# Behavioral Effects of Low Doses of DDT (35703)

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(Introduced by J. Reilly)

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Early reports of dichlorodiphenyltrichloroethane (DDT) poisoning in animals described such signs as tremors, augmented reflexes, nervousness, sensitivity to stimuli, and general irritability (1). These effects indicated that DDT may have a considerable impact on the emotional state of an animal. However, in spite of the apparent central activity of DDT, until recently little effort had been made to experimentally define the qualitative effects that this compound exerts on behavioral systems.

Over the past several years, the resurgence of interest in DDT has revealed that this widely used and much investigated pesticide exhibits some very interesting and specific effects on neuronal tissue. Bleiberg *et al.* (2) suggested that DDT, through the action of some of its metabolites, might cause a functional, pharmacological denervation of the cholinergic system. More recently, DDT has been shown to possess the rather unique ability to disrupt the neuronal transport mechanism of potassium ions (3–5). These effects of DDT are highly suggestive of an association of this compound with central neuronal inhibition. The cholinergic system, the function of which may be attenuated by DDT metabolites, is generally considered to comprise a considerable part of the central inhibitory systems (6–9). Moreover, the antagonism of the $K^+$ flux by DDT is further suggestive evidence of an effect on central inhibition, since $K^+$ has been shown to exert an integral function in the development of inhibitory postsynaptic potentials in higher centers of the nervous system, especially in the hippocampal portion of the limbic system (10).

The present study describes the influence of DDT on behaviors intimately associated with central inhibitory systems, such as habituation (11) and attenuation of ongoing behavior in response to stress (8). In order to characterize more generally the state of the central nervous system in the presence of DDT, the pattern of maximum electroshock seizure has also been investigated.

*Materials and Methods. Animals.* Adult male albino mice (Flander's Research Farm), weighing an average of 27 g, were used. Food and water were provided *ad libitum* and all animals were housed in groups of 15–20 per cage in a constant-temperature environment.

*Maximum electroshock seizure (MES).* Maximum electroshock seizure was induced by a single shock of 30 mamp/0.2 sec delivered via corneal electrodes. The durations of the following components of the seizure pattern were measured: tonic flexion, tonic extension, and clonus.

DDT in corn oil was administered orally at 0, 0.25, 0.50, 1.0, 10.0, or 25.0 mg/kg to a total of 86 mice, and the MES pattern was observed for each group at 1, 4, and 24 hr after dosing.

*Open-field activity.* Exploratory behavior, generally regarded as an index of emotionality in animals (12, 13), was measured by the use of a large rectangular box (10 × 18 × 6 in.) with a marked grid floor. Single doses of DDT in corn oil were administered orally at 0, 1.0, 10.0, or 25.0 mg/kg body weight to a total of 50 mice. Twenty-four hours later, each mouse was tested individually in the open-field apparatus. The average number of squares traversed per minute for a 5-min period was used as exploratory activity.

As an index of habituation to the novel environment, the difference between the average activity of the first 2 and the last 2 min was calculated. A large difference (the aver-

age activity of the last 2 min being smaller) was taken to imply a rapid suppression of motor exploration, *i.e.*, a high degree of habituation; in contrast, a smaller difference would indicate relatively little motor suppression or habituation to the novel environment. The use of a decline in exploratory activity over a period of time as an index of habituation is based on the method of Carlton (14).

*Passive avoidance.* Fear-induced suppression of motor activity (passive avoidance) was measured in a standard passive avoidance situation (15). The apparatus consisted of a small chamber (3.5 × 6.5 in. with a grid floor) adjoining a larger chamber (13.5 × 13.5 in. with a grid floor) with a door between them. Four hours after orally dosing the mice with DDT in corn oil at 0, 0.5, 1.0, or 10.0 mg/kg (a total of 80 mice), each mouse was placed in the small chamber facing away from the door. The door was immediately opened, thereby activating a timer. The time (latency) to enter the larger chamber was recorded. As soon as the animal entered the large chamber, the door was closed and a shock of 8 mamp/0.2 sec was delivered through the grid floor. Mice were retested on the next day. All data were statistically analyzed using the Student *t* test.

