Long-Term Behavioural and Biochemical Effects Following Prolonged Treatment with a Neuroleptic Drug (Flupenthixol) in Rats

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Abstract. The effects of long-term treatment (36 weeks) with a neuroleptic drug (flupenthixol) were investigated behaviourally and biochemically in rats. Sixteen rats were trained on a DRL (differential reinforcement of low rate) 15-s schedule until stable responding was obtained. During the following 36 weeks 9 rats were injected weekly with flupenthixol dissolved in Viscoleo® [4 mg/kg (i.m.)] and seven rats received Viscoleo® alone. During this period the animals were not run on the DRL schedule. Retesting on DRL 7 weeks after the last drug injection yielded highly significant differences between the flupenthixol-treated animals and the controls. Thorough neurological examinations of the animals just preceding the retesting period also revealed some deficits in the flupenthixol-treated animals. At sacrifice, 14–18 weeks after the last drug injection, levels of homovanillic acid (HVA) were measured in the corpus striatum and total 3-methoxy-4-hydroxyphenylglycol (MOPEG) in the rest of the forebrain. The results indicate a nonsignificant increase of 25% in the dopamine metabolite HVA, while the noradrenergic metabolite MOPEG was significantly decreased by 14% in experimental animals. The possibility of persistent functional and biochemical effects produced by prolonged treatment with a neuroleptic drug is highlighted in the results presented here.

Key words: DRL’ 15 schedule - Flupenthixol - Longterm treatment - Homovanillic acid - 3-Methoxy-4-hydroxyphenylglycol

Various deficits, especially tardive dyskinesias, are known to follow long-term treatment with neuroleptic drugs (Crane, 1968; Gerlach and Thorsen, 1976). The effects usually noted tend to be peripheral motor effects and these often disappear or are reduced when medication is withdrawn. However, Pakkenberg et al. (1973) have presented anatomical data from rats indicating irreversible effects upon the number of nerve cells in the corpus striatum following long-term treatment (1 year) with the neuroleptic drug perphenazine enantate. More recent findings (Fog et al., 1977) indicate, however, that even a tenfold increase in dose level for a shorter period of 6 months in somewhat younger animals does not necessarily produce this cell loss. Moreover, since cell loss, if evident, might not appear until long after serious reduction in nervous transmission, the present study also included a series of neurological examinations both during and after drug treatment as well as determination of the amount of metabolites produced by two of the major transmitter substances, noradrenaline and dopamine, at sacrifice, well after the drug treatment.

There is also the possibility that long-term treatment with neuroleptics may produce a deficit in learned behaviour. For instance, Ahlenius et al. (1975) demonstrated that young rats, nursed by mothers that received Pimozide on the first 7 days after delivery, demonstrated a deficit in avoidance responding 4 weeks later. It was therefore considered of interest that the present study, in comparison with previous investigations, should include a test of retention deficits in a learned activity following training on a relatively demanding schedule of positive reinforcement. The DRL (differential reinforcement of low rate) schedule was chosen since it requires lengthy training to a stable acquisition level and since it makes demands on the animal's ability to pace its responding in order to maximize reinforcement. The requirement of low rate also makes rate changes highly visible during retest and quickly demonstrates any inability to withhold responses (Ferster and Skinner, 1958).

The experiment was purposely designed to provide separate and adequately spaced periods of behavioural...
training, drug treatment, withdrawal, and behavioural retest before the final anatomical and biochemical examinations were made.

MATERIALS AND METHODS

Sixteen male Wistar rats weighing approximately 200 grams each were used. The rats were housed individually in wire mesh cages, placed in an inner basement room, with constant 65±% relative humidity and constant temperature (20 – 21°C). Food and water were available ad libitum except as noted below. The general experimental design is shown in Table 1. Throughout the whole experiment the weight of each rat was taken at least once a week.

**Behavioural Test Method**

The animals were trained to lever-press on a DRL schedule in a standard Lehigh Valley Electronics automated Skinner-box, which was connected to a specially constructed solid-state apparatus controlling the relevant parameters in the schedule. The DRL 15-s schedule insured that response (lever depressions) with an inter-response time of less than 15 s were not reinforced, while the first response emitted 15 s or more after the preceding response was always reinforced. Reinforcement consisted of a water reward (0.1 ml), which was available in a dipper presented for 2.5 s. When the dipper was up a light was also present just over the dipper hole. Session time during all experiments was 20 min, and the animals were tested twice a week with an intersession interval of 3 – 4 days.

