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TIMID SINGLY-HOUSED MICE: THEIR VALUE IN PREDICTION OF PSYCHOTROPIC ACTIVITY OF DRUGS

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1 About 45% of singly-housed male mice showed timidity (alert postures, running away, defensive postures) instead of aggression on interactions in pairs with group-housed male mice, though their partners did not show any aggression. The isolation-induced timidity was stable in repeated interactions. Timid mice also showed locomotion (walking across cage and rearing) and a small amount of sociable activity (sniffing, following partners and climbing over them).

2 Diazepam (5 mg/kg), chlordiazepoxide (20 mg/kg), chlorpromazine (7.5 mg/kg) and barbitone (60 mg/kg) given orally inhibited the isolation-induced timidity without reducing other motor activities in the timid mice. Imipramine lessened timidity only in a dose (80 mg/kg) which also decreased other components of behaviour in the timid isolates. (+)-Amphetamine and lysergic acid diethylamide (LSD) increased the timid response.

3 Comparison of the inhibition of timid activities with changes in other behaviour occurring at the same time seems a better measure of selective timidity-reducing effects of drugs than the rota-rod test.

4 Diazepam (5 mg/kg) increased sociable and locomotor activities. Barbitone (20 and 60 mg/kg) increased sociable activities; however, the higher dose also evoked some aggression in timid mice.

5 Behaviour of timid singly-housed male mice seems to be a good measure for prediction of activity of drugs in relieving anxiety as well as for detection of aggression-evoking and sociability-increasing effects of drugs.

Introduction

It is well-known that individually-housed male mice can show defensive-escape activity as well as aggressive behaviour on interaction with strange males (Scott & Fredericson, 1951). The defensive-escape activity of singly-housed mice has been considered to be a mere passive submissive response coerced by the more aggressive partner. However, it has been found recently (Cairns & Nakelski, 1971; Kršiak & Borgesová, 1973) that some male mice housed singly for several weeks show alert and defensive postures, exaggerated escape responses and squeaking instead of aggression even on interaction with completely non-aggressive male mice. The first aim of the present paper was to discover whether isolation-induced timidity in mice can be used as a measure of a drug's effectiveness in relieving anxiety.

It has always been difficult to assess the relative specificity of behavioural actions of drugs in animal models. Usually some measure of 'neurotoxicity' such as decreased spontaneous motor activity, ataxia, loss of righting reflex, disruption of rotating rod performance are used for assessing the selectivity of the action of a drug on behaviour. However, there is still no established rationale for selection of different neurotoxicity measures as reference effects for judging this specificity (Cook & Kelleher, 1963). Furthermore, neurotoxicity is ascertained mostly in different animals with another history and in other experimental conditions which can greatly change their sensitivity to a drug. Therefore, an attempt was made in the present paper to assess the selectivity of the timidity-reducing effect of drugs by comparing this with effects on other components of behaviour occurring in the same animals and tests. The rota-rod test, which is a common measure of neurotoxicity in experiments on singly-housed mice (e.g. Janssen, Jageneau & Niemegeers, 1960; Sofia, 1969; Valzelli, 1973) has also been used.

Apart from the defensive-escape activities, timid isolates show locomotion (walking across cage and rearing), a small amount of sociable activity (sniffing and following partners and climbing over them), and after repeated

interactions some aggressive behaviour. The third aim of the present paper was to ascertain the value of non-timid behavioural activities in assessing the psychotropic activity of drugs.

Methods

Subjects, housing and apparatus

Male albino random-bred Swiss mice weighing 18-20 g at the beginning of the experimental housing were used. They were housed singly in self-cleaning cages or in groups of 20. The cages used for the individual housing had solid metal walls 13 cm high with wire-mesh floors (8 x 16 cm) which were placed on trays with wood shavings. Except on experimental days, the isolates were not handled throughout the isolation period. The mice kept in groups were housed in standard plastic cages 25 cm high with solid bottoms (22 x 38 cm) covered with wood shavings. All mice were housed in a natural day-and-night cycle at temperatures ranging from 22 to 24° C. Food and water were available permanently *ad libitum*.

The mice were observed in transparent cages (20 x 30 x 20 cm) with wood shavings on the floor and open tops. The observations were performed in a quiet experimental room from 08 h 30 min to 16 h 00 min under moderate artificial dispersed lighting.

Procedure

Social interactions were started after 3-5 weeks of isolation, always involving one singly-housed and one group-housed mouse in the observational cages. The isolates were allowed 30 min adaptation in the observational cages before the group-housed partners were introduced; interactions ended after 4 minutes. The observational cages were cleaned and their floors were covered with new wood shavings after each interaction.

Altogether 3-5 interactions were repeated one week apart with 355 pairs of singly- versus group-housed mice. Each isolate was paired with the same group-housed partner throughout the whole experiment. The isolates were given drugs or water orally 30 min before each interaction in a randomized order according to the Latin square design (each mouse served as its own control). The Wilcoxon matched pairs signed-ranks test (Siegel, 1956) was used for statistical evaluation. The group-housed mice were given only water.