*Results. Maximum electroshock seizure pattern.* The effects of DDT on MES seizure pattern are shown in Fig. 1. Each of the seizure parameters of the DDT groups were compared to those of the control group; the values are expressed as percentage of control. In general, DDT treatment resulted in a shortening of the tonic flexor duration and prolonged tonic extensor period. An attenuation of clonus tended to occur but this, generally, did not reach statistical significance. Surprisingly, these changes induced by DDT, especially involving tonic flexion and tonic extension, were more apparent at the lower than at the higher doses.

Alteration of the seizure pattern was already evident 1 hr after dosing. The peak effects of DDT appeared at 4 hr after administration and were still apparent at 24 hr.

*Open-field activity.* The effects of DDT on exploratory activity in and habituation to a novel environment are shown in Table I. Twenty-four hours after an oral dose of DDT at 25 mg/kg, exploration was significantly increased compared to that of the control mice given only corn oil. This same dose also significantly attenuated the animals' habituation to the open-field chamber. The lower doses had no significant effect on either exploration or habituation.

*Passive avoidance.* As shown in Table II, DDT treatment at all three doses 4 hr before the preshock trial and 24 hr before the post-

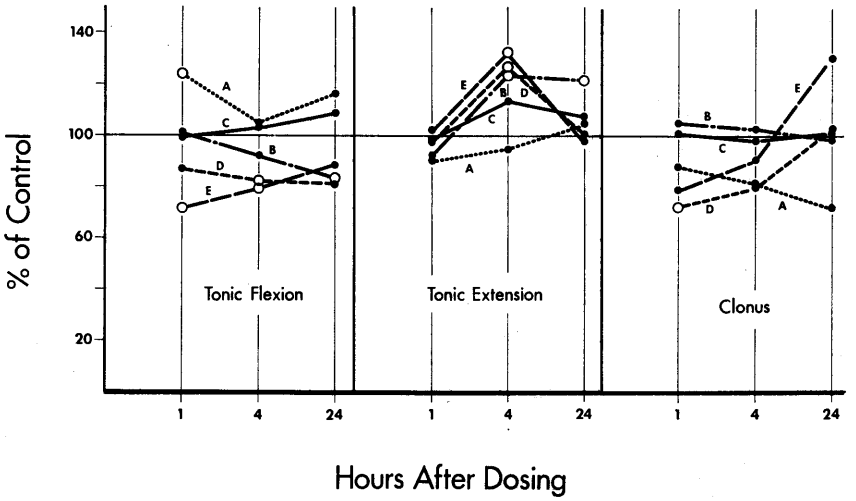


FIG. 1. Time course of the effect of DDT on maximum electroshock seizure pattern. Large circles indicate those values which are significantly different from control values ($p<.05$). Dosages, in mg/kg: A, 25; B, 10; C, 1.0; D, 0.5; E, 0.25.

TABLE I. Effect of DDT on Open-Field Exploration and Habituation of Mice at 24 Hours Postdosing.[a]

| DDT, mg/kg | Exploration[b] | Habituation[c] |
|---|---|---|
| 0 | 40.8 ± 1.6 | 21.3 ± 2.2 |
| 1 | 39.4 ± 3.3 | 17.0 ± 2.0 |
| 10 | 42.6 ± 2.5 | 21.8 ± 2.2 |
| 25 | 47.6[d] ± 3.4 | 12.8[e] ± 2.7 |

[a] Total of 50 mice used, 20 controls and 10 per test group.

[b] Average number of squares crossed per minute ± SE.

[c] Difference between first 2-min and last 2-min averages of exploratory period ± SE.

[d] $p < .05$.

