Br. J. Pharmac. (1974), 51, 447-451

THE EFFECT OF PHASE-SHIFT ON THE PASSIVE AVOIDANCE RESPONSE IN RATS AND THE MODIFYING ACTION OF CHLORDIAZEPOXIDE

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1 In rats trained to a 12 h light-12 h dark cycle, advancing the phase by 6 h produced a resynchronization of the 24 h variation in passive avoidance response (PAR) which was completed after 10 days.

2 The attainment of the new steady state was preceded by a period of disruption which was greatest 5 days after phase-shift.

3 The presence of chlordiazepoxide (62.5-500 μg/ml) in the drinking water during the days after phase-shift produced a dose-dependent lessening of the disruptive effect of phase-shift, and a more rapid adaptation to the new light-dark cycle.

Introduction

Most physiological functions exhibit a circadian rhythm which under normal circumstances is synchronized to a 24 h cycle by environmental stimuli. It is well known that the response to phase-shift (the sudden alteration of the external time cues or zeitgebers, for instance by transmeridian flight) is a resynchronization of the circadian rhythm to the new zeitgebers. The time taken to resynchronize varies with the individual and with the particular rhythm studied (Hauty & Adams, 1966a, b; Weitzman, Kripke, Goldmacher, McGregor & Nogiere, 1970). Transients characteristically precede the attainment of the new steady state (Pittendrigh, 1960), and there is a growing awareness that during this period psychological and behavioural functions as reflected in decision time, concentration, reaction time, mental agility and reaction to stress, may be adversely affected (Hauty & Adams, 1966a, b; Klein, Bruner, Holtmann, Rehme, Stolze, Steinhoff & Wegmann, 1970; Klein, Wegmann & Hunt, 1972; Hale, Hartman, Harris, Williams, Miranda & Hosenfield, 1972; Conroy, 1972).

As far as we are aware the only published report on the use of drugs to alleviate the adverse and possible dangerous effects of phase-shift is that of Ehrenstein, Schaffler & Muller-Limmroth (1972) who used oxazepam in an attempt to offset the effects of shift-working on day-time sleep patterns.

In the experiments reported in this paper, the passive avoidance response (PAR) was chosen as a simple test of learning and retrieval which has been demonstrated to display a 24 h rhythm (Davies, Navaratnam & Redfern, 1973).

The essential feature of a passive-avoidance situation is that, at the first trial, the animal is given a noxious stimulus which is associated with a specific situation. At subsequent trials, the animal can avoid the noxious stimulus by avoiding the associated situation. It was thought particularly suitable for this investigation because it involves a one-trial learning procedure and subjects the animals to minimal training. The effect of phase-shift on the PAR and the influence of chlordiazepoxide (librium) on this response are reported.

Methods

Male Sprague-Dawley rats weighing 120-150 g at the beginning of the experiment were housed in environmental cabinets (described in detail by Hillier, Davies & Redfern, 1973) which provided controlled conditions of light and which excluded sound below 74 dB at all audible frequencies. By this means different groups of animals could be exposed to different light cycles at the same time; in this paper, the middle of the dark phase has always been taken as 00 h 00 min and the middle of the light phase as 12 h 00 min and all other times have been transformed accordingly. All animals were allowed food and water *ad libitum*

and were allowed 14 days acclimatization before the experiment.

Phase-shift

The normal lighting cycle was 12 h light (06 h 00 min-18 h 00 min) and 12 h dark (18 h 00 min-06 h 00 min). The phase-shift used in all our experiments was an advance of 6 h made at 12 h 00 minutes.

Passive-avoidance

The apparatus, first described by Burešova, Bureš, Bohdanecky & Weizz (1964), consisted of a large, light compartment (280 x 280 mm) connected by an opening to a small, dark compartment (240 x 165 mm) which had a grid floor that could be electrified. On initial exposure the animals were allowed to explore for 3 minutes. The time spent in the dark compartment during this initial exploratory period was always between 160 s and 180 s and did not vary significantly with clock hour. At the end of this period the animals were confined to the dark compartment for 30 s and shocked (0.3 mA, 55 V a.c., one shock of 50 ms every 2 s, not scrambled). Twenty-four hours later the time spent in the light compartment was recorded during a second 3 min period of exploration. In our experience behaviour on retrial is of two distinct kinds; some animals remain in the light compartment the whole time, while others spend almost the entire time in the dark compartment. We have therefore found it convenient to assume an arbitrary cut-off point. Animals spending more than 90 s in the light compartment were assumed to have acquired the passive avoidance response and the results are expressed as the percentage of animals in each group having so 'learned'.

The Chi-square test was used to assess the statistical significance of the difference between groups. Comparisons of proportions of correct responses for individual treatments and for the collective control groups were made and the Chi-square statistic calculated on one degree of freedom.

