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INTERACTION BETWEEN DELTA-9-TETRAHYDRO-CANNABINOL AND KINDLING BY ELECTRICAL AND CHEMICAL STIMULI IN MICE

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Summary—Mice were kindled to produce minimal convulsions by repeated application of either electrical or chemical stimuli. Electrical kindling involved the use of corneal electrodes; chemical kindling involved the use of pentylenetetrazol or picrotoxin. Delta-9-tetrahydrocannabinol (delta-9-THC) appeared to be capable of enhancing kindling to all three stimuli. In the studies with electroshock and pentylenetetrazol, in contrast to those with picrotoxin, a single exposure to delta-9-THC sufficed to facilitate the subsequent kindling, and the enhancement of kindling persisted after withdrawal of the cannabinoid. In the case of electrical kindling, the cannabinoid may promote the phenomenon by decreasing the convulsion threshold. Because the threshold was not lowered to all kindling stimuli, however, other mechanisms must be involved. In general, the data indicate that even a single dose of delta-9-THC can promote the development of long-lasting elevations of CNS excitability.

Key words: delta-9-tetrahydrocannabinol, kindling, enhancement of kindling, convulsions.

Delta-9-THC has been shown to produce a variety of depressant and excitatory effects in the CNS (Paton, 1975). The diversity of effects is illustrated by the anticonvulsant and convulsant properties of the drug. For example, delta-9-THC is anticonvulsant in a variety of seizure tests (Karler and Turkanis, 1981), but it can also precipitate convulsions induced by cobalt and iron in epileptic rats (Chiu, Olsen, Borys, Karler and Turkanis, 1979; Turkanis and Karler, 1982), in epileptic beagles (Feeney, 1979), and in a strain of rabbits (Martin and Consroe, 1976). The drug can also produce more subtle proconvulsant effects in several electro- and chemoshock tests; that is, delta-9-THC can, in certain seizure models, lower the convulsion threshold (Karler, Cely and Turkanis, 1974).

In the present study, the interaction between delta-9-THC and various seizure models was extended to include the influence of the drug on kindling produced by electrical and chemical stimuli. Kindling is a phenomenon in which repeated treatment (e.g. once daily) with a subconvulsant electrical or chemical stimulus results in the development of overt convulsions (Racine, 1978); the reaction represents an increase in sensitivity caused by repeated exposure to the stimulus. The interaction between THC and kindling is of potential significance because the kindled state represents long-term changes in excitability of the CNS, and kindling has also been proposed as a model of the epilepsies (Racine, 1978).

METHODS

Experimental animals and drugs

Male CF-1 mice, weighing 20–25 g, were used throughout the experiments. They were housed in groups of about 25, fed *ad libitum*, and maintained on a 12-hr light–dark cycle in which the light cycle corresponded to daylight. Suspensions of delta-9-THC were prepared, as previously described (Turkanis, Cely, Olsen and Karler, 1974), in an isotonic sodium-chloride vehicle, containing 3% Tween 80. The other drugs were dissolved directly in isotonic sodium-chloride solution. In all experiments, the cannabinoid was administered intraperitoneally, and its effects on, or interactions with, either development of kindling or electrical or chemical convulsive thresholds, were determined at its time of peak concentration in brain, 2 hr (Karler, Borys and Turkanis, 1982). The relatively small (25 mg/kg) and large (150 mg/kg) doses of delta-9-THC used in the following experiments were taken from the present authors' previous observations of the doses required to cause excitation in different test systems (unpublished observations).

Kindling procedures

Animals were kindled electrically to induce minimal convulsions by stimulation with conventional, silver-corneal electrodes and a constant-current stimulator (Wahlquist Instrument Co., Salt Lake City, Utah; Sangdee, Turkanis and Karler, 1982). The corneal-electrode stimulation technique was identical to that frequently used in rodents for the administration of a conventional electroconvulsive shock. This technique of kindling with external electrodes differs from the traditional use of electrodes implanted in specific areas of brain; however, the results with the two different approaches appear to be indistinguishable (Sangdee *et al.*, 1982). The kindling

was produced by sinusoidal, 60-Hz, 2.6–3.5 mA, 2-sec stimuli, which are subthreshold for minimal convulsions in most naive mice. The specific intertest interval and current used are given below in the Figure legends; the current in each experiment was determined experimentally. The end-point for electrically-kindled minimal convulsions was jaw or front-paw clonus.

