacological, and behavioral effects of Cannabis and its constituents have used a number of modes of administration. Included among these are intraperitoneal (IP), intramuscular, intracerebral, and intravenous injections, oral administration, and inhalation. Very few studies have compared these different routes in a direct fashion (e g. [7, 8, 9]), and only one report has examined them with respect to tolerance. In a recent study [1] it was found that the rate of tolerance development in pigeons was approximately the same whether Δ^9-tetrahydrocannibinol (THC) was injected orally, intramuscularly, or intravenously.

In this present work, a related problem pertaining to the question of cross-tolerance between different modes of Cannabis administration will be investigated. The question is of interest from an applied point of view as certain experimental procedures necessitate the changing from one mode of drug administration to another (e.g., examining drug effects during pregnancy and in the offspring [6]) and, additionally, some drug-users alternate between different modes of marihuana intake From a theoretical point of view the issue is germane to the problem of the role of conditioning in the development of tolerance [16] According to this theory, anticipatory responses to the drug effects serve to attenuate such effects and manifest themselves by a diminished response to the drug As a large component of the anticipatory responses are elicited by the administration procedures, altering the procedures would be predicted to attenuate tolerance effects.

METHOD

Animals

Eight male and 8 female naive Wistar rats, approximately 60 days of age were used All animals were housed individually with food and water available ad lib

Apparatus

The activity sensor utilized was a modification of one described by Remington and Anisman [15] Essentially it consisted of an 8 W speaker measuring 20 cm in dia covered by a 0.012 cm mylar sheath. A constant air pressure was produced between the speaker and the sheath by sealing the mylar between two "O" rings attached to the metal frame of the speaker. By placing an animal on the mylar sheath, air pressure displacement induced a deflection in the speaker cone and varied the electromagnetic flux. The speaker was attached to a variable calibrated transformer, thereby allowing for an adjustment of sensitivity of the apparatus. The speaker was housed in a cylindrical tube of clear plastic 20 cm high and was connected, via the transformer to one channel of a 10 channel Beckman type CE polygraph. Pen deflections of the polygraph corresponded directly with the intensity and frequency of the animal's movements.

The inhalation chamber consisted of a transparent, plastic rectangular box (30 cm high × 20 cm wide × 15 cm deep) with a grid floor Underneath the floor were 3 openings. Through 2 of these a mixture of smoke and air

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could be pumped by means of a respirator previously described [7]. Through the third opening the smoke could be evacuated by connecting it to the expiration valve of the respirator. Animals were placed in and removed from the apparatus via a hinged lid.

Drugs

Cannabis sativa, provided by the Health Protection Branch of Health & Welfare Canada, containing 1.1% THC and unknown quantities of other cannabinoids was used in the experimental conditions whereas Cannabis placebo was used in the control conditions. The placebo had all cannabinoids removed and then was rehydrated to the same consistency as the experimental drug. Both forms of Cannabis were used to make filter tipped cigarettes containing 0.6 g of the plant. For IP injections synthetic THC dissolved in dehydrated alcohol (0.2 g/ml) was suspended in a mixture of 5% propylene glycol and 95% tween 80–saline (0.5 ml tween/47 ml saline) One mg THC was dissolved in 0.25 cc vehicle solution.

Procedure

After pilot work which took into consideration such factors as the rate of burning of the cigarettes, level of emotionality of the rat, and the general effectiveness of the procedures, the following parameters were employed the animal was exposed to the smoke in the closed box for 9 min with a period of 10 sec at the 4½ min mark during which the hinged lid was opened to dilute the smoke with air At the end of this time, less than 5% of the Cannabis material remained. The respirator was set at 45 cycles/min, 20 cm pressure, 1 1 ratio of inspiration to expiration, and a 50/50 mixture of air and smoke. The connection between the cigarette and respirator was such that the smoke did not pass through the filter portion of the cigarette but was blown directly into the smoke chamber As a maximum 50% of the THC in the cigarette was actually delivered to the animal [4,17] the amount of THC each rat was exposed to was a maximum of 3 3 mg (16 5 mg/kg). Although the quantification of the amount of THC received by an animal can only be approximated there was a considerable degree of standardization between animals

The eight rats of each sex were randomly divided into experimental and control groups and each animal was placed on the activity sensor for 3 min. This was considered baseline activity. The subsequent experimental period lasted for 36 days and consisted of rats in the experimental group being exposed to Cannabis smoke and rats in the control group being exposed to smoke from placebo material 16 times – once every 48 hr. Fifteen min after every third exposure to smoke each animal was placed on the movement sensor for 3 min. Two days after the 16th inhalation trial all animals were given 4 mg/kg THC IP and 20 min later were placed on the activity sensor for 3 min.

RESULTS

Using an analysis of variance with sex and drugs as between variables and testing days as a within variable no differences between groups occured either on the baseline day nor the first day of Cannabis or placebo inhalation There was, however, a significant decrease in activity on the first inhalation day when contrasted to the baseline record $F(1,12) = 7.58$, $p = 0.018$.

The lack of a drug × day interaction indicates that both Cannabis smoke and placebo initially suppress activity (Fig. 1). However, as can be seen in the graphical representation of the results, the rate of tolerance development was much more rapid in the placebo group than in the experimental group. By the third day on the activity sensor (fourth inhalation) the placebo group did not differ significantly from its baseline activity whereas the experimental group was still significantly slower $F(1,6) = 5.69$, $p<0$ 05 By the last 2 activity recordings (inhalation 13 and 16) the experimental and control group did not differ from one another nor from their baseline scores indicating the development of tolerance. When both groups were then given 4 mg/kg THC they differed significantly in their response. The control animals significantly decreased their activity $F(1,6) = 5.70$, $p<0.05$ relative to treatment Day 16 whereas the experimental animals actually increased their activity $F(1,6) = 11.36$, $p<0$ 02. In this latter analysis there was an effect of sex × drugs $F(1,6) = 17$ 96, $p<0.005$. The female experimental animals demonstrated tolerance between the two modes of administration by not changing their activity when switched from inhalation to IP injections whereas the male experimental animals demonstrated their tolerance by actually increasing their activity. Both sexes of the placebo group, when injected with THC, significantly decreased their activity.

DISCUSSION

The principle findings of this study are that inhalation of Cannabis containing cannabinoids reduces activity in rats, that tolerance develops to this behavior, and that cross-tolerance between Cannabis smoke and IP injections of THC is demonstrable.

It is apparent that exposure to placebo smoke also has a pronounced reducing effect upon activity. Unlike the Cannabis containing cannabinoids however, there is a very rapid rate of return to baseline levels and no cross-tolerance between the inhaled placebo material and injected THC was evident. The factors (pharmacological or other) underlying the diminution in activity following the initial exposure to placebo cannot be specified at this time.

The increased activity in male rats after they were given a single injection of 4 mg/kg THC following inhalations of Cannabis parallels the observation of Potvin and Fried [14] that increased activity, in males relative to controls, occurred following chronic IP injections of THC In the present work this increment in activity was not observed in the female rats although they were clearly tolerant to the drug effect on this behavioral measure The differences between the sexes may be due to the increased sensitivity of female rats to THC (e.g. [3]). It has been shown that low acute doses of THC has stimulant properties (e g. [5,13]) Therefore, to a tolerant male rat the effects of 4 mg/kg may manifest themselves by increased activity whereas in the tolerant female rat this dose may be of a high enough level so as not to potentiate this activity

The occurrence of cross-tolerance between the 2 modes of drug administration indicates that attenuation to THC can occur in the absence of conditioning cues which persistently precede the systemic effects This differs from a number of reports [2, 11, 16] in which tolerance to the analgesic effects of morphine have been shown to be dependent upon consistent stimulus events present at the time of drug administration. These discrepant results may

