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Characterisation of the phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze

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Abstract. In the elevated plus-maze test of anxiety the scores of control animals remain stable over repeated tests. However, a single prior exposure to the plus-maze renders an animal insensitive to the anxiolytic effects of chlordiazepoxide. This phenomenon of "one-trial tolerance" persisted even when the two trials were separated by as much as 2 weeks. It has previously been shown that the drug state of the animal on trial 1 is not important to the development of the phenomenon, but one-trial tolerance did not develop if a very high dose (75 mg/kg) of chlordiazepoxide was given on trial 1; it is suggested that this is due to the amnesic effects of the drug. The learning on trial 1 was not specific to a particular plus-maze and tolerance could be observed even when the maze on trial 1 was made from different material. The crucial experience on trial 1 was experience of an open arm of the maze. Whereas tolerance could be obtained as a result of a previous plus-maze experience, there was no evidence of an anxiogenic withdrawal response when rats were tested the following day undrugged. The phenomenon of one-trial tolerance is explained within our recently proposed two-factor theory of benzodiazepine dependence; it is suggested that one-trial tolerance provides a method for studying the mechanism underlying the development of tolerance to anxiolytic effects, independently from the mechanism underlying the development of withdrawal responses.

Key words: Benzodiazepines – Tolerance – Learning – Benzodiazepine dependence

Tolerance develops at different rates to the different behavioural effects of the benzodiazepines and develops more slowly to the anxiolytic than to the sedative or anticonvulsant effects (File 1985). In general, 3 weeks of daily injections are needed to demonstrate tolerance in animal tests of anxiety (Vellucci and File 1979; Cooper et al. 1981; Gonzales et al. 1984; Treit 1985; Soderpalm 1987; File and Baldwin 1989), including the elevated plus-maze (File et al. 1987).

However, there is evidence that a single experience of the plus-maze can significantly reduce the anxiolytic effects of chlordiazepoxide in the mouse (Lister 1987). Similarly, in the rat a single experience in the plus-maze abolished the anxiolytic effects of chlordiazepoxide (File 1990). The drug state of the rats on trial 1 was not important for the development of "one-trial tolerance", and it could be demonstrated in rats that had been undrugged on trial 1, or had received either chlordiazepoxide or the benzodiazepine antagonist, flumazenil. It cannot be explained simply by assuming that the plus-maze had become less anxiogenic with repeated testing, since this should have been reflected in a change in the control scores over trials, and this was not seen in either the mouse (Lister 1987) or the rat (File 1990). The phenomenon depends on an interaction between the experience on trial 1 and an action at the benzodiazepine receptors on trial 2. Behaviourally, one 5 min exposure to the plus-maze produces the same apparent tolerance as 21 daily CDP injections.

The following experiments were designed to further characterise the phenomenon of one-trial tolerance. Experiment 1 examined the duration of the effect of the initial exposure to the plus-maze, i.e. how long the interval between trial 1 and trial 2 could be to still show a significant reduction of the anxiolytic effect of chlordiazepoxide on trial 2. The results showed that the phenomenon persisted even with an inter-trial interval of 2 weeks. Thus, it appeared likely that a learning process was involved. Experiments 2–4 examined the nature of the experience on trial 1 that was crucial to the phenomenon. Experiment 5 was designed to determine whether an anxiogenic "withdrawal" response would be apparent when rats were tested undrugged after demonstrating one-trial tolerance.

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Methods

Animals. Male hooded Lister rats (Olac, Bicester) were housed in groups of five in a room with lights on from 0700 to 1800 hours; food and water were freely available.

Drugs. Chlordiazepoxide hydrochloride (7.5 mg/kg, Roche Products Ltd) was dissolved in distilled water; control rats received water injections. All injections were IP in a volume of 2 ml/kg, 30 min before testing in the plus-maze, where appropriate.

Apparatus. The plus-maze was made of wood and had two open arms (50 × 10 cm) and two enclosed arms of the same size with walls 40 cm high; it was elevated 50 cm above the ground. In experiment 2 an identical size plus-maze made from metal was used. Each rat was placed in the central square (10 × 10 cm) and observed remotely using a video camera for the number of entries into each type of arm (all four paws defining an entry) and the time spent in open and closed arms.

Statistics. The data were analysed by analysis of variance, with the least significant difference test for differences between individual groups, where appropriate.

Procedure. Each rat was placed in the centre of the plus-maze and allowed 5 min free exploration; the maze was cleaned after each trial. The measures that reflect an anxiolytic action in this test are the percentage of entries made onto open arms and the percentage of time spent on the open arms (Pellow et al. 1985). The rats were tested in an order randomised for drug treatment between 1330 and 1630 hours. When rats were tested repeatedly in the plus-maze the inter-trial interval was 24 h, except for experiment 1 when it was 1 or 2 weeks. When the initial treatment was an injection alone, the interval between first and second treatments was 24 h, except for experiment 1 when it was 1 or 2 weeks.

