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Some studies on the influence of chlordiazepoxide on the social interaction of golden hamsters *(Mesocricetus auratus)*

T. B. POOLE

Department of Zoology, University College of Wales, Aberystwyth, Wales

Summary

1. The effects of a 'minor' tranquillizer (chlordiazepoxide) on the social behaviour of male golden hamsters were investigated.

2. Thirty-six hamsters were divided into pairs. Each pair was placed in an arena measuring 1 m^2; under these conditions the hamsters fought, but once a winner and loser emerged the relationship between the two animals became stabilized. The influence of 50 mg/kg chlordiazepoxide on this stable relationship was investigated and compared with normal behaviour in the arena.

3. Under the influence of chlordiazepoxide, hamsters showed a significant reduction in 'aggression', 'defensive fighting' and an increase in 'investigation'.

4. When the data for winners and losers were analysed independently, the drug was seen to influence losers more than winners.

5. It is postulated that the primary influence of chlordiazepoxide is to reduce fear and as a result 'investigation' is probably affected indirectly as a behaviour pattern suppressed in an individual showing fear of its opponent.

Introduction

Chlordiazepoxide is known to have a taming effect on some wild animals where it has been claimed (Hentschele, 1961) that it reduces aggression. The following experiments were designed to investigate the effects of a taming dose on the intraspecific aggressive behaviour in the golden hamster.

Chance & Silverman (1964) and Silverman (1965, 1971) have demonstrated the value of an ethological approach to drug testing. Using brown laboratory rats, Silverman (1965, 1966a, b) compared the fighting behaviour of normal animals with that shown by drugged rats. He demonstrated conclusively that amylobarbitone, amphetamine, chlorpromazine and other drugs produced major changes in the occurrence of certain behaviour patterns during and after fighting.

However, it has been shown (Poole, 1972) that in polecats individual variations in aggression are considerable. Preliminary experiments suggested that this was also true of hamsters. Consequently it was considered necessary to take individual variations into account (see Silverman, 1971), particularly since these variations are an important behavioural factor, both in man and in many naturally occurring populations of animals.

In the experiments to be reported, male hamsters were placed in an unfamiliar environment in which their social interactions with an unfamiliar individual could be observed (see also Payne & Swanson, 1970). Under these conditions hamsters fight, and one is usually submitted to the stress of losing. A variety of behavioural patterns can be observed and the effects of a drug on these can be studied. A factor favourable to this ethological method is that many of the elements of social behaviour are stereotyped and common both to hamsters (Dieterlin, 1959) and many other rodents (Grant & Mackintosh, 1963). Because they are stereotyped they are easy to observe and record without ambiguity. Therefore, it was felt that such an approach to the investigation of the effects of tranquillizers was justified.

Methods

Preliminary experiments with hamsters showed that, typically, fighting could be divided into three phases, an investigatory period when the two individuals behaved similarly showing investigatory and low intensity aggressive and defensive behaviour, a fight period when both animals showed high intensity aggressive and defensive behaviour, and a post-fight period when a 'winner' and a 'loser' emerged. In the post-fight period the behaviour of both was distinct and this was therefore referred to as the 'post-fight dichotomy'.

Once a post-fight dichotomy has been established it generally remains stable, and can be observed if the same animals are placed together on a second occasion 24 h or even a week later. This predictable behaviour has been utilized in the experimental procedure: after the post-fight dichotomy had stabilized, one animal was given chlordiazepoxide and its behaviour compared with that shown in its previous encounter. The animal therefore acted as its own control.

The most complex types of social interaction described in this work are the two main forms of fighting; equal fighting in which attempts to bite are combined simultaneously with attempts to avoid being bitten and unequal fighting in which one individual merely defends itself against attack. For each individual, therefore, fighting has been classified either as that involving marked aggression ('fighting') or that which is defensive only ('defensive fighting').

Procedure

Animals The 36 golden hamsters used in this study were of the CLGN agouti type and had been isolated in cages $20 \times 20 \times 30$ cm for 4 weeks before the experiments began. The animals were experimentally 'naïve'. Males only were used (to avoid variations in aggression, shown by Payne & Swanson (1970) to be related to the female oestrous cycle) and all were over three months old at the time of the experiments. They were fed *ad lib.* on a dry diet of MRC 41 B pellets and a daily slice of carrot.