Results

In all figures the times shown on the abscissae are the times of retrial. When the PAR was measured at either 06 h 00 min or 18 h 00 min, the trial was always carried out immediately after the change in illumination. The initial exploratory period was always 24 h before retrial, thus avoiding any possibility of retention deficit at intermediate intervals (the 'Kamin effect', for instance see Holloway & Wansley, 1973) complicating the results.

The effect of phase-shift on the passive avoidance response

The normal 24 h rhythm in PAR shows a peak in the middle of the light phase (12 h 00 min) and a trough in the middle of the dark phase (00 h 00 min) and we have previously presented evidence to show that this variation reflects a 24 h rhythm in some aspect of learning and retention, and does not simply result from the fact that some animals were taken from a dark environment into the brightly lit apparatus while others were already accustomed to the light at the time of trial (Davies *et al.*, 1973). The effect of phase-shift was observed by measuring the PAR at two clock hours, 00 h 00 min and 12 h 00 minutes.

In Fig. 1, the PAR at 12 h 00 min for 12 days after phase-shift is compared to the responses at 12 h 00 min and 18 h 00 min before phase-shift. It can be seen that by 10 days after phase-shift the PAR is not significantly different from the new 'expected' level (equivalent to 18 h 00 min in non-phase-shifted animals). It is also clear that the attainment of the new steady state is not smooth but is preceded by a period of disruption which is maximal on day 5.

The same effect is seen in Fig. 2 in which the PAR at 00 h 00 min for 13 days after phase-shift is compared to the responses at 00 h 00 min and 06 h 00 min before phase-shift.

The effect of chlordiazepoxide on phase-shift

Since the greatest disruptive effect of phase-shift was apparent after 5 days, the PAR at this time was used to measure the effects of chlordiazepoxide on phase-shift.

Chlordiazepoxide was administered in the drinking water. Solutions of 62.5, 125, 250 and 500 μg/ml were made by dissolving chlordiazepoxide base in a small volume of 0.1N HCl and diluting to volume with tap water. The solutions were kept in light-proof bottles and were renewed every 24 hours.

Figure 3 shows the effect of phase-shift in animals receiving chlordiazepoxide for the period between phase-shift and the retrial of the PAR at 12 h 00 min 5 days later. At each dose level, three groups of ten animals were tested: phase-shifted animals receiving chlordiazepoxide and phase-shifted animals receiving tap-water (both groups tested at 12 h 00 min) and non-phase-shifted animals receiving chlordiazepoxide tested at 18 h 00 minutes.