Experiment 1. Rats were randomly allocated between groups that received only a prior injection of chlordiazepoxide (7.5 mg/kg, $n = 8$/group) and those that were given an injection of chlordiazepoxide (7.5 mg/kg) and a prior exposure to the plus-maze ($n = 9$/group). Half of the rats were tested in the plus-maze 1 week after their initial injection or plus-maze test, the other half were tested 2 weeks later; in all cases the plus-maze test took place 30 min after IP injection with chlordiazepoxide (7.5 mg/kg).

Experiment 2. Rats were randomly allocated to control ($n = 9$) and chlordiazepoxide ($n = 13$) groups. All rats were given two trials in the plus-maze, 24 h apart. For the control group both trials were undrugged (i.e. after water injections); on trial 1 the chlordiazepoxide group received 75 mg/kg and on trial 2 the rats received 7.5 mg/kg.

Experiment 3. Rats were randomly allocated between control and chlordiazepoxide (7.5 mg/kg) groups ($n = 8$/group). On trial 1 each rat was given a 5 min trial in the metal plus-maze, 30 min after the appropriate IP injection. Trial 2 took place 24 h later in the wooden plus-maze, 30 min after the appropriate injection.

Experiment 4. Rats were randomly allocated among a control group and three chlordiazepoxide groups with different prior experience conditions ($n = 8$/group). The control group received two plus-maze trials undrugged, in the normal fashion. The chlordiazepoxide groups received either a normal plus-maze trial, or were trapped in one enclosed arm or in one open arm. In each of these groups the first trial lasted 5 min and took place 30 min after an IP injection of chlordiazepoxide (7.5 mg/kg). All the rats were tested 24 h later in the plus-maze 30 min after an IP injection of chlordiazepoxide (7.5 mg/kg) or water, as appropriate.

Experiment 5. Rats were randomly allocated to the control, the "tolerance", and the "withdrawal" groups ($n = 8$/group). All animals received three trials in the plus-maze, with an inter-trial interval of 24 h. Rats in the control group received all three trials undrugged; those in the tolerance group received three trials after chlordiazepoxide (7.5 mg/kg); those in the withdrawal group received the first two trials after chlordiazepoxide (7.5 mg/kg) and the third after an injection of distilled water.

Results

Chlordiazepoxide (7.5 mg/kg) did not change the total number of arm entries (control = 16.3 ± 0.3, chlordiazepoxide = 16.1 ± 1.7) and therefore only the data for the two measures reflecting anxiety (% number of entries onto open arms and % time spent on open arms) will be presented for each experiment.

Figure 1 shows the scores for rats tested in the plus-maze with chlordiazepoxide (7.5 mg/kg) 1 or 2 weeks after a prior experience of the plus-maze or only a prior injection of chlordiazepoxide. At both the inter-trial intervals and for both the measures of anxiety there were significant differences between the group with the prior plus-maze experience and the group with only a prior injection, indicating the development of one-trial tolerance [$F(1,15) = 9.1$ for % number and $= 19.5$ for % time for the 1-week group; and $= 5.1$ for % number and 10.3 for % time for the 2-week group, significance levels shown in Fig. 1].

Table 1 shows the plus-maze scores on trial 2 for the control group and for the chlordiazepoxide group; the scores for the latter group are shown separately for the rats that were very inactive during trial 1 (≤ 2 arm entries) and the rest who had scores equal to the control rats. There were significant differences between the groups [$F(2,19) = 14.3$ for % number and $= 20.4$ for % time, $P < 0.0005$ in both cases] and both the chlordiazepoxide groups showed scores significantly higher than the controls. Thus, if trial 1 is experienced with a very high dose of chlordiazepoxide then the phenomenon

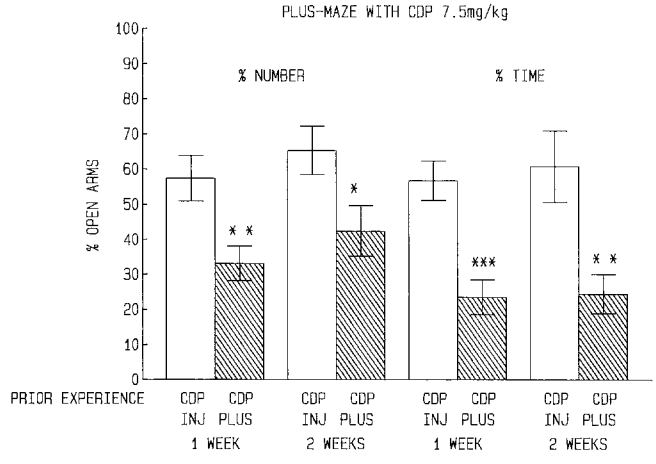

Fig. 1. Mean (± SEM) percentage of entries made onto open arms and percentage of time spent on the open arms of an elevated plus-maze by rats tested with chlordiazepoxide (7.5 mg/kg) 1 or 2 weeks after a previous injection of chlordiazepoxide (*INJ*) or a previous plus-maze test (*PLUS*). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, comparing INJ and PLUS groups