Lighting schedule Golden hamsters are nocturnal animals with peaks of activity immediately after dawn and before dusk. In the laboratory they had been maintained for at least 14 days under a modified diurnal rhythm (0.00–12.00 white light, 12.00–24.00 dim red lighting). Experiments were carried out during one of the periods when they normally showed activity under this régime (i.e. between 14.00 and 17.00).

Apparatus The apparatus consisted of a 1 m^2 arena with 30 cm high walls which were painted matt white to prevent the hamsters responding to their own reflections. The arena was lit by two 500 W photo-flood bulbs suspended 1.5 m above the arena to enable an Eumig 'C5' single lens reflex ciné camera to be used; pilot experiments under photo-flood versus dim red lighting had established that the photo-flood lighting had no detectable effect on the hamsters' social behaviour in the arena.

Experimental procedure For each experiment, two animals were placed simultaneously in the arena, and the behaviour of both was recorded for ten minutes. Written notes were taken, and any rapid sequences of behaviour were also filmed; the hamsters showed no apparent reaction either to camera noise or to the presence of experimenter and cameraman.

Determining experiments

Eighteen pairs of male golden hamsters were used. Each pair met daily in the arena for ten minutes until a stable relationship had developed so that two consecutive encounters were similar. In the first of these 'determining' encounters, the animals, being unfamiliar with one another, fight and this determines their status. Consequently it was necessary to arrange 3–4 encounters to obtain two consecutive similar ones. Only one of the eighteen pairs of hamsters failed to fight and this pair was therefore excluded from subsequent experiments.

Drug experiment

Two hours before each experiment both hamsters were given one level 2 ml teaspoonful of Glaxo 'Complan' (a high protein milk food) mixed with enough water so that the mixture just covered the bottom of a 5 cm Petri dish; the hamsters invariably drank all of this mixture. Chlordiazepoxide, 50 mg/kg, was administered orally to the experimental animal via this medium. A check was made to ensure that both control and experimental animals had completely consumed the liquid.

The main aim of the experiment was to discover the precise effects on social behaviour of the dose of chlordiazepoxide which would produce a taming effect on hamsters. To select an appropriate dose level, doses of 20, 30, 40 and 50 mg/kg were administered in pilot experiments and their effects on the tameness of the individual were recorded. Undrugged hamsters and those given 20, 30 and 40 mg/kg showed a clearly defined response when the experimenter placed a hand in the animal's cage. This response took the form of biting, running away or adopting a sideways offensive posture. Hamsters given a dose of 50 mg/kg, however, approached the human hand, climbed on to it and ran up the arm; 50 mg/kg was therefore adopted as a dose which gave a distinct taming effect yet had no adverse effects on mobility or co-ordination.

An orally administered dose was preferred to an injection because the latter procedure disturbed the hamsters, made them nervous of the experimenters and might have influenced their subsequent behaviour in the arena.

Analysis of data

Behavioural elements in the form of 'acts' and 'postures' were classified according to the terminology of Grant & Mackintosh (1963). A third category of behaviour termed 'interactions' has now been included. An interaction is defined as a behaviour pattern or a rapid sequence of behaviour patterns, dependent upon a specific reaction from the opponent. Thus it is a joint action carried out by two animals and is dependent upon social co-operation. Examples of interactions are ' nose ' (in which both hamsters adopt a similar posture simultaneously) and ' chase ' (involving flight by one animal and tracking by the other).

In order to reduce these elements to a manageable number for analysis and interpretation some of them have been combined into categories which represent functional groups. For example, the group ' movement towards ' contains the elements ' approach ' and ' follow '; the effect of such behaviour is to reduce the distance between the two individuals. Similar criteria have been observed in other categories of behaviour, the most complex being ' fear '; each of the elements of behaviour listed under ' fear ' depends upon the position of the opponent and the freedom of movement available in the particular circumstances. Table 1 shows the categories of behaviour used in the analysis of the results.

TABLE 1. *Behavioural categories used in analysis of the data*

Name of category	Constituent elements
Aggression	attack, chase
Attempted mounting	attempted mount
Attend	attend
Biting	bite, allogroom
Defensive fighting	behaviour of defender in an unequal fight (evasion of attack without retaliation)
Defensive postures	upright defensive posture, sideways defensive posture
Fear	flee, freeze, full submissive posture
Fighting	equally matched fight, or behaviour of aggressor in unequal fight (offensive fighting)
Investigation	investigate, nose, sniff
Movement away	avoid, retreat
Movement towards	approach, follow
Offensive postures	sideways offensive posture, upright offensive posture

Two encounters were judged to be similar by the Kruskal–Wallis analysis of variance (Siegel, 1956) when the proportions of offensive or defensive behaviour shown by the individual were approximately equal for both encounters. The existence of a stable post-fight dichotomy was, therefore, based on numerical criteria ; it was also easy to identify subjectively.

