

## THE INTEGRITY OF THE SOCIAL HIERARCHY IN MICE FOLLOWING ADMINISTRATION OF PSYCHOTROPIC DRUGS

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1 Mice in small groups develop a despotic type of social hierarchy, a feature of which is to resist alteration through the medium of psychotropic drugs. This makes a rapid pharmacologically induced change in the social hierarchy impossible.

2 Patrolling the territory and a certain level of social interaction are both critical factors in maintaining the phenomenon of inertia in the social hierarchy. Psychotropic drugs (diazepam, droperidol and mescaline) altered both these factors to a varying degree and also displayed a differing ability to maintain the inertia of the social hierarchy.

3 A drug-induced alteration in the level of aggression in a subordinate mouse in a group of three does not cause an alteration in its social position.

4 Chronic administration of diazepam, droperidol or mescaline, all of which alter the level of aggression in different ways, can result in an inversion of the social hierarchy where a competitive rival is present in the group of mice. The rate of inversion of the social hierarchy depends on the type of pre-existing social hierarchy.

5 It is suggested that the ability of psychotropic drugs to maintain the inertia of the hierarchy be used as an index of their effect upon certain types of species-specific behaviour; in particular aggression.

### Introduction

Mice establish a dominance hierarchy in a relatively short period when placed in a small enclosure (Crowcroft, 1966; Mackintosh, 1970; Poole & Morgan, 1973; Novikov & Kiadanov, 1978) and these relationships are a convenient subject for pharmacological experimentation (Cutler, Mackintosh & Chance, 1975; Ely, Henry & Jarosz, 1975; Poshivalov, 1976; Valdman, 1978). However, it is not clear to what extent the pharmacological effects depend on extra-drug factors, such as social environment and specific interrelationships, in particular, the stability of the dominance-subordinate (DS) relationships and the type of hierarchical organization in animal groups. The aim of the present work was to study the character of hierarchical structures in model populations of laboratory mice (in groups of 3, 4 or 12) and the controlled pharmacological regulation of the DS relationships. The present study was also intended to examine: (1) the possibility of inverting DS relationships by acute and chronic inhibition of aggressive behaviour in dominant animals, or by increasing the levels of aggressive behaviour in subordinate animals, and (2) how the psychotropic drug effects depend on the type of hierarchical organization in the groups of animals.

### Methods

A total of 200 randomly bred CC57W male laboratory mice weighing initially 16 to 18 g were used. At first they were caged in groups of 10 in stock boxes measuring 40 × 25 × 15 cm. They were maintained at a temperature between 22 and 24°C under a reversed lighting regime, with strong white light from 18 h 00 min to 09 h 00 min and dim red light for the remaining period. Water and standard briquette diet was available *ad lib*. The observations were carried out at uniform times between 09 h 00 min and 16 h 00 min. There were four series of experiments and apart from experimental days the mice were not handled.

### Experiment 1

Thirty-six spontaneously aggressive mice were chosen from the groups by successive selection of the dominant mouse from each home cage (10 mice in each cage). The selected mice were housed singly in separate cages and subdivided into nine groups consisting of 4 mice each. DS relationships were studied in a specially constructed communication device, 50 × 50

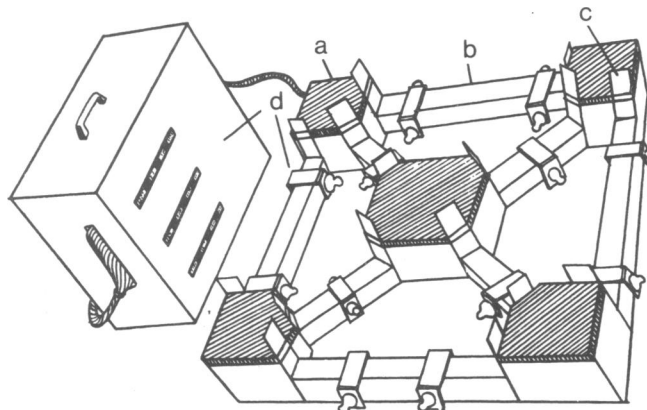


Figure 1 Communication device: (a) boxes, (b) tubes, (c) passages, (d) registration system.