Table 1. Mean (±SEM) percentage number of entries made onto open arms and percentage of time spent on the open arms during trial 2 in an elevated plus-maze. Rats either received both trials undrugged (CON), or received chlordiazepoxide (CDP: 75 mg/kg on trial 1 and 7.5 mg/kg on trial 2). The data for the CDP group are shown separately for the rats that were very inactive on trial 1 and for those that were more active

	% No. on open arms	% time on open arms
CON	12.2±4.8	7.6±3.5
CDP (7.5 mg/kg)		
Inactive Tr 1	36.4±1.7**	31.7±2.8**
Active Tr 1	41.6±3.7**	42.0±4.8**

** $P<0.01$, compared with control group

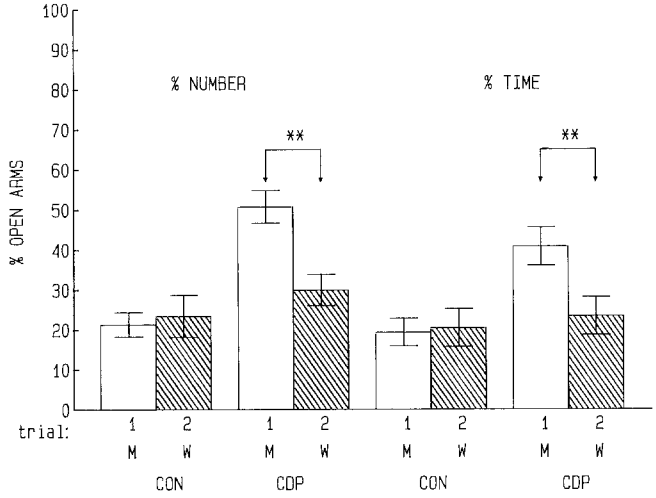

Fig. 2. Mean (±SEM) percentage of entries made onto open arms and percentage of time spent on the open arms of an elevated plus-maze on two successive trials, with trial 1 in a metal maze (*M*) and trial 2 in a wooden maze (*W*) by rats injected with distilled water (*CON*) or chlordiazepoxide (*CDP* 7.5 mg/kg). **$P<0.01$, comparing trials 1 and 2

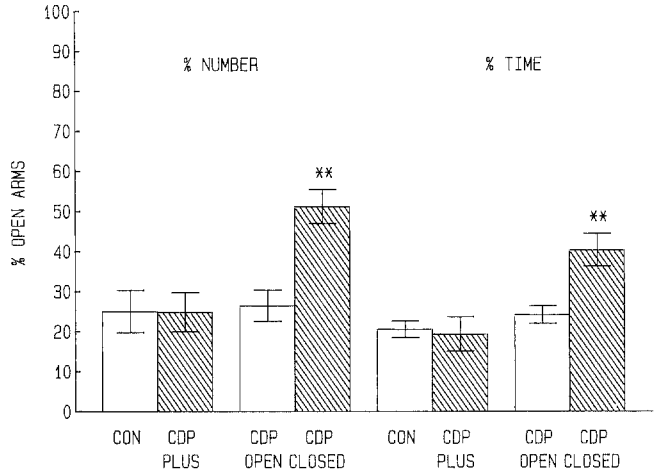

Fig. 3. Mean (±SEM) percentage of entries made onto open arms and percentage of time spent on the open arms of a plus-maze for control animals with one previous trial in the plus-maze (*CON*), for rats treated with chlordiazepoxide (7.5 mg/kg) with a previous trial in the plus-maze (*CDP PLUS*), with previous experience of an open arm (*CDP OPEN*), and with previous experience of a closed arm (*CDP CLOSED*). **$P<0.01$, compared with controls

Table 2. Mean (±SEM) % number of entries onto, and % time spent on, open arms of the plus-maze on three successive trials by rats injected each day with water (CON), or with chlordiazepoxide (CDP 7.5 mg/kg, "tolerance" group). Also shown are the data for the "withdrawal" group (CDP on days 1 and 2, CON on day 3)

Trial	% No. on open arms			% time on open arms		
	1	2	3	1	2	3
CON	15.4 ±3.6	15.6 ±3.4	14.5 ±4.3	16.2 ±3.3	17.3 ±1.9	16.0 ±2.4
Tolerance	56.3** ±4.5	28.9 ±3.3	30.9 ±4.4	40.5** ±5.2	17.0 ±3.2	25.2 ±7.1
Withdrawal	45.8** ±3.8	18.8 ±4.7	16.6 ±2.7	38.6** ±3.5	14.0 ±6.9	17.4 ±1.9

** $P<0.01$, compared with control group

of "on-trial tolerance" does not develop. There were no differences between the two chlordiazepoxide groups and thus the level of activity on trial 1 was not crucial.

Figure 2 shows that whereas the scores of the control rats did not change from trial 1 to trial 2 (even though there was also a change from metal to wooden plus-maze), the scores of the chlordiazepoxide-treated rats did show a significant decrease over trials [$F(1,7)=21.6$ for % number of entries; $F(1,7)=15.9$ for % time on open arms; in both cases $P<0.005$].

Figure 3 shows the scores in the plus-maze when rats were tested with chlordiazepoxide (7.5 mg/kg), but following different trial 1 experiences in the plus-maze. It can be seen that only the group previously trapped in an enclosed arm had scores significantly higher than the controls; both the other groups showed the phenomenon of one-trial tolerance. Thus, experience on an open arm is sufficient to mediate this phenomenon.

It can be seen from Table 2 that on trial 3 in the plus-maze the scores of the withdrawal group did not differ significantly from the controls. Thus, whilst the phenomenon of one-trial tolerance can be seen on trials 1 and 2, there was no evidence that a "one-trial withdrawal" effect could be observed.

Discussion

The results from experiment 1 show that even when the trials in the plus-maze are separated by as much as 2 weeks, the phenomenon of one-trial tolerance can be observed. This very long-lasting effect suggests that some kind of learning is mediating the change observed on trial 2. The results of experiment 2 showed that the phenomenon did not occur if trial 1 took place under the influence of a very high dose of chlordiazepoxide. This dose was strongly sedative and all the rats were sleeping in their home cage just prior to the plus-maze test; however, not all the rats remained inactive in the plus-maze. The failure to find a difference between the scores of previously active and inactive rats suggested that the

reason for these rats not demonstrating one-trial tolerance was not simply because they were asleep throughout the trial. It is likely that it was due to the amnesic effects of the benzodiazepines (Lister 1985). Experiment 3 showed that the learning was not specific to a particular plus-maze and that it could be observed even if trial 1 was in a plus-maze made from different material. Experiment 4 showed that the trial 1 experience that is crucial to the development of one-trial tolerance is that of an open arm.

Experiment 5 found no evidence for a one-trial withdrawal phenomenon, thus suggesting that this behavioural procedure has separated the phenomena of tolerance and withdrawal. We suggest that this phenomenon of one-trial tolerance can be explained within the same theoretical framework as the tolerance that develops after chronic benzodiazepine administration. We have recently proposed (File and Hitchcott 1990) that during benzodiazepine treatment two independent adaptive mechanisms are triggered that gradually lead to the development of tolerance to the anxiolytic effects and to anxiogenic responses on withdrawal of the drug. The phenomenon of one-trial tolerance may provide a way of studying changes solely in the mechanism underlying tolerance. The suggestion is that the behavioural experience of the plus-maze induces the same change in one adaptive mechanism as does a period of chronic daily injections, but that it does not induce any change in the mechanism underlying withdrawal responses. A change in only one mechanism would result in no change in the scores of control animals with repeated testing, but would be revealed by the failure to respond to benzodiazepine administration. It would also be predicted that there would be no decrease in scores when the rats are tested undrugged on trial 3 (i.e. no spontaneous withdrawal response). These results were confirmed in the withdrawal group of experiment 5, see Table 2.

So far we have explored the phenomenon of one-trial tolerance to the anxiolytic effects of benzodiazepines only in the plus-maze. We have not explored whether there is cross-tolerance from the plus-maze to other tests of anxiety, but because of the apparent specificity of trial 1 experience (of the open arms) we would not expect this. We have found that there is no cross-tolerance from the effects of benzodiazepines in the plus-maze to their effects on bicuculline seizure thresholds (unpublished data). Although the phenomenon of one-trial tolerance may be test specific, it may not be a phenomenon specific to benzodiazepines. We have some initial data indicating that the effect can be obtained with barbiturates, although the phenomenon was less striking. It remains to be explored whether it can be observed with other anxiolytics, particularly those not acting at the GABA-benzodiazepine receptor complex.

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