Animals were kindled chemically to produce minimal or maximal convulsions by repeated administration of pentylenetetrazol. The doses of pentylenetrazol for kindling were 35–45 mg/kg (s.c.) for minimal and 85 mg/kg (s.c.) for maximal convulsions. The frequency of administration and the dose are given below in the Figure legends; the dose in each experiment was determined experimentally. The end-point for minimal convulsions was jaw or front-paw clonus; for maximal convulsions, hind-limb extension. Animals were also kindled to produce minimal convulsions with picrotoxin (1 mg/kg, s.c.).

Tests of electroshock and chemoshock convulsive thresholds

The test for electroshock threshold involved minimal convulsions caused by the same 60-Hz, 2-sec stimulus used in the kindling experiments. In the pentylenetetrazol threshold tests, the minimal doses required to produce both minimal and maximal convulsions were determined by administering the drug through a tail vein, with the use of a Harvard apparatus infusion pump. The flow rate for pentylenetetrazol was 0.54 ml/min (3.0 mg/min); for picrotoxin, 0.54 ml/min (0.81 mg/min). All drug solutions given intravenously contained heparin (final activity, 10 units/ml).

Experimental design and analysis of data

In the experiments designed to measure the influence of delta-9-THC on the development of kindling, groups of 25–50 animals were subjected to the kindling procedures, and the percentage of animals exhibiting convulsions at each stimulus time was determined; the percentages were plotted as a function of the day of stimulation. In these experiments, the frequency of administration of the various stimuli was selected arbitrarily. Because the interstimulus interval can affect the rapidity of kindling (Sangdee *et al.*, 1982), the specific interval for each type of experiment was selected to yield a kindling rate which either could be measurably enhanced or depressed by delta-9-THC.

In the tests of electroshock threshold, groups of 35–50 mice were used and current–response curves were determined by the "staircase" method (Finney, 1971). The median-effective values (CD_{50}s) and their 95% confidence limits were derived from such curves, and the CD_{50}s were compared statistically by a relative potency test ($P < 0.05$; Litchfield and Wilcoxon, 1949).

The tests of chemoshock threshold involved 20–25 animals for each test. The mean doses of drug needed to produce minimal or maximal convulsions for the control and delta-9-THC-treated groups were determined and compared statistically by a *t*-test ($P < 0.05$; Snedecor and Cochran, 1976).

RESULTS

Figure 1 represents the influence of treatment with delta-9-THC on alternate days on the development of electrically-induced kindled minimal convulsions. The results demonstrate that the small dose of the cannabinoid retarded, whereas the large dose promoted, the development of the phenomenon. In addition, the findings showed on day 16, three days after withdrawal of drugs, a continuous development of kindling for each of the two groups previously treated with drug. Thus, the drug-induced changes in the development of the kindling process persisted following withdrawal of drug.

In the large dose, delta-9-THC not only enhanced the development of convulsions but caused the unique appearance of maximal convulsions to electrical stimulation. Although these data are not shown in Fig. 1, by the 9th day of the experiment about one-half of the animals treated with the large dose,

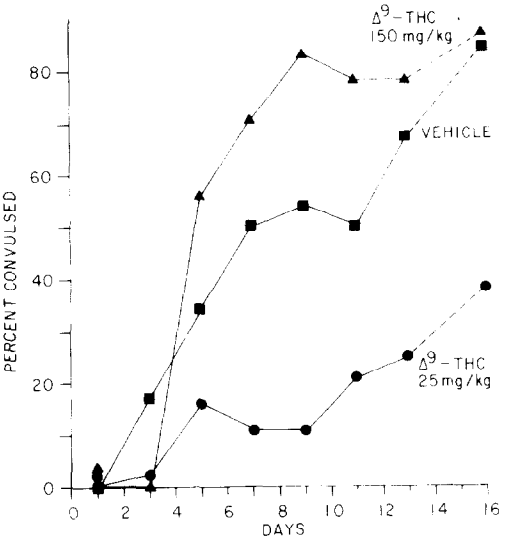

Fig. 1. Influence of delta-9-THC on electrical kindling to produce minimal convulsions. Three groups of 25 male mice were given either cannabinoid or vehicle (i.p.) every other day for 13 days; on each treatment day at the peak time of concentration in the brain (2 hr), the mice were subjected to a corneal electroshock-kindling stimulus (2 sec, 3.5 mA, 60 Hz) in order to determine the percentage of animals kindled to produce minimal convulsions. Animals were withdrawn from treatment after day 13; on day 16, the animals were given only electroshock. The solid lines represent the percentages of animals kindled in the presence of delta-9-THC or vehicle; the dashed lines, those kindled after withdrawal from delta-9-THC or vehicle. The treatment data were significantly different from the controls, as determined by the many-one rank statistic ($P < 0.01$; Steel, 1959).