In each encounter only one of the two hamsters was drugged, the individual selected being chosen at random. For each drugged hamster, statistical tests were carried out on every behavioural category to compare the pre-drugged with the drugged encounter.

In order to discover whether the proportions of the different behavioural categories had changed under the influence of the drug, the occurrence of each one was expressed as a percentage of the number of all social categories occurring. For each individual the differences in behaviour were estimated by subtracting the percentage non-drugged behaviour from the percentage drugged behaviour.

An inspection of the results suggested that individuals of different status might react differentially to the drug. The data were therefore divided into three categories for ' winners ', ' losers ' and ' intermediates ' ; these divisions were based upon the status of the individual during the post-fight dichotomy and before the administration of the drug. Winners were defined as individuals which showed the behavioural element aggression $>2{\cdot}0\%$ and fear $<2{\cdot}0\%$, while losers showed fear $>2{\cdot}0\%$ and aggression $<2{\cdot}0\%$; individuals falling into neither of these categories were classified as intermediates.

Results

Amongst the 17 drugged animals, 5 were winners, 9 losers and 3 intermediates.

The differences between the behaviour of the drugged hamsters as compared with their normal behaviour are shown in Table 2. Statistically significant values for the Wilcoxon test (see (Siegel, 1956) were obtained for an increase in investigation ($P<0{\cdot}005$) and a decrease in defensive fighting ($0{\cdot}05>P>0{\cdot}02$) and aggression ($0{\cdot}05>P>0{\cdot}02$).

TABLE 2. *The influence of chlordiazepoxide on the post-fight behaviour of hamsters. Changes in behaviour are expressed as percentages of social behaviour recorded*

	Wilcoxon test						Sign test		
	Combined data			Data for losers			Data for winners		
	Drugged minus control % change			Drugged minus control % change			Drugged minus control % change		
Behaviour patterns	median	range	*P*	median	range	*P*	median	range	*P*
Aggression	−0·6	−40·0, +2·6	0·05				−4·5	−40·0, −2·1	0·062
Investigation	+11·8	+0·5, +21·7	0·005	+11·8	+0·8, +21·7	0·01	+10·2	+5·4, +19·4	0·062
Movement towards				+8·2	−2·1, +18·2	0·02			
Fear				−12·5	−41·4, +12·0	0·05			
Defensive fighting	−0·8	−20·6, +2·1	0·05	−5·5	−20·6, +2·1	0·05			
Defensive postures				−6·8	−16·0, +2·5	0·02			
No. of individuals	17			9			5		

The effect of the drug appeared to be related to the post-fight status of the individual, and Table 2 shows also the results obtained by independently analysing the data for individuals of different status.

Whilst the results for intermediates were too few to be useful in comparison, it is clear that winners and losers differed in their response to the drug. Table 2 shows that the drug appeared to have its greatest effect on the losers in reducing defensive postures, fear and defensive fighting and in increasing investigation and movement towards. The results for the winners, however, failed to show statistically significant values but the sample was small and near-significant levels ($P=0{\cdot}062$) were obtained for increased investigation and decreased aggression. It is clear from the column headed ' combined data ' in Table 2 that there was also a statistically significant decline in aggression in the data for winners and intermediates combined (losers showed no aggression).

Although statistically significant results were obtained overall, hamsters showed a considerable amount of individual variation in response to the drug. For example, one hamster changed from an aggressive individual (aggression 40%, fear 0%) to a timid one (fear 34%, aggression 0%), while in another animal the amount of fear was reduced from 53% to 11% ; this hamster also showed an increase in investigation of its opponent from 3% to 25%. Further evidence of individual differences is provided by the range values given in Table 2.

To check on the similarity between the first and second determining experiments by comparison with the drug test experiments, a Kruskal–Wallis analysis of variance for the three encounters (determining experiment 1, determining experiment 2 and drug test experiment) was performed; this showed that, whereas the determining experiments resembled one another as a class for all behavioural categories, the drug test experiments, as a class, differed from the determining experiments for those behaviour patterns which had been shown to be influenced by the drug.

Discussion

Previous work on the influence of tranquillizers on aggression in rats (Chance & Silverman, 1964; Silverman, 1965, 1966a, b) compared the behaviour of drugged animals with a separate group of controls injected with saline. In the present experiments, as in those of Silverman (1971), the behaviour of the individual was stabilized and then each individual was used as its own control.