[e] $p < .01$.

shock trial tended to decrease the average latencies to enter the stress chamber during the postshock trial compared to the entrance latencies of the control group. However, because of the variance in response latencies, only the 10 mg/kg group differed statistically from the control postshock latency.

*Discussion.* The behavioral and physiological effects described for DDT in this work may indicate an antagonism of central inhibitory systems by DDT, *i.e.*, the development of disinhibition. In the presence of DDT the development of habituation to a novel environment was shown to be significantly retarded. Habituation is generally agreed to be based on central inhibitory systems (11) and to be dependent on an adequately functioning cholinergic system (14).

TABLE II. Effect of DDT on Passive Avoidance in Mice.[a]

| DDT, mg/kg | Preshock trial latency, sec[b] ± SE | Latency of postshock trial, sec[c] ± SE |
|---|---|---|
| 0 | 8.4 ± 0.9 | 26.5 ± 9.4 |
| 0.5 | 6.6 ± 0.7 | 19.8 ± 9.3 |
| 1.0 | 5.6 ± 0.6 | 16.9 ± 5.6 |
| 10.0 | 7.4 ± 0.9 | 10.1[d] ± 1.4 |

[a] Total of 80 mice used, 20 per group.

[b] DDT was given 4 hr before preshock trial.

[c] Postshock trial was given approximately 24 hr after DDT.

[d] $p < .05$.

Facilitation of overall exploratory activity by DDT demonstrated in this work may also indicate a release of motor activity curbed by some anxiety-induced internal inhibition (16).

That DDT exerts a substantial disinhibitory effect was indicated by its ability to antagonize the motor depression induced by the stress of an electroshock punishment. This is not due to an enhanced exploratory activity, since equivalent doses did not alter open-field exploration at a similar time after dosing. Preliminary work in this laboratory has also shown that this effect is not due to an impairment of memory, since a comparable low dose of DDT did not impair acquisition or retention in an active avoidance situation. This effect of DDT takes on additional importance in view of the fact that, according to Stein (8), a substantial effect on passive avoidance situations is produced only by minor tranquilizers.

The qualitative alterations of MES seizure patterns by DDT were also in keeping with an antagonistic effect on inhibitory systems. The decreased duration of tonic flexion and prolongation of tonic extension reflect an overall increase in brain excitability (17). This general increase in excitability may be partially explained by the ability of DDT to induce repetitive neuronal discharge, thereby contributing to maximum central neuronal facilitation. However, the possibility that removal of inhibition may contribute to this central excitation is indicated by the tendency for clonus to be depressed; this seizure component is thought to be due to an active inhibitory system (18). The more pronounced effect of DDT on MES seizure at the lower doses may represent a relatively select action of this agent on some central neuronal system. At the higher doses the more general and complex actions of DDT may effectively mask the facilitation of MES seizure at the lower doses. In fact, an attenuation of MES seizure was found by Woolley (19) at doses of DDT slightly higher than those employed in the present experiment.

The present study indicates that DDT at doses well below toxic levels facilitates central excitatory processes. This central facilita-

tion may involve a disinhibition, *i.e.*, a release from central inhibitory control, rather than an exclusive direct stimulation of excitatory neurons. The degree of cholinergic involvement in this effect could be tested by employing selected pharmacological agents.

*Summary.* Several behavioral and neurophysiological parameters were altered in mice acutely dosed with low doses of DDT. Open-field exploratory activity was significantly enhanced 24 hours after an oral dose of DDT at 25 mg/kg. Concomitantly, the animals' ability to habituate to the open-field situation was attenuated. In a passive avoidance test DDT, at doses lower than 25 mg/kg, alleviated the stress-induced motor depression. Selected changes in the maximum electroshock seizure pattern reflected an increase in brain excitability. The possibility is advanced that DDT facilitates the central excitatory process, at least partially, by a disinhibitory mechanism.

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