cm (Figure 1) (Poshivalov, 1977; 1978). Following three successive control exposures in the apparatus (one a day), a stable social hierarchy was established in all the groups in less than three days. Prior to testing one mouse was placed in each of the four boxes on the periphery of the apparatus. The animals were given a 15 min adaptation period in the boxes before the box passages were opened and the mice were allowed to move freely all over the apparatus for 30 min. During this period the unrestrained behaviour was assessed at 5 min intervals on a scale devised by Poshivalov (1978a). The number of newly formed aggregations of 2, 3 or 4 mice, the time of their formation and their duration, the latency to the first fight, the cumulative number of fights and the direction of attack for each mouse were recorded. There were special labels on each mouse by which the dominant mouse could be recognised. Two observers assessed the animals' behaviour simultaneously. The following drugs were used: diazepam (2.5 mg/kg), droperidol (2.5 mg/kg) and mescaline (30 mg/kg). The drugs were administered intraperitoneally in a dose volume of 0.1 ml per 10 g body weight, 30 min before each experiment. These anxiolytic, neuroleptic and psychodysleptic drugs were administered either only to the dominant or only to the subordinates.

#### Experiment 2

Longer term experiments with the same drugs were performed over a period of seven days on six selected groups of 4 mice. The mice were put into the communication device and their behaviour was assessed according to the same criteria as before. Six control groups of animals (0.9% saline, 0.1 ml per 10 g body weight) were assessed in the same way over a period of 14 days.

#### Experiment 3

Groups of 12 male mice were transferred from a home cage into an 'open field box' (50 × 50 × 40 cm). The floor covering was changed before each exposure. Three control exposures had been carried out in the 'open field box' since the formation of each group and its social structure in the home cages. The total number of social interactions, fights, chases and directions of attack were recorded for 30 min. The social structure of the group was also determined (i.e. which mouse was the dominant, subdominant and subordinate). A partial hierarchy was formed in all the groups. In every group the dominants were injected with diazepam 2.5 mg/kg for seven days. Six intact groups acted as controls (0.9% saline, 0.1 ml per 10 g body weight) for 14 days.

#### Experiment 4

Twenty four groups of 3 mice (triads) were formed. A transitive linear system of subordination ( $\alpha$ - $\beta$ - $\gamma$ ) was created artificially through a special selection and training. The middle chain of this system was the focus of this experiment. Male- $\beta$  was injected with amphetamine, 0.5 mg/kg (to increase species specific aggression) in 12 groups or diazepam, 2.5 mg/kg (to decrease aggression). All drugs were injected 30 min before the experiments. Following these injections two successive exposures were carried out on the neutral territory. Ethological analysis techniques were used for the assessment of the animals' behaviour (Grant & Mackintosh, 1963; Silverman, 1965; 1966) using ECS (Ethograph Computer System, Poshivalov, 1977; 1978b). Non-parametric Wilcoxon tests were used for statistical evaluation.

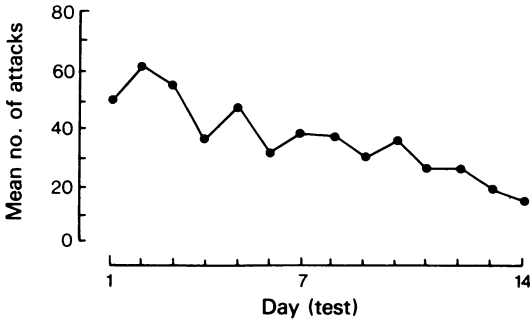


Figure 2 Changes in total number of dominant mouse's attacks in tetrads.

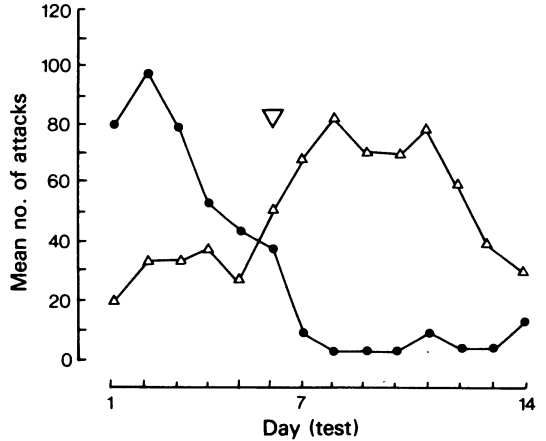


Figure 3 Changes of total number of dominant's and rival's attacks in groups of twelve mice: (●) attacks of dominant; (△) attacks of rival; (▽) time of DS relationships inversion.

Results

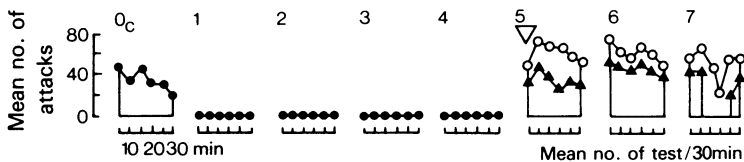
Social behaviour of male mice in control exposures

When a group of 4 aggressive male mice were introduced into the communication apparatus, distinct phases of behaviour could be discerned: (1) obligatory exploration of the territory; (2) conflict interactions (establishment of dominant-subordinate relationships); (3) maintenance of DS relationships. Two types of dominance hierarchy were found in the experiments: (A) despotic dominance, characterized by fierce unilateral attacks by the dominant against the subordinates, without fights between the subordinates, which is typical of groups of 4 and (B) partial hierarchy, where the dominant attacks other members of the group and one of the subordinates (subdominant) attacks another one and reciprocal attacks are possible, which is typical of groups of 12 mice. The dominant showed a high frequency of attacks, intimidating all the subordinates, but the distribution of the attacks was not uniform, with the most active animals being attacked more often. The total number of attacks varied with time, the attack frequency falling off with each trial (Figure 2). An inverse ratio was found between the number of the dominant's attacks and the number of upright and sideways defensive postures shown by the subordinates. The dominant's approach provoked squeaking from the subordinates as they assumed an upright defensive or submissive posture, which may inhibit the dominant's attacks. The presence of the dominant dramatically inhibited the activity of the subordinates all over the territory, which was reflected in the decrease in the number of newly formed aggregations of 2 and 3 males, and in the prolongation of the existence of old aggregations of subordinates; the subordinates not infrequently congregated in one of the boxes of the apparatus. Patrolling by the dominant, with or without infrequent attacks, successfully maintained a definite level

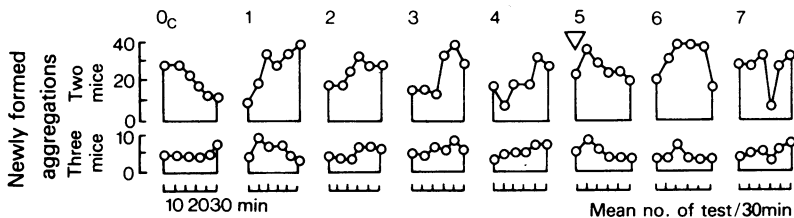
of fear in the subordinates, which was manifested as various elements of escape and flight. In the groups of 12, the dominant had a strong rival, attacking other animals and responding with reciprocal attacks to those of the dominant. Although the total number of attacks in groups of 12 was high, the number of attacks directed at any one individual was less than in tetrads, hence the dominant did not succeed in intimidating every mouse to the same degree. The control experiments demonstrated the maintenance of a stable social hierarchy in tetrads for two weeks or more (Figure 2), and in the groups of 12 mice a spontaneous inversion of the DS relationships sometimes occurred on the sixth day (Figure 3).

Experiment 1: Acute effects of diazepam, droperidol and mescaline on DS relationships in tetrads

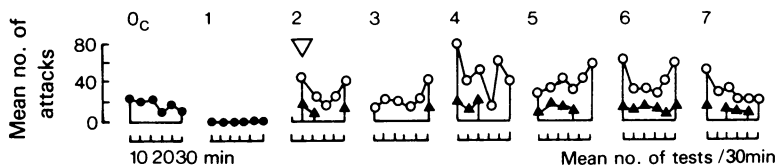
A single injection of diazepam (2.5 mg/kg) to the dominant in a tetrad resulted in a sharp decrease in the total number of aggressive elements shown (Figure 4); the number of attacks decreased significantly ( $n = 9, T = 0, P < 0.05$ ). However some forms of intraspecies sociability, that is various forms of social grooming and social investigation of the partners, were unaffected. As long as the dominant patrolled the territory, even if he did not attack them, the subordinates assumed upright or sideways defensive postures, or showed escape or flight behaviour ( $n = 9, T = 0, P < 0.05$ ). The patrolling of the dominant stimulated motor activity in the subordinates, resulting in a rise in the number of newly formed aggregations of 2 to 3 mice (Figure 5). A single droperidol injection (2.5 mg/kg) resulted in a sharp



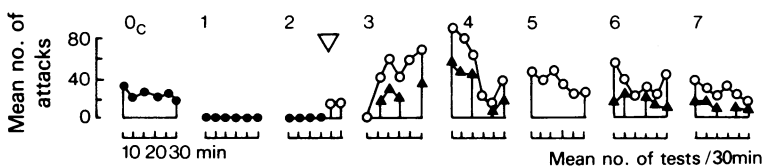
**Figure 4** Acute and chronic effects of diazepam on DS relationships in tetrads: (●) attacks of dominant; (○) attacks of rival; (▲) attacks of rival directed only to the first dominant; (O<sub>c</sub>) control exposure; (1–7) exposure to drugs; (▽) time of DS relationships inversion.



**Figure 5** Acute and chronic effects of diazepam on newly formed aggregations in mouse tetrads; symbols as in Figure 4.



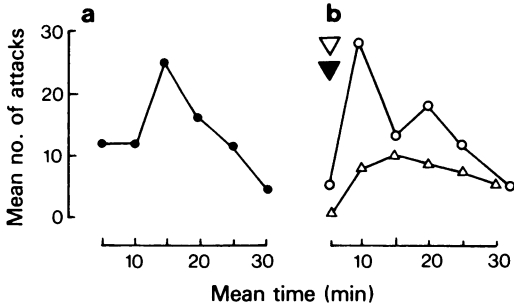
**Figure 6** Acute and chronic effects of droperidol on DS relationships in mouse tetrads; symbols as in Figure 4.



**Figure 7** Acute and chronic effects of mescaline on DS relationships in mouse tetrads; symbols as in Figure 4.

inhibition of the dominant's aggression (Figure 6), sociability and patrolling the territory, and also a decrease in locomotion ( $n = 9, T = 0, P < 0.05$ ). Neither fights among subordinates nor attacks on the dominant were observed. Only a small number of defensive postures in the presence of the dominant were recorded in the initial phases of the interaction. When the dominant did not patrol the territory, there was a rapid subsidence of fear in the subordinates, and their exploratory and motor activity in the apparatus rose. Mescaline (30 mg/kg) caused a reliable decrease in the

dominant's aggression (Figure 7), sociability and patrolling activity without provoking any significant changes in the structure of DS relationships within the observed intervals of time. In summary, acute administration of an anxiolytic, neuroleptic or psychodysleptic drug to the dominant animal did not cause a rapid inversion of DS relationships in the groups with a despotic type of hierarchy. The same doses of diazepam, droperidol and mescaline injected to all subordinates in a group of 4 did not affect the established DS relationships. Diazepam, by relieving fear and



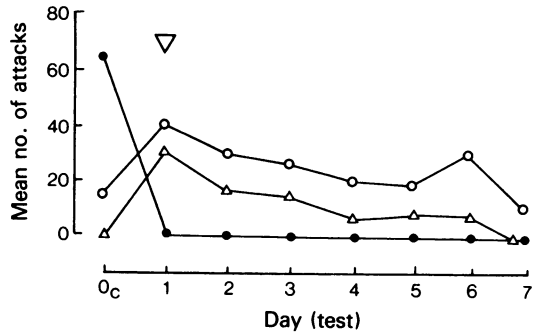
**Figure 8** Acute effect of aggressive-male introduction on DS relationships in tetrads (with dominant mouse treated with diazepam); symbols as in Figure 4. (a) control, (b) drug exposure; (▼) time of aggressive-male introduction; (▽) time of DS relationships inversion.

disinhibiting the motor activity of the subordinates, evoked an increase of conflict interactions, i.e. an increase in attacks by dominants on subordinates ( $n = 9, T = 0, P < 0.05$ ). Droperidol and mescaline inhibited the subordinates' motor activity, but it was difficult to obtain further inhibition against the background of the fear-suppressed activity; thus the structure of the DS relationships and the structure of activity did not change significantly.

*Experiment 2: The effect of chronic administration of diazepam, droperidol and mescaline on DS relationships in tetrads*

Repeated administration of the drugs to the dominants at the same daily doses resulted in inversion of the DS relationships in tetrads, but with a different time course for each drug. Only the fifth diazepam injection (Figure 4), or the third mescaline injection (Figure 7) to the dominant caused DS inversion, as a result of which a rival became dominant in the group. The patrolling of the territory by the dominant served to maintain his status without aggressive attacks, while stimulating escape and defensive behaviour in the subordinates. The subordinates exhibited prolonged defensive upright and submissive postures. They never attempted to attack or chase the dominant, even though the dominant had ceased to be aggressive. It is this that manifests itself as the 'inertia of DS relationships' in groups with the despotic type of dominance hierarchy. In these experiments a male mouse of similar rank in the tetrads was chased and attacked more frequently than the others. Indeed the data show that at the moment of inversion and in the subsequent period the number of attacks by the rival towards the first dominant rose (Figure 4, diazepam and Figure 7, mescaline).

The phenomenon of inertia of DS relationships

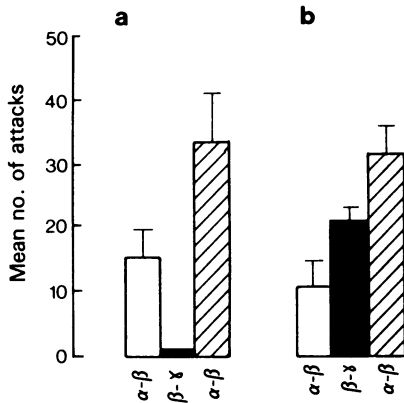


**Figure 9** The effects of diazepam on DS relationships in groups of 12 mice; symbols as in Figure 4.

only applies within groups, since the introduction into the tetrad of an intact highly aggressive male mouse, from another group and with experience of a high number of victories, resulted in a rapid change in the pre-existing DS relationships, with an increased frequency of attacks towards the diazepam-treated dominant ( $n = 6, T = 0, P < 0.05$ ) (Figure 8). In the case of droperidol a longlasting sedation led to a dramatic drop in the level of patrolling and social interaction by the dominant. The DS relationships then changed very rapidly after the second injection of the drug (Figure 6). Irrespective of the type of psychotropic drug (diazepam, droperidol or mescaline), the pharmacological inversion of DS relationships passed through a short transition from despotic dominance to partial hierarchy with a new dominant, the rival. In this phase there were no attacks on the male, but there were 'trial of strength' interactions with the subordinates. It was only after this that despotic dominance with a new dominant occurred.

*Experiment 3: The effects of diazepam on DS relationships in groups of 12 mice*

In experiments 1 and 2 it was found that diazepam maintained the phenomenon of inertia of the DS relationships in the tetrads. It was therefore decided to continue the study of these drug effects in larger groups of mice with different social structures. The presence of the diazepam-treated dominant in a group of 12 mice with a partial hierarchy resulted in a decrease in the number of aggressive interactions in the group (particularly attacks), a decrease in the ability of the dominant to maintain his dominance and rapid inversion of the DS relationships. The rival (sub-dominant) might start to attack the dominant after the first injection of diazepam (Figure 9). Such attacks were observed on the first day of injection of diazepam (2.5 mg/kg) in four groups out of six, and subsequently in all the groups.



**Figure 10** Acute effects of diazepam (a) and amphetamine (b) on frequency of attacks in linear triads: open columns—male- $\alpha$  attacked male- $\beta$ ; solid columns—interactions of male- $\beta$ , treated by diazepam (a) or amphetamine (b) with male- $\gamma$ ; hatched columns—male- $\alpha$  attacked male- $\beta$ , treated by drugs (a and b). Vertical lines show s.e. mean.

*Experiment 4: The effects of psychotropic drugs on DS relationships in a linear triad*

A linear hierarchy was created artificially in selected groups of three mice. An amphetamine treated male- $\beta$  showed an increase species specific aggression towards the subordinate male- $\gamma$  (Figure 10) ( $n = 12$ ,  $P < 0.05$ ). In the interactions between male- $\alpha$  and male- $\beta$ , the amphetamine-treated male- $\beta$  lost reliably to male- $\alpha$ , and the stimulation of locomotion in male- $\beta$  by amphetamine provoked a greater number of attacks from the male- $\alpha$ . In male- $\beta$  and male- $\gamma$  interactions the suppression of aggression, with a rise in sociability, in the diazepam-treated male- $\beta$  in relation to  $\gamma$ , did not however cause an inversion of the DS relationships between male- $\alpha$  and male- $\beta$  ( $n = 12$ ,  $P < 0.05$ ). In fact the inability of male- $\beta$  to assume defensive postures quickly enough, or to develop escape or flight in response to the attacks of male- $\alpha$  resulted in an increase in the cumulative number of attacks from the male- $\alpha$  (Figure 10). Pharmacological intensification or reduction of intraspecies aggression towards the subordinate animal does not alter the hierarchical position of the mouse in the middle of the chain (male- $\beta$ ) in a transitive linear system; that is, attacks by the male- $\beta$  directed against the dominant- $\alpha$  were not observed.

## Discussion

Crowcroft (1966) emphasized the particular role of

patrolling, which he considered to be a critical factor in the maintenance of DS relationships and territory in a competitive social environment. If after administration of a drug the dominant still maintains the same level of sociability and patrols the territory, its position in the hierarchy in small groups of mice may be kept up for some time without any open conflicts or fights 'a phenomenon of DS inertia'. The sight and odour of the dominant become a negative conditioned stimulus for the subordinates, and is sufficient to evoke escape activity and submissive postures. Under these conditions the subordinate does not take up or adequately appreciate the limitations of the dominant, which are different after injection of the different drugs (diazepam, droperidol or mescaline). A certain time must elapse before they realise, on the basis of their own experience or through extinction of fear, that the dominant is no longer able to maintain the hierarchical structure by means of attacks. The power factor (the learning of subordination) takes place under the conditions of interaction in small groups, since escape, defensive and submissive behaviour at the approach of the dominant is most clearly shown in those animals which are most frequently and fiercely attacked.

The experiments with repeated administration of diazepam, droperidol and mescaline show that the inertia of DS relationships is a phenomenon, possessing a definite time characteristic, which is a function of the extent to which the dominant has been able to intimidate the subordinates. In the despotic type of groups, DS relationships constitute an inert 'psychological factor' withstanding the effect of psychotropic drugs, and preventing any rapid pharmacological alteration in the established DS relationships. This may account for the results of Apfelbach & Delgado (1974), who showed that only prolonged suppression of the dominant's activity by chlordiazepoxide was capable of causing an inversion of the DS relationships in monkey tetrads. The present results demonstrate various effects of diazepam, droperidol and mescaline on the social rank of animals. The administration of drugs only to the subordinates in a tetrad does not affect the structure of the relationships, as the inhibition or stimulation of activity does not in itself change the rank of a subordinate. Neither does an increase in aggression (resulting from a small dose of amphetamine given to a subordinate) towards another mouse in a transitive linear triad result in an alteration in its position in the hierarchy because of the rigidity of the DS relationships.

In contrast, inversion of the DS relationships can be evoked, in the presence of a competitive rival, through the cumulative effect of drugs during prolonged administration. The results obtained also show that the rate of drug-induced DS inversion depends not only on the specific properties of the drug (tran-

quilliser, neuroleptic or psychodysleptic) but also on the character of the hierarchical organization. In the groups with a despotic type of organization the rate of diazepam-induced inversion is slower, than in the groups with a partial type of organization. Thus the same suppression of species-specific aggression, may finally produce different results at the level of the population.

The phenomenon of DS inertia is due to a system of connections established exclusively within the given group, since the introduction of a strong rival into a group causes rapid dissolution of the pre-existing DS organization and a change of dominant animal.

The inertia of hierarchical relationships must be taken into account when assessing the capability of a psychotropic drug to change the social rank of an

animal. The tranquilliser blocked the ability of the dominant to use aggression as a means of maintaining the hierarchy. In such cases the dominant may yield his dominant position to a stronger rival, or after chronic administration, may lose his dominant position within his own group. It would seem that the prolonged administration of any drug (neuroleptic, tranquilliser, sedative, etc.) which tends to suppress aggressive reactions, will result in the dominant suffering a non-specific drop in rank. Thus psychotropic drugs are capable of both maintaining DS inertia and causing DS inversion. This quality may be used as an integrative measure of the selectivity of a drug's action on behaviour, in particular aggression, and also for evaluating the effect of psychotropic drugs on the normalization and restoration of social behaviour.

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