Expression of the results as percentages enables the determining and drug experiments to be compared and it also eliminates any effect which the drug may have on overall social activity. Thus, information was obtained on the relative frequency changes of the different types of behaviour.

The use of the three categories, winners, losers and intermediates, revealed significant differences in the animal's responses to chlordiazepoxide which were not apparent previously. Thus, hamsters which are losers in non-drug conditions respond to their opponent by showing fear, defensive fighting and defensive postures, types of interaction referred to as 'intimidation'. In addition, intimidated hamsters do not normally show investigation or movement towards the opponent, types of behaviour which can be regarded as social approaches. It was found that chlordiazepoxide changes the behaviour of losers by decreasing intimidation and increasing social approaches; as these two types of behaviour are mutually incompatible, a drug which affects either of them will conversely affect the other indirectly. Consequently a drug which increases social approaches may be expected to decrease intimidation and *vice versa.*

Although the statistical tests indicate that chlordiazepoxide induces an increase in social contact, it is apparent that this effect was most marked in the losers.

It seems possible therefore that the primary behavioural effect of chlordiazepoxide may be to reduce intimidation (or anxiety) and that its effect on social contact may be indirect. Such a hypothesis would explain the reduction of the amount of behaviour indicating intimidation (i.e. fear, defensive postures and defensive fighting) in the losers and its failure to affect movement towards in the winners.

If this view of the action of the drug is correct then the tendency to an increase in investigation shown by the winners may perhaps be explained either as a specific effect of chlordiazepoxide on investigation or, alternatively, it may be that the drug reduces anxiety and that even winners normally show some anxiety in the presence of their opponent.

One important aspect in studying the effects of a drug on animal behaviour is the possibility of extrapolation to human behaviour. The case for this has been recently emphasized by Steinberg (1970). On the basis of the results reported here it may be hypothesized that a human being in a stressful situation would show less fear and avoidance of the situation after taking chlordiazepoxide. Consequently the human subject might be expected to show more positive activity normally inhibited by a state of anxiety or fear. If winning a fight can be regarded as an indication of the hamsters' confidence, then, on the basis of these results, it could be expected that chlordiazepoxide will have less effect upon the social behaviour of a non-anxious person than on one who is anxious. These findings are in agreement with those of Barrett & DiMascio (1966), whose data indicated that a 15 mg dosage four times per day of chlordiazepoxide reduced anxiety in 'high anxious' student volunteers but did not have this effect on 'low anxious' subjects.

As regards the methodology of the experiments reported in this paper, it was chosen primarily because it was possible to obtain positive results with a small sample of animals despite considerable individual variations in behaviour. The animal's behaviour can be standardized, classified into categories and, furthermore, no specialized apparatus is needed apart from a ciné camera and suitable lighting.

The results also suggest that chlordiazepoxide reduces the aggression of male golden hamsters towards males of their own species and it has been claimed (Hentschele, 1961) that it reduces aggression in zoo animals.

Aggression observed in the male golden hamsters took the form of an active approach to the opponent and spontaneous attacks on it; in contrast, however, the form of aggression found in captive wild animals is usually what Hediger (1950) has termed the 'critical reaction', which is primarily a fear response. Hediger argued that, in attacking a man, the wild animal is adopting the only form of self-defence available when flight and avoidance are impossible. It seems likely therefore that what is regarded as a reduction of aggression in zoo animals is, in reality, a reduction of the tendency to self defence. In this situation, therefore, it is the anxiety-reducing property of the drug which is probably the most significant factor rather than its influence on aggression.

In addition, Hoffmeister & Wuttke (1969) have shown that 10 mg/kg chlordiazepoxide decreases defensive aggression in cats, while having no effect upon spontaneous attacking behaviour. These results are in general agreement with the data obtained for hamsters and they also support the hypothesis that chlordiazepoxide primarily reduces self-defence rather than aggression in zoo animals.

These examples underline the importance of studying the effects of a given drug under a range of environmental conditions. It is also important to investigate the influence of the drug on a number of different species, for preliminary studies on the shrew *Crocidura russula*, for example (Poole, unpublished observations), indicated that large doses (e.g. 200 mg/kg) of chlordiazepoxide administered either orally or subcutaneously had no detectable influence upon their social behaviour. This last finding suggests that the effects of chlordiazepoxide are, to some extent, species-specific.

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