EFFECT OF SUBLETHAL DDT ON A SIMPLE REFLEX IN BROOK TROUT

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Abstract

Brook trout were conditioned in about 30 trials to exhibit the propeller-tail reflex, using electric shock as the unconditioned stimulus and light as the conditioning stimulus. When the trout were first exposed for 24 hours to sublethal DDT (20 ppb), more than half could not be conditioned at all; the remainder required significantly more trials than did the untreated ones. Three to four weeks following initial testing, the DDT effect had largely disappeared. It seemed likely that the DDT was acting upon central nervous system structures. The general ecological implications of the results are discussed.

Introduction

Fish which have been exposed to very low doses of DDT may seem perfectly normal to the casual observer. Experimentation, however, often reveals profound physiological and behavioural disturbances. Changes have been observed in temperature selection (Ogilvie and Anderson, 1965; Javaid, 1967), lower lethal temperature (Elson and Kerswill, 1966; Elson, 1967), lateral line function (Anderson, 1968), EEG pattern of the cerebellum (Aubin and Johansen, 1969), and the cold-blocking temperature of a simple reflex (Anderson and Peterson, 1969).

Recent work suggests that instrumental learning may also be affected by sublethal DDT (Anderson and Peterson, 1969). Brook trout, exposed for 24 hours to 20 ppb DDT, could not be trained to exhibit a simple visual conditioned avoidance response. The results suggested that short-term memory was being affected. However, the inability of the fish to become conditioned in these experiments was because they evidently failed to make an association between the doorway connecting the two compartments in the shuttle box apparatus and the unconditioned stimulus (US), electric shock. (Change of light intensity between the two compartments was the conditioning stimulus (CS).) The possibility therefore existed that either sensory or motor impairment, or both, was the cause for the failure to establish learning. At any rate, the results did not seem to warrant the generalization that learning in the usually accepted sense, i.e. changes in the central nervous system, was being affected by the DDT.

The experiments reported here deal with the effect of sublethal DDT on a conditioned response involving an extremely simple reflex (the propeller-tail reflex). The greatly reduced complexity of the sensory and motor apparatus required to exhibit this response would make any observed changes in the rate of learning much more likely to be a function of the central nervous system.

Materials and Methods

Mild stimulation of the gular region of fish results in a weak propeller-like movement of the tail (von Holst, 1934). This simple reflex was elicited using mild electric shock as US. Light was the CS and the experiment was designed to test whether previous exposure to DDT affected the rate of establishment of the conditioned response. The apparatus and experimental protocol are depicted in Figure 1.

The CS was delivered from a Grass P8 stimulator and consisted of a 500 m sec train of square wave pulses (1.5 m sec and approximately 2.5 volts each at a rate of 400 per sec). The learning criterion was 5 consecutive correct responses, a "correct response" being the reflex appearing after the CS but before application of the US. If conditioning was not established by 100 trials, the experiment was discontinued.
The fish were brook trout (Salvelinus fontinalis Mitchell) and were approximately one and one-half years old at time of testing. Weight ranged from 110 to 180 g. They were fed DDT-free beef liver once daily. To minimize the amount of detritus present, the fish were not fed for 3 days prior to and during the period of treatment. Fish were exposed to 20 ppb of DDT for 24 hours in 9 liters of continuously aerated water in a glass jar, one fish per jar. The temperatures for acclimation, exposure, and testing were the same, 12±1.0°C. DDT was added to the water in 0.45 ml acetone. Control fish were treated the same as were the experimental ones, except that no DDT was dissolved in the acetone. All testing was done in clean water immediately following the 24 hour exposure to DDT.

Results

Of the 16 control fish, all fish, with two exceptions (at 51 & 90 trials) took no more than 50 trials to be conditioned (Figure 2). The average was 29 trials. In contrast, 10 of the 16 DDT-treated fish failed to become conditioned after 100 trials and the remainder took 60 or more trials, the average being 76. Clearly DDT treatment prior to training markedly affected the rate of learning of the propeller-tail reflex.

It had been planned to re-test all of the fish at various intervals following first training to investigate the permanency of the DDT effect. Unfortunately the survival of fish following the initial experiments was not good. The cause seemed to be stress imposed upon the fish by the holding apparatus. Scale loss of the fish after removal from the apparatus was particularly heavy.

The results from fish which did survive, however, are revealing. All re-testing was done from 3 to 4 weeks following the trials. Of the 4 surviving untreated fish, the average number of trials at first testing was 28, and in all cases but one the fish took 8 or 9 fewer trials at second testing; in the case of the exception, the fish took 11 more trials than initially, bringing the overall average to 27. Of the 9 surviving DDT-treated fish, 4 at first testing had not been trained by 100 trials; the remainder had an average of 73 trials. But at second testing, while one of the original 4 was still not trained by 100 trials, all the rest were trained in significantly fewer trials, the average being 38.

Discussion

Classical conditioning (of the propeller-tail reflex) is made markedly more difficult by prior exposure of the fish to sublethal DDT. Unlike the previous work dealing with instrumental learning, the chances of motor impairment being responsible for the results seem remote. However, it was observed that the US could elicit the propeller-tail reflex at a lower voltage for the DDT-treated fish than for the control ones. It seems unlikely, however, that a heightened sensitivity of receptors (if real) could account for the drastic decrease in learning rate. A more likely possibility to us is that the DDT is directly affecting the normal functioning of the CNS, perhaps in the region of the optic tectum.

This work, and that reported elsewhere on the sublethal effects of DDT, suggest that insecticide pollution may be even more harmful to fish than the well-publicized lethal effects which have been reported. Learning, for example, is probably a critical element in such behavioural activities as territorial defence and migration. Any pollutant which might affect such behaviour patterns is obviously of potential ecological significance.
To the extent that insecticide-induced changes in behaviour may adversely influence the survival of the species, the term "sublethal", applying to the concentrations used, is clearly a misnomer, or at any rate may imply to some that no biological effects of consequence are involved. Perhaps "subacute" would be a more appropriate term. As re-emphasized recently by Macek (1968), no-effect levels, rather than no-kill levels, should serve as the basis for establishing water quality criteria.

The fact that the effects of the 24-hour exposure to DDT in these experiments had largely disappeared within 3 to 4 weeks is of course encouraging. The only disquietening aspect is the knowledge that in nature there are many areas where fish are unable to avoid continuous exposure to low doses of insecticides.

References


The Effect of 20 ppb DDT on the Learning Ability of Brook Trout

Figure 2

Number of Trials in Blocks of 10

Per Cent of Fish in Group

- Controls
- DDT Treated

Figure 1

Stimulus Schedule
Light - Conditioned Stimulus (CS)
Shock - Unconditioned Stimulus (US)

CS (5 sec) Light Off (50-70 sec) CS (5 sec)
US (1/2 sec) US (1/2 sec)

Figure 1