In the beginning of training all rats were deprived of water 24 h before the experimental session. For seven of the animals the 24-h deprivation schedule was not sufficient to produce criterion responding, and for these animals a fixed amount of water, based on maintenance of normal body weight, was supplied each day (except prior to an experiment), with a total of about 75 ml/week. The criterion for stability in responding was that during the last six sessions the number of reinforced responses should not vary by more than 10. In order to control for individual differences in reaching the level of response stability, the rats were divided into four subgroups (N = 5, 4, 5, 2), which required 22, 21, 45, and 41 training sessions respectively. The first two subgroups were on the 24-h deprivation schedule and started the drug treatment schedule at the same time (see below), while the last two subgroups were delayed due to a longer training period and were tested on the ‘fixed-water’ schedule. The possible influence of these differences has been taken into account when analysing the results (see below).

In deciding which animals would act as experimentals or controls the following rules were used: Within subgroups, a rank order priority list was made on the basis of the mean numbers of reinforced responses from the six criterion sessions. The rats with ‘even’ priority numbers became controls, and the ‘odd’ numbers became the experimentals.

**Drug Treatment Schedule**

When training was completed, the experiments (N = 9) were injected weekly for 36 weeks with flupenthixol decanoate (4 mg/kg of 1.6% dissolved in a special oil, Viscole® oleum vegetable tenue, i.m.). Control animals (N = 7) were injected with Viscole® alone. Injections were aimed at the biceps femoris muscle of the hindleg alternating between left/right leg.

**Neurological Examinations**

A brief weekly neurological test was performed 2 days after each injection during the treatment period. The procedure included three items: (1) the rat was placed on a table for 2 min, and it was observed whether normal coordinated movement of the whole body and legs occurred; (2) the animal was put on a wire net, which was then tilted 45° while body balance and behaviour were observed; (3) the animal was placed on a very thin rod, its holding and balancing attempts were observed and righting reactions were seen when the animal dropped from the rod.

The longer postdrug neurological tests were made 6 weeks after the last drug injection. These tests included many items, each of which was given one of the following scores: (1) not normal; (2) doubtful or very weak; (3) moderate; (4) good or strong; (5) very good or unusually strong. The procedure was performed under single blind control conditions. The items in the test were: appearance (the general appearance of the fur, eyes, and body was scored); activity (the animal was put on the floor and locomotion with all four legs and normal movement of the whole body was scored. Vocalization during handling was also scored); coordination and rotation (the rat was put on top of a thick rod and its coordination was scored regarding the use of all four legs and tail in maintaining a precarious balance. The animal was then placed on top of a thin metal rod which it could grasp but where it could not maintain balance for long, finally being forced to hang from the rod and drop down to the table. Its holding attempts together with righting reactions during the fall were scored. The rat was then dropped while lying on its back in the experimenter’s hand and again the righting reaction was scored. The rat was hung by the tail and tendency to rotation was scored. Finally the rat was rotated towards right and left while lying on its back and head-orientation to both sides was scored). Resistance to passive movement (the rat was held on its back in the experimenter’s hand, and passive resistance to flexion was scored for each leg). Placing and hopping reactions (all four legs were scored individually for abduction, adduction, lateral and medial hopping as well as for placing to proprioceptive and tactile stimulation). Cutaneous sensory reactions (the legs, body, and head were gently pricked with a needle and withdrawal or flinching reaction scored. A light puff of air from a pipette was directed around the eyes, ears, and vibrissae and reactions were scored).

**The DRL-Retest Method**

Seven weeks after the last drug/vehicle injection, retesting began on the DRL schedule. All animals were retested under deprivation conditions similar to those during their training. The rats received from 10 – 18 retest sessions. After retesting was completed, the animals received water ad libitum until sacrifice and biochemical analysis 2 weeks later.

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Table 1. The general experimental design

<table>
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<th>Training</th>
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<th>Pause</th>
<th>Retest</th>
<th>Pause</th>
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<td>5–9 weeks</td>
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