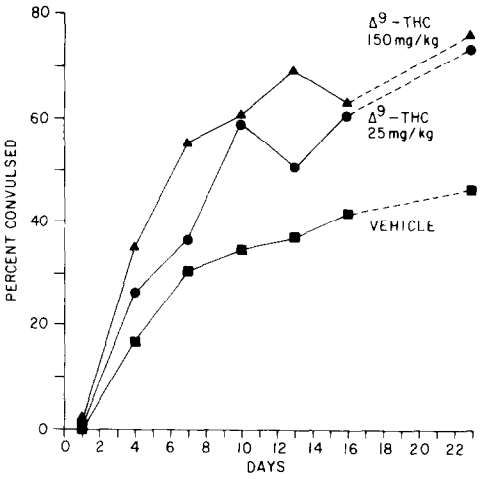

Fig. 2. Effects of delta-9-THC on kindling to minimal convulsions induced by pentylenetetrazol. Three groups of 25 animals were given cannabinoid or vehicle (i.p.) every third day for 16 days; on each day of treatment at the peak time of concentration in the brain (2 hr), the mice were given the kindling stimulus of pentylenetetrazol (40 mg/kg, s.c.) in order to determine the percentage of animals kindled to minimal convulsions. Animals were withdrawn from treatment after day 16; on day 23 the animals were subjected only to the kindling stimulus in order to determine the percentage of kindled animals after the withdrawal of the cannabinoid. The solid lines represent the percentages of animals kindled in the presence of delta-9-THC or vehicle; the dashed lines, those kindled after withdrawal from delta-9-THC or vehicle. The treatment data were significantly different from the controls, as determined by the many-one rank statistic ($P < 0.01$; Steel, 1959).

that were classified as kindled, were manifesting maximal convulsions. Because only minimal convulsions can normally be produced with this electrical stimulus (Sangdee *et al.*, 1982), the appearance of maximal convulsions must have been the consequence of the presence of the cannabinoid. On the 11th day of the experiment, however, there was a precipitous drop in the percentage of animals manifesting maximal convulsions, even though all animals which were considered to be kindled continued to exhibit minimal convulsions. The development of maximal convulsions and their sudden disappearance was a reproducible observation in three identical experiments.

The data in Fig. 2 illustrate the effect of delta-9-THC on kindling to produce minimal convulsions with pentylenetetrazol (40 mg/kg, s.c., daily). In this case both the small and the large doses of cannabinoid produced enhancement. As was the case with the electrical experiments, upon withdrawal of the treatment with delta-9-THC (day 16), the effect on kindling persisted (day 23). Although the data are not shown, in an identical experimental design, similar effects of the drug were also observed when pentylenetetrazol (85 mg/kg, s.c., daily) was used to increase the sensitivity of animals to maximal convulsions.

The influence of the large and small doses of delta-9-THC on kindling with picrotoxin is shown in Fig. 3. In this case, the results were the reverse of those seen in the electrical experiment (Fig. 1): the large dose inhibited and the small dose enhanced the development of kindling. On day 16, two days after withdrawal of the cannabinoid, the percentages of animals convulsing to the stimulus of picrotoxin were approximately equal in the three groups; that is, both effects of delta-9-THC disappeared after withdrawal.

The data shown in Fig. 4 illustrate the influence of a single exposure to delta-9-THC on the subsequent kindling to electrically-induced minimal convulsions. On the first day of the experiment, the animals were given the dose of delta-9-THC (150 mg/kg) that facilitated the kindling shown in Fig. 1. After the administration of the drug, animals were electroshocked and a greater number in the cannabinoid group convulsed than did those in the control group. After day 1, the drug was withdrawn and the animals were subjected only to electroshock on days 2 through to 7. As can be seen, even a single exposure to the drug caused enhancement. After day 7, daily electrical stimulation was suspended for 15 days, at which time the animals were again electroshocked to determine how many remained kindled. Essentially

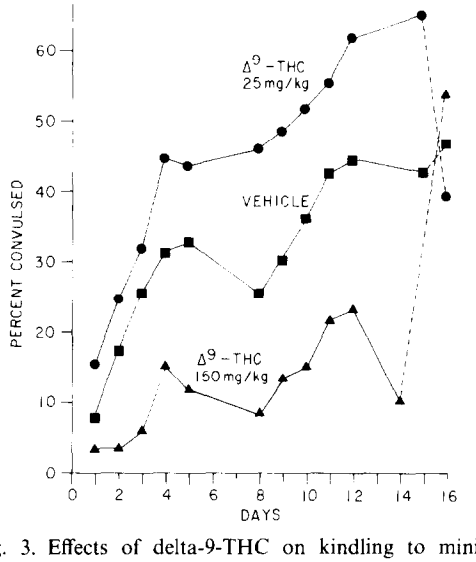

Fig. 3. Effects of delta-9-THC on kindling to minimal convulsions produced by picrotoxin. Three groups of 25 mice were given cannabinoid or vehicle (i.p.) once daily, 5 days/week for 2 weeks; on each treatment day at the time of peak concentration in brain (2 hr), the mice were given the picrotoxin-kindling stimulus (1 mg/kg, s.c.) in order to determine the percentage of animals kindled to minimal convulsions. On day 14 the animals were withdrawn from treatment; on day 16 the animals were subjected only to the kindling stimulus in order to determine the percentage of kindled animals after the withdrawal of the cannabinoid. The solid lines represent the percentages of animals kindled in the presence of delta-9-THC or vehicle; the dashed lines, those kindled after withdrawal from delta-9-THC or vehicle. The treatment data were significantly different from controls, as determined by the many-one rank statistic ($P < 0.01$; Steel, 1959).

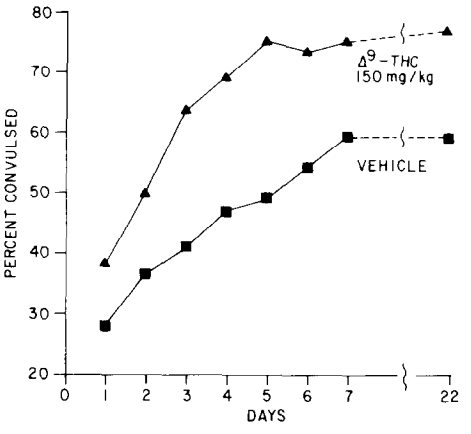

Fig. 4. Effect of a single exposure to delta-9-THC on the rate of electrical kindling. Two groups of 39 mice were subjected once daily for 7 days to a corneal electroshock-kindling stimulus (2 sec, 2.6 mA, 60 Hz). On day 1, the animals were pretreated 2 hr prior to the electroshock with either 150 mg/kg of delta-9-THC or vehicle. On days 2–7, both groups were only given electroshock. Both groups were withdrawn from electroshock treatment on day 7; on day 22 each group was again given electroshock to determine the persistence of the enhanced sensitivity to the kindling stimulus. The treatment data were significantly different from the controls, as determined by the Mann–Whitney *U*-test ($P < 0.025$; Siegel, 1956).

the same number of animals convulsed on day 22 as did on day 7.

An experiment comparable to that used for Fig. 4 was designed to show the effect of a single exposure to delta-9-THC on kindling to produce minimal convulsions by pentylenetetrazol (Fig. 5). Here again, a single exposure (day 1) to the cannabinoid subsequently increased kindling by pentylenetetrazol. Again, after 15 days of withdrawal from daily treatment with pentylenetetrazol, the animals were given the convulsant to determine the persistence of the increased sensitivity to convulsions. As in the case of the electrical experiment (Fig. 4), most of the animals remained kindled. The results of a single exposure to delta-9-THC on electrical and pentylenetetrazol stimuli contrast with those from a similar experiment with picrotoxin. In the latter case, a single exposure to the cannabinoid exerted no effect on the subsequent kindling.

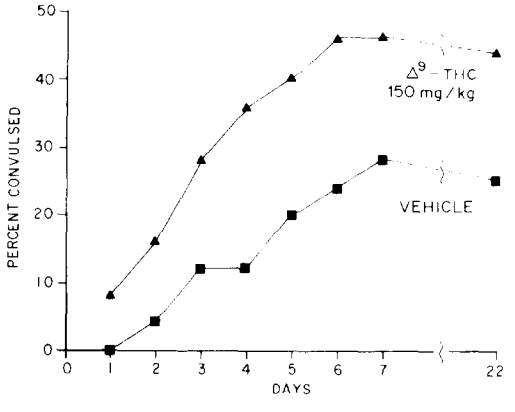

Fig. 5. Effect of a single exposure to delta-9-THC on the rate of pentylenetetrazol kindling. Two groups of 25 mice were kindled to pentylenetetrazol by the once daily administration of 35 mg/kg (s.c.) for 7 days. On day 1, animals were pretreated 2 hr prior to the chemoshock with either 150 mg/kg of delta-9-THC or vehicle. On days 2–7, both groups were only given chemoshock with pentylenetetrazol. Both groups were withdrawn from treatment with chemoshock on day 7; on day 22 each group was again given chemoshock to determine the persistence of the enhanced sensitivity to the kindling stimulus. The treatment data were significantly different from the controls, as determined by the Mann–Whitney *U*-test ($P < 0.025$; Siegel, 1956).

Table 1 includes the results of the influence of the large and small doses of delta-9-THC on the thresholds for convulsions induced by the electrical and chemical stimuli used in the kindling studies: the large dose of the cannabinoid lowered the threshold for the electrically-evoked convulsions, whereas the small dose raised the threshold. In comparable threshold studies with pentylenetetrazol, the large dose of cannabinoid had no effect on the threshold for minimal convulsions, but the small dose decreased the threshold. Although the data are not shown, the opposite effects were obtained for the threshold for maximal convulsions caused by pentylenetetrazol: the large dose decreased the threshold, while the small dose was ineffective. The influence of delta-9-THC on the threshold for minimal convulsions caused by picrotoxin differed markedly from that seen in the studies with pentylentetrazol. With picrotoxin, the large dose of cannabinoid significantly increased the threshold, an anticonvulsant effect compared with the proconvulsant interactions with pentylenetetrazol. No effect on the convulsive threshold for picrotoxin was observed with the small dose of delta-9-THC.

DISCUSSION

The data presented indicate that delta-9-THC can enhance kindling to either electro- or chemoshock. The results of the interaction with kindling again illustrate the excitatory potential of this cannabinoid, although its interactions with the different stimuli appeared to be different. In the cases of electroshock and pentylenetetrazol, delta-9-THC caused an effect on kindling that persisted for several days after withdrawal from the treatment with the cannabinoid. The persistence of the effect is in striking contrast to the results from the study with picrotoxin, in which withdrawal from the cannabinoid resulted in reversal of the effect on kindling; that is, after withdrawal of drug, the number of animals kindled in the treatment and control groups was similar.

The effects of delta-9-THC on the seizure threshold for the various types of kindling stimuli suggest a possible mechanism by which the drug enhances kindling. In two of the tests the drug lowered the convulsion threshold, which may account for the enhancement: on the one hand, the large dose of delta-9-THC lowered the electrical convulsion thresh-

Table 1. Influence of delta-9-THC on convulsion thresholds to electrical and chemical stimuli

Threshold test	Minimal convulsion threshold
	Median-effective currents and 95% confidence limits (mA)
Electrical, 60 Hz, 2 sec	
Control	3.05 (2.9–3.2)
Delta-9-THC, 25 mg/kg	3.30 (3.2–3.4)*
Delta-9-THC, 150 mg/kg	2.70 (2.6–2.8)*
	Means and standard deviations (mg/kg, i.v.)
Pentylenetetrazol	
Control	44 ± 8
Delta-9-THC, 25 mg/kg	37 ± 5†
Control	51 ± 8
Delta-9-THC, 150 mg/kg	49 ± 6
	Means and standard deviations (mg/kg, i.v.)
Picrotoxin	
Control	39 ± 4
Delta-9-THC, 25 mg/kg	41 ± 5
Control	31 ± 6
Delta-9-THC, 150 mg/kg	38 ± 8†

*Significantly different from control, as determined by the method of Litchfield and Wilcoxon (1949; $P < 0.05$). †Mean value is significantly different from that of the control, as indicated by a t-test ($P < 0.01$).

old and facilitated kindling; on the other hand, the small dose, which elevated the convulsion threshold, retarded the reaction. In this particular study, the effect of the drug on the threshold correlated with that on kindling. A decrease in threshold suggests that delta-9-THC raised the probability of an afterdischarge (AD) to a given stimulus; such an effect may provide the basis for the enhancement caused by the cannabinoid because the afterdischarge is known to be essential for the development of the kindling reaction (Racine, 1978). A reduction in threshold cannot, however, explain all of the observed increases in kindling by delta-9-THC. In the case of pentylenetetrazol both the small and large doses of the cannabinoid promoted kindling, but only the small dose lowered the threshold. Because the threshold was not lowered in all instances, the effect must involve additional phenomena. Another possible explanation for the facilitation by delta-9-THC of kindling without an effect on threshold hinges on the ability of the drug to affect the afterdischarge directly; that is, the cannabinoid can prolong the duration of the afterdischarge (Turkanis and Karler, 1981). In this respect the cannabinoid mimics the kindling process because as sensitivity to convulsions develops, the afterdischarge becomes prolonged (Racine, 1978).