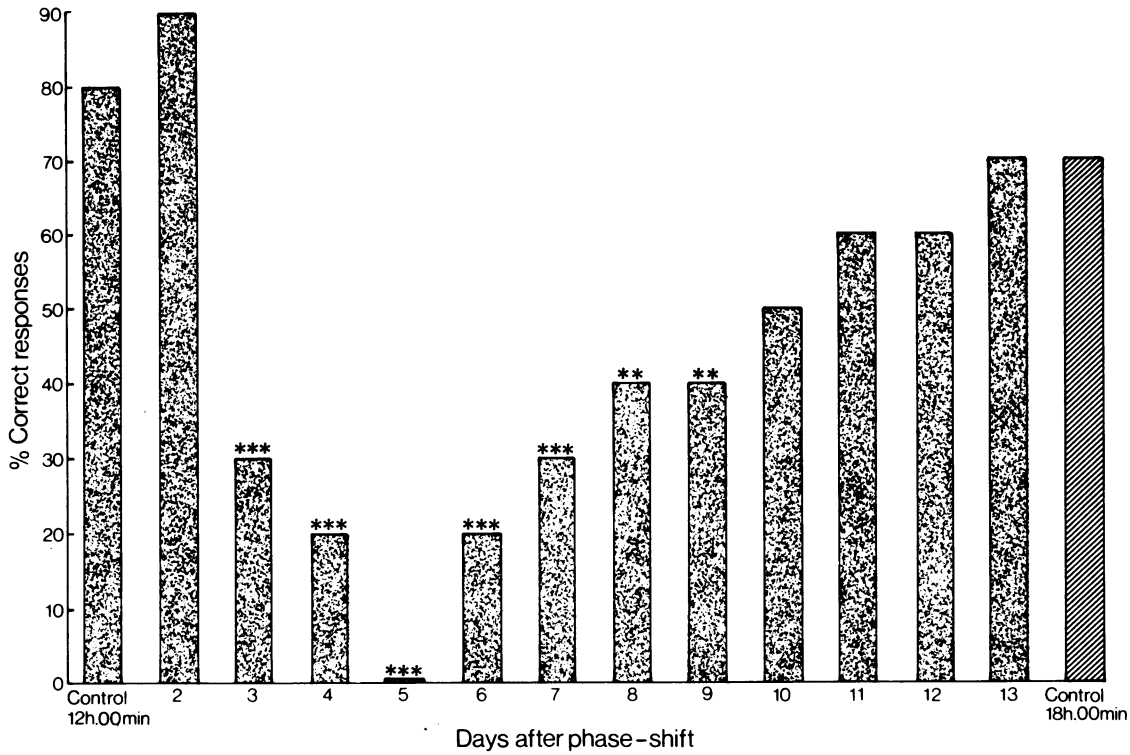

Fig. 1 The effect of phase-shift on the passive-avoidance response at 12 h 00 minutes. The percentage correct responses before phase-shift and for 13 days thereafter is compared to the percentage correct responses at 18 h 00 min before phase-shift (hatched column). Differences from the 18 h 00 min response, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

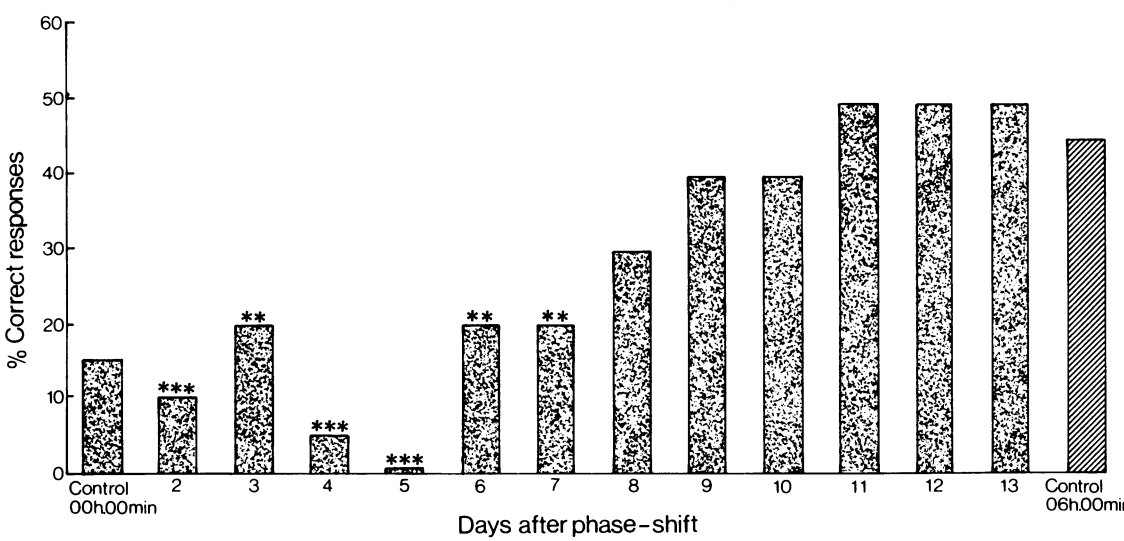

Fig. 2 The effect of phase-shift on the passive avoidance response at 00 h 00 minutes. The percentage correct responses before phase-shift and for 13 days thereafter is compared to the percentage correct responses at 06 h 00 min before phase-shift (hatched column).

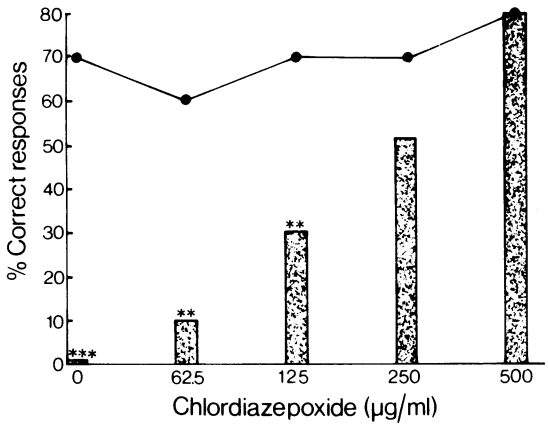

Fig. 3 The effect of chlordiazepoxide on phase-shift. The columns show the passive avoidance response at 12 h 00 min on the 5th day after phase-shift in chlordiazepoxide-treated animals compared to that in phase-shifted animals receiving no chlordiazepoxide. The passive avoidance response in chlordiazepoxide-treated non-phase-shifted animals measured at 18 h 00 min in also shown (•).

As in the experiment shown in Fig. 1, the phase-shifted animals which did not receive chlordiazepoxide consistently failed to acquire the PAR. The non-phase-shifted animals receiving chlordiazepoxide showed a PAR of between 60% and 80% which was independent of dose and did not vary significantly from the response of control non-phase-shifted animals, showing that in the doses used, chlordiazepoxide did not directly affect the PAR.