Measures

The incidence of the following behavioural acts and postures similar to those described by Grant & Mackintosh (1963) was recorded by a keyboard-counter system:

Sociable activities: Social sniff—sniffing the partner's head, body, genitals or tail. *Climb*—the mouse places its forepaws on the partner's back, mostly in the shoulder region, and usually sniffs this area at the same time (Grant & Mackintosh called this Attempted Mount). *Follow*—following the partner by a quiet walking.

Timid activities: Alert posture—a sudden interruption of all movements with eyes and ears being directed towards the other mouse (Attend). *Escape*—a rapid running or jumping away from the opponent (Retreat and Flee). *Defence*—the mouse responds to the partner's social behaviour by raising the forepaws, hunching the back or by rearing up on the hind legs with the head up and forelegs extended (Defensive or Submissive upright posture).

Aggressive activities: Attacks—a fierce lunging at the partner from various sides often associated with biting. *Aggressive unrest*—walking around the partner (Walk round, mince) or on its own axis (Circle) walking to and from the partner (To-fro) and chasing the partner. *Tail rattle*—rapid vibrations of the tail were classified as an ambivalent activity reflecting both aggressive and flight tendency.

Locomotion (non-social activities): *Walk across cage*—any walking which is apparently not related to the partner. *Rear*—the mouse stands only on his hind legs and usually sniffs air or walls at the same time.

The interobserver reliability of the recorded items was satisfactory, as determined by two observers recording independently behaviour of 18 mice in interactions lasting 200 seconds. The r_S values ranged from 0.7 to 0.8. Observers did not know which kind of treatment was given to the tested animals.

Apart from the listed items, five other activities were also observed in the tested animals: approach (walking to the partner), leave (walking away from the partner), crouch (all the ventral surface from the chin to the tail is pressed to the floor, with the back moderately hunched and eyes semiclosed or closed while the other mouse usually climbs at the same time), self-groom (the mouse licks or scratches its body or wipes its face with forepaws) and resting (the mouse sits quietly on all fours, moves occasionally with the head or forepaws without orientation to the partner). However, these activities were not included in the present results for the following reasons: approaching

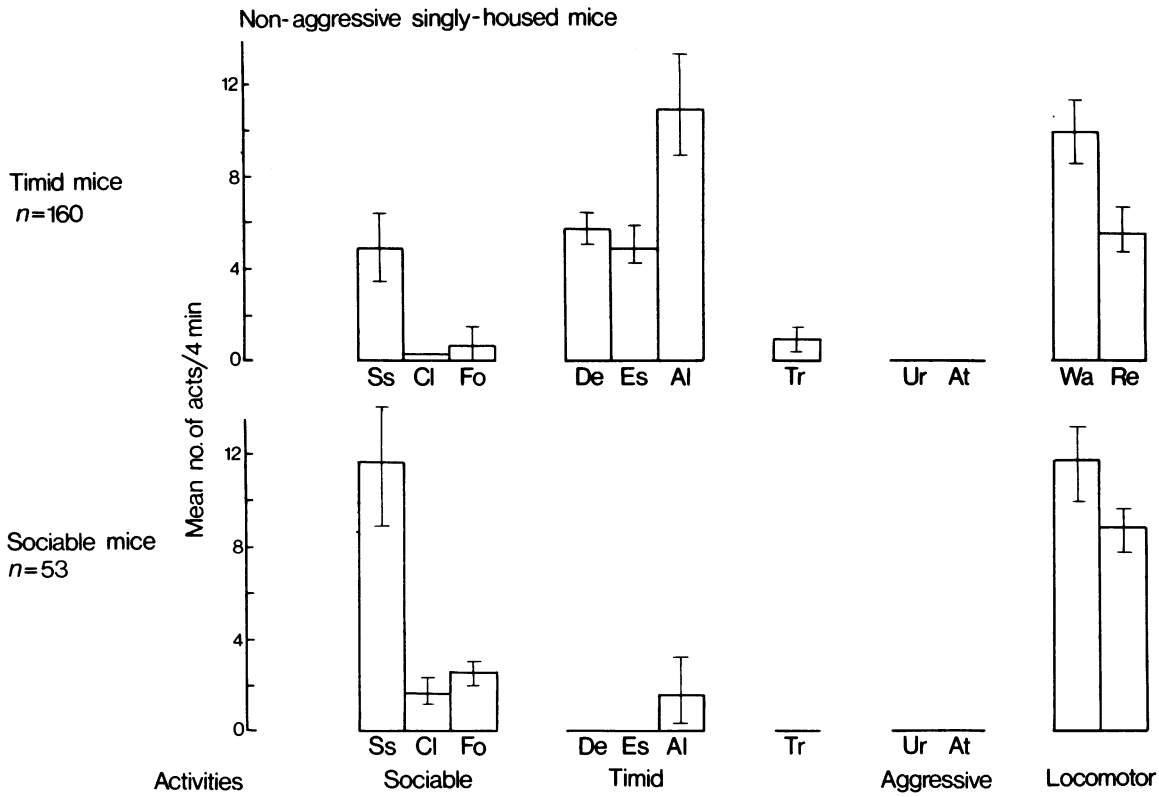

Figure 1 Behaviour of non-aggressive singly-housed male mice in the control paired interaction with non-aggressive group-housed male mice. Code for abbreviations: Ss = social sniffing, Cl = climbing over partner, Fo = following partner, De = defