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Effects of Two Anxiolytics on Distraction, Habituation and Dishabitiuation

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FILE, S. E. *Effects of two anxiolytics on distraction, habituation and dishabituation.* PHARMAC. BIOCHEM. BEHAV. 7(2) 105–109, 1977. – Rats were engaged in a baseline activity of licking and their distraction to tone stimuli measured by an interruption in licking. Habituation, distraction to a stimulus change and dishabituation (the return of distraction to the original tone) were studied. Ethanol (0.4 and 0.8 g/kg) and chlordiazepoxide (7.5 mg/kg) produced significantly faster habituation, a result that cannot be attributed to changes in the baseline response rate, or to differences in the initial distraction to the tone. All groups showed 24 hr retention to the tone when they were tested in the same state in which they were habituated. There was transfer of habituation between drugged and undrugged states for rats injected with chlordiazepoxide, but rats habituated undrugged showed no retention if tested after injections of ethanol. Chlordiazepoxide impaired the specificity with which the physical parameters were coded, whereas ethanol improved the specificity of coding. Neither drug affected dishabituation.

Distraction Habituation Dishabituation Anxiety Stimulus Specificity
Chlordiazepoxide Ethanol

IN HUMANS an increase in anxiety leads to slower habituation of the vasoconstriction and galvanic skin responses to tone stimuli [9, 10, 12]. This slower habituation may be a direct result of increased anxiety or it may be secondary to the changes produced by anxiety in the baseline response levels. In order, therefore, to see whether changes in anxiety directly affect the rate of habituation it is necessary to select a situation in which the baseline response is unaffected by anxiety.

If an increase in anxiety does lead to slower habituation then drugs that reduce anxiety should produce faster habituation. Experiment 1 was designed to investigate the effects of 2 anxiolytics, ethanol and chlordiazepoxide (CDP), on distraction in rats to a tone stimulus and their effects on the rate of habituation to this stimulus. It also examined the extent to which this habituation was state-dependent [13]. The doses of the two drugs were based on those found to be effective in increasing exploration during the first trial in a holeboard [4], an effect possibly due to anxiety reduction.

Experiment 2 was designed to investigate whether these drugs affected the specificity with which the physical parameters of the stimulus were coded during habituation. This was tested by measuring the distraction produced when features of the habituated stimulus were changed. Undrugged rats are distracted by changes in the frequency or intensity of an habituated tone stimulus [2,6]; if the original stimulus is then presented again dishabituation will be reflected in a return of distraction to this stimulus. The phenomenon of dishabituation has been demonstrated in this way for the galvanic skin response in humans (e.g., [15]). A second purpose of Experiment 2 was to examine the effects of anxiolytics on dishabituation, a phenomenon which is said to reflect the process of sensitization [7].

EXPERIMENT 1

METHOD

Animals

Sixty male hooded rats (*Rattus norvegicus*) from Olac Ltd (Bicester), 350 g in weight, were housed in groups of 6 in a room maintained at 25°C in an 11 hr light: 13 hr dark cycle. The rats were given an initial 48 hr period of water deprivation, and thereafter received water during and immediately following the test, in sufficient quantity to maintain a steady body weight. Food was available ad lib.

Apparatus

The test box, 19 × 19 × 26.5 cm, was enclosed in an acoustically insulated chamber. A slit in the end wall gave access to a water spout and a drinkometer recorded the rat's licking. Tones were delivered via a loudspeaker positioned in the lid of the test box at the water spout end. The tone used was 7 kHz, 75 dB (re 0.0002 dynes. cm^{-2}) for 9 sec.

Drugs

Chlordiazepoxide hydrochloride (Roche Products Ltd) was dissolved in deionised water to give a concentration of 2 mg/ml.

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Spectroscopically pure absolute ethanol (BDH chemicals Ltd) was dissolved in deionised water to give a concentration of 0.16 g/ml (20% v/v).

Half the control rats tested with each drug group received injections of deionised water equal in volume to the lower drug dose, and half received injections equal in volume to the higher drug dose.

All injections were given intraperitoneally 30 min before testing.

Procedure

Thirty rats were randomly allocated ten each to control, ethanol (0.4 g/kg) and ethanol (0.8 g/kg) groups and, likewise, 30 rats were randomly allocated to control, CDP (5 mg/kg) and CDP (7.5 mg/kg). Two control groups were included because testing with ethanol and CDP took place consecutively.

On the first day following the water deprivation period no injections were given and each rat was placed in the test box and given free access to the water spout for 20 min. The next day, 30 min after the appropriate injection, each rat was placed in the test box and its 200th lick switched on a control period for 9 sec. The number of licks made in this control period (A) was counted. Following this period the next 20th lick switched on the tone for 9 secs and the number of licks made during the tone (B) was counted. Control and tone periods alternated until habituation criterion was reached. This was taken as 3 successive tone presentations producing a distraction ratio of $\frac{A-B}{A} \leqslant 0.10$. All rats reached criterion on the first test day, within 20 min.

The following day the rats were tested to see whether the habituation was state-dependent or whether it would transfer between drugged and undrugged states. Thus those previously habituated drugged were now tested undrugged and those previously habituated undrugged were randomly allocated 5 each to be tested after injections of ethanol (0.4 g/kg), ethanol (0.8 g/kg), CDP (5 mg/kg) and CDP (7.5 mg/kg). A series of control and tone periods again alternated until the rats once more reached habituation criterion.

RESULTS

There were no significant differences in the rate of licking between the control and drugged groups. In 9 sec the two control groups made a mean of 47.5 ± 0.9 and 49.8 ± 1.4, the ethanol (0.4 and 0.8 g/kg) groups means of 45.8 ± 1.0 and 46.3 ± 1.1 respectively, and the CDP (5 & 7.5 mg/kg) groups means of 47.4 ± 0.9 and 47.1 ± 0.8 respectively. There was no change in the rate of licking during the test session. Thus, any changes in distraction and habituation produced by the drugs are not secondary to changes in the baseline response rate.

Table 1 shows the distraction ratios to the first tone presentation for the control and drugged groups. None of the differences in distraction was significant. However, both doses of ethanol and the higher dose of CDP produced significantly faster habituation; a change in habituation that cannot be attributed to changes in the initial distraction response.

Rats that had initially been habituated after injections of ethanol (0.4 and 0.8 g/kg) were tested the following day undrugged, and had mean distraction ratios of 0.25 ± 0.08 and 0.23 ± 0.07 respectively, showing some recovery of distraction from the previous day's criterion level of < 0.10. The rats initially habituated after injections of CDP (5 and 7.5 mg/kg) also showed some return of distraction when tested undrugged, and had mean distraction ratios of 0.17 ± 0.06 and 0.37 ± 0.09 respectively. The trials taken by these rats to rehabituate in the undrugged state are shown in Table 2; in all cases rehabituation was significantly faster than the rate of initial habituation shown by the undrugged control rats.

The rats initially habituated undrugged were tested the

TABLE 1

MEAN AND SEM DISTRACTION RATIO TO FIRST TONE PRESENTATION AND MEAN AND SEM TRIALS TO HABITUATE (N = 10/group)

	Ethanol			CDP		
	Control	0.4 g/kg	0.8 g/kg	Control	5 mg/kg	7.5 mg/kg
Experiment 1						
Distraction Ratio	0.56 ± 0.10	0.44 ± 0.08	0.46 ± 0.08	0.48 ± 0.06	0.46 ± 0.10	0.56 ± 0.09
Trials to Habituate	13.0 ± 2.6	6.4† ± 1.7	6.5† ± 1.6	13.5 ± 1.5	14.0 ± 1.6	6.5‡ ± 1.0
Experiment 2						
Distraction Ratio	0.54 ± 0.05	0.51 ± 0.08	0.50 ± 0.08	0.51 ± 0.06	0.43 ± 0.10	0.36 ± 0.11
Trials to Habituate	11.0 ± 1.5	7.1* ± 1.6	4.1‡ ± 0.8	8.5 ± 1.1	8.0 ± 1.4	3.9‡ ± 0.8

*$p<0.05$, one-tailed t-test.
†$p<0.05$, two-tailed t-test.
‡$p<0.01$, two-tailed t-test.

TABLE 2

MEAN AND SEM TRIALS TO REHABITUATE FOR RATS REHABITUATING IN A STATE DIFFERENT FROM THAT IN WHICH THEY WERE FIRST HABITUATED

State in which 1st habituated (N = 10/group)		Trials to rehabituate when drugged
Ethanol	0.4 g/kg	5.9 ± 1.8*
	0.8 g/kg	5.5 ± 2.2*
CDP	5 mg/kg	5.9 ± 1.8†
	7.5 mg/kg	5.3 ± 2.1†
State in which rehabituated (N = 5/group)		Trials to rehabituate for rats 1st habituated undrugged
Ethanol	0.4 g/kg	4.6 ± 2.6
	0.8 g/kg	4.8 ± 2.6
CDP	5 mg/kg	2.2 ± 1.2§
	7.5 mg/kg	2.2 ± 1.4‡

*$p<0.05$, †$p<0.01$. Two-tailed t-tests compared the trials to rehabituate undrugged with the trials to habituate for the rats initially habituated undrugged.

‡$p<0.05$, §$p<0.001$. Two-tailed t-tests compared the trials to rehabituate drugged with the trials to habituate for the groups originally habituated after injections of ethanol (0.4 and 0.8 g/kg) and CDP (5 and 7.5 mg/kg) respectively.

following day after drug injections. They also showed some return of distraction with ratios of 0.27 ± 0.08, 0.28 ± 0.08, 0.16 ± 0.06 and 0.14 ± 0.06 for those rehabituating after injections of ethanol (0.4 and 0.8 g/kg) and CDP (5 and 7.5 mg/kg) respecitvely. The rats rehabituating after ethanol injections did not reach criterion significantly faster than did the rats originally habituated after ethanol injections; but, those rehabituating after CDP injections did reach criterion faster than the rats originally habituated after CDP injections (see Table 2).

Thus there was little evidence of state-dependent habituation with CDP, whereas animals habituated undrugged showed no retention if retested after injections of ethanol. The data from these state-change groups can be compared with the data from Experiment 2, where all the rats were tested the following day in the same state in which they had been habituated.

EXPERIMENT 2

METHOD

Animals

Sixty male hooded rats were housed and deprived as in Experiment 1.

Apparatus

The same test chamber was used. The following tones were presented, all were 9 sec in duration: 7 kHz 75 dB, 13 kHz 75 dB, 7 kHz 85 dB and a 7 kHz 75 dB tone that was pulsed, i.e. 1 sec on, 1 sec off.

Drugs

As in Experiment 1.

Procedure

Rats were randomly allocated 10 each to the same groups as in Experiment 1 and the procedure for the first 2 days followed that in Experiment 1, the rats habituating to a tone of 7 kHz 75 dB.

The day after reaching habituation criterion each rat was given the same injection as before and first presented with the 7kHz 75 dB tone, to check that it was still habituated to this tone. Each rat then received a series of tone presentations in which a novel tone was presented (to test for the specificity of habituation) and then the original tone was presented again (to measure dishabituation). The original tone was then presented until it again produced no distraction and then the next novel tone was given. In this way 3 novel tones were presented to each rat and dishabituation was measured on 3 occasions. One of the novel tones represented a change in frequency from the original tone, another an intensity change and the third was a change from a continuous to a pulsed tone. The order of presentation of the 3 novel tones was randomly determined for each rat.

RESULTS

The initial distraction to the 7 kHz 75 dB tone and the trials to habituate to this tone are shown in Table 1. The results replicate the finding in Experiment 1, that ethanol and CDP do not change the initial distraction, but do lead to more rapid habituation.

The specificity of the stimulus coding should reflect whether the more rapid habituation seen in the drugged groups was at the expense of specificity of coding of the stimulus parameters. Table 3 shown the % of novel stimulus presentations that produced distraction (i.e. a ratio > 0.10) in each group of rats. It can be seen that after injections of CDP distraction occurred only half as often as it did in the control rats, suggesting that CDP impaired the specificity of stimulus coding. This was supported by the mean distraction ratios to stimulus change. For each rat a mean distraction ratio to the 3 novel stimulus presentations was calculated and the group means are shown in Table 3. Both doses of CDP produced lower distraction ratios to stimulus change than was shown by the control rats (see Table 3). Thus the more rapid habituation in the CDP groups did seem to be at the price of a less specific stimulus coding. The results with ethanol contrast with the CDP results. The ethanol groups showed more distraction responses to the novel stimuli and higher distraction ratios, significantly so for the 0.8 g/kg group. Thus ethanol produces rapid habituation without any loss of specificity and, at the higher dose, with an increase.

Table 3 also shows the group means for the dishabituation produced by these stimulus changes. None of the drug groups differed significantly from the controls.

DISCUSSION

Since increased anxiety leads to slower habituation in humans [9, 10, 12] the more rapid habituation found in this experiment after injections of ethanol and CDP is consistent with an interpretation in terms of reduced anxiety. CDP has also been reported to produce more rapid habituation in Siamese fighting fish [1], and more rapid habituation to light flashes in rats [16]. Although the effects of ethanol on startle habituation were not studied

TABLE 3

THE % OF NOVEL STIMULUS PRESENTATIONS THAT PRODUCED A DISTRACTION RESPONSE IN EACH GROUP OF RATS, THE MEAN DISTRACTION RATIO TO STIMULUS CHANGE, AND THE MEAN DISHABITUATION. (N = 10/group)

Group	% Distraction	Mean Distraction to Stimulus Change	Mean Dishabituation
Control	53	0.27 ± 0.08	0.27 ± 0.11
CDP (5mg/kg)	27	0.11* ± 0.04	0.13 ± 0.03
CDP (7.5 mg/kg)	27	0.11* ± 0.03	0.11 ± 0.03
Control	49	0.24 ± 0.03	0.21 ± 0.04
Ethanol (0.4 g/kg)	61	0.32 ± 0.05	0.27 ± 0.04
Ethanol (0.8 g/kg)	72	0.40† ± 0.05	0.27 ± 0.02

*$p<0.10$, two-tailed t-test.
†$p<0.02$, two-tailed t-test.

acute doses of ethanol (from 0.5 g/kg) did reduce startle amplitude [14]. Because neither drug changed the rate of licking or the initial distraction to the tone, in this situation at least, the more rapid habituation was not secondary to changes in response level. The unchanged initial distraction also makes it unlikely that the drugs were changing either sensory thresholds or stimulus salience. It is therefore proposed that reduced anxiety leads to more rapid habituation primarily because stimuli are more readily classified as irrelevant.

In the case of CDP the more rapid habituation was accompanied by a loss in the specificity with which the physical parameters were coded. Iwasaki *et al.* [8] have reported that CDP impairs the ability to integrate stimulus cues when these are presented sequentially. This deficit may account for one of the effects of CDP in the holeboard. When objects were placed under the holes CDP impaired the between-day habituation [4], perhaps because of a deficit in integrating the very complex set of stimuli from day to day. Certainly there was no indication of either CDP or ethanol producing more rapid habituation of exploration. This is further evidence that habituation of exploration and habituation of distraction have different mechanisms [18], but would also support the suggestion that the critical difference between the two situations might lie in the stimulus analysis involved and not in the different responses [3].

There was no evidence from Experiment 2 that sensitization, reflected in dishabituation, was affected by either ethanol or by CDP and so the more rapid habituation cannot be attributed to a reduction in sensitization (a process that leads to an increase in responding with stimulus repetition). These two drugs are the only ones that have been found to improve habituation in this test situation. But in a very similar test, hippocampal lesions transecting the fimbria, but sparing the cingulum, resulted in more rapid habituation [11]. It is extremely interesting that rats injected with ethanol were not only able to habituate more rapidly, but were able to code the physical parameters of the stimulus at least as accurately as undrugged rats. These results are probably restricted to relatively low doses of ethanol. Low doses of ethanol produce EEG activation [17], in contrast to signs of drowsiness at higher doses, and certainly the doses used in this experiment were too low to produce decreased motor activity [5].

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