It can be seen that phase-shifted animals receiving chlordiazepoxide for the 5 days after phase-shift showed a dose-dependent improvement in the acquisition of the PAR and that the response of the animals receiving the two highest doses of drug did not differ significantly from that of the non-phase-shifted animals tested at 18 h 00 minutes. These animals appeared therefore to have adapted to the new light-dark cycle after 5 days and it was of interest to see whether this was a temporary effect dependent on the presence of the drug, or whether a permanent resynchronization had occurred. Further groups of rats were therefore subjected to phase-shift and provided with a solution of chlordiazepoxide (500 μg/ml) for 5 days thereafter. The PAR was then measured in different groups of phase-shifted animals every two days and the results are shown in Figure 4. As

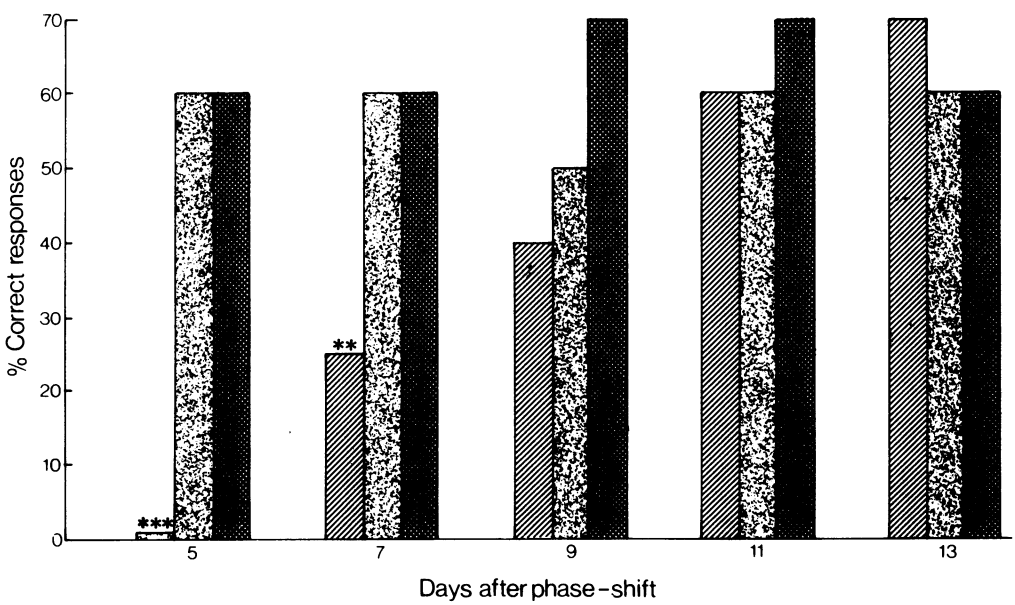

Fig. 4 The effect of chlordiazepoxide on phase-shift. The passive avoidance response at 12 h 00 min in chlordiazepoxide-treated, phase-shifted animals (stippled columns), and phase-shifted control animals (hatched columns) is shown up to 13 days after phase-shift. The significance from the passive-avoidance response at 18 h 00 min in non-phase-shifted control animals (cross hatched columns) is shown by asterisks.

already shown in Fig. 3 the drug-treated, phase-shifted animals gave the new 'expected' PAR after 5 days, and this response was found not to vary significantly up to 13 days after phase-shift.

Discussion

The results of these experiments show that the effects on the passive avoidance response of a 6 h positive phase-shift can apparently be prevented by treatment with chlordiazepoxide. Since dosing animals several times a day would inevitably have introduced an additional time-cue, we chose to administer the drug in the drinking water. The animals were not given a choice, but the presence of drug in the drinking water did not measurably affect fluid intake. However the disadvantage of this method is the absence of an exact measure of the dose administered. If no drug were lost by spillage or degradation, and all the animals in each group drank the same volume daily, then it can be calculated from the volumes used that the daily doses of chlordiazepoxide varied between 7.5 mg/kg and 60 mg/kg. Clearly this will be an overestimate. It is also to be expected that the rate of ingestion, and therefore the plasma concentration of drug, varied with clock-hour. It would be of interest to know whether it is necessary to administer the drug over the prolonged period used here, or whether a single dose at the time of phase-shift would produce the same effect.

The only previous report of drug treatment of the effects of phase-shift appears to be that of Ehrenstein *et al.* (1972) who showed that day-sleep in night-working nurses was prolonged by oxazepam. Experiments in progress in our laboratory suggest that other benzodiazepines have the same effect as chlordiazepoxide on PAR after phase-shift. Two questions to be answered, therefore, are: is the effect reported here in any way specific to the benzodiazepines, and can the effect be extended to other parameters known to be affected by phase-shift? When these questions have been answered, we may be in a better position to judge whether the results of these experiments have any relevance to the effects of transmeridian flight in man.

Librium (chlordiazepoxide) was kindly supplied by Roche Products Limited.

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(Received February 18, 1974)