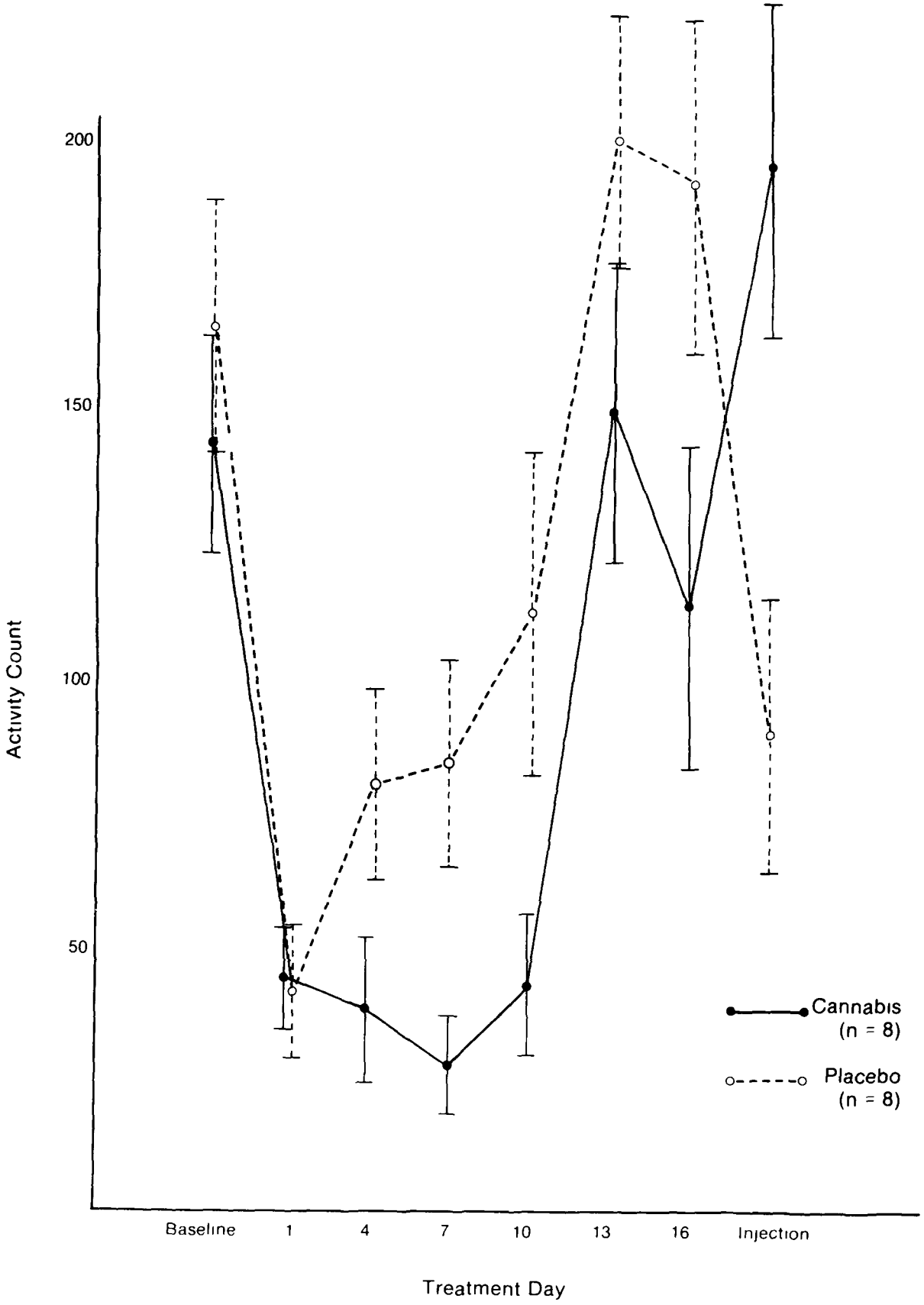

FIG. 1. Activity counts and standard errors during baseline, following 16 inhalations, and after 1 injection of 4 mg/kg Δ^9-THC. The male and female data are combined.

reflect basic differences in the mechanisms of tolerance to THC and morphine (or opiates in general) for tolerance development differs in a number of respects in these two drugs. For example, narcotic antagonists disrupt behavior in morphine tolerant animals but not in THC tolerant animals and cross-tolerance is not evident between morphine and THC [12] An alternate and more pragmatic explanation accounting for the finding of tolerance to THC but not to morphine in the absence of consistent preadministration cues is that the presence of these drug relevant cues augment the rate of tolerance development [10]. That is, if tolerance fails to manifest itself after a limited number of trials (e.g. 4 in Siegel's [16] study) that does not mean that the basic neurophysiological mechanisms underlying drug attenuation are not present but rather that they are masked or are of insufficent strength to be observed unless augmented by conditioned cues. This latter interpretation is consistent with findings from a number of studies reviewed elsewhere [5] that the rate of tolerance development to THC is accelerated by the opportunity or necessity to make compensatory responses while under the drug's influence

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