The results of the interaction between delta-9-THC and the kindling reaction are consistent with previous observations that indicate that the drug has both excitatory and depressant properties (Paton, 1975; Turkanis and Karler, 1981). In the present studies the anticonvulsant activity of the drug was also apparent in the instances in which kindling was decreased; conversely, the proconvulsant activity was evident in those cases in which the phenomenon was increased. The ability of the drug to promote the reaction, however, is of particular significance toxicologically because kindling is known to involve relatively long-lasting changes in excitability of the CNS (Racine, 1978; Sangdee *et al.*, 1982). The data demonstrate that delta-9-THC can, therefore, contribute to relatively persistent alterations in CNS excitability; even a single dose is capable of enhancing these excitatory changes. Although the extrapolation of these results to man is difficult, the data suggest that marijuana may interact with environmental or pathological factors to increase CNS excitability.

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REFERENCES

Chiu P., Olsen D. M., Borys H. K., Karler R. and Turkanis S. A. (1979) The influence of cannabidiol and delta-9-tetrahydrocannabinol on cobalt epilepsy in rats. *Epilepsia* **20:** 365–375.

Feeney D. M. (1979) Marihuana and epilepsy: Paradoxical anticonvulsant and convulsant effects. In: *Marihuana: Biological Effects. Advances in the Biosciences, Vols 22 and 23* (Nahas G. G. and Paton W. D. M., Eds), pp. 643–657. Pergamon Press, Oxford.

Finney D. I. (1971) *Probit Analysis*, 3rd edn. Cambridge University Press, Cambridge.

Karler R. and Turkanis S. A. (1981) The cannabinoids as potential antiepileptics. *J. clin. Pharmac.* **21:** 437S–448S.

Karler R., Cely W. and Turkanis S. A. (1974) Anticonvulsant properties of delta-9-tetrahydrocannabinol and other cannabinoids. *Life Sci.* **15:** 931–947.

Karler R., Borys H. K. and Turkanis S. A. (1982) Influence of 22-day treatment on the anticonvulsant properties of cannabinoids. *Naunyn-Schmiedebergs Arch. Pharmac.* **320:** 105–109.

Litchfield J. T. Jr and Wilcoxon F. (1949) A simplified method of evaluating dose-effect experiments. *J. Pharmac. exp. Ther.* **96:** 99–113.

Martin P. and Consroe P. (1976) Cannabinoid induced behavioral convulsions in rabbits. *Science* **194:** 965–967.

Paton W. D. M. (1975) Pharmacology of marijuana. *A. Rev. Pharmac.* **15:** 191–220.

Racine R. (1978) Kindling: The first decade. *Neurosurgery* **3:** 234–252.

Sangdee P., Turkanis S. A. and Karler R. (1982) Kindling-like effect induced by repeated corneal electro-shock in mice. *Epilepsia* **23:** 471–479.

Siegel S. (1956) Chapter 6. The case of two independent samples. In: *Nonparametric Statistics for the Behavioral Sciences*, pp. 119–121. McGraw–Hill, New York.

Snedecor G. W. and Cochran W. G. (1976) In: *Statistical Methods*, 6th edn. Iowa State University Press, Ames.

Steel R. G. D. (1959) A multiple comparison rank seem test: treatments versus control. *Biometrics* **15:** 560–572.

Turkanis S. A. and Karler R. (1981) Electrophysiologic properties of the cannabinoids. *J. clin. Pharmac.* **21:** 449S–463S.

Turkanis S. A. and Karler R. (1982) Central excitatory properties of delta-9-tetrahydrocannabinol and its metabolites in iron-induced epileptic rats. *Neuropharmacology* **21:** 7–13.

Turkanis S. A., Cely W., Olsen D. M. and Karler R. (1974) Anticonvulsant properties of cannabidiol. *Res. Commun. chem. Path. Pharmac.* **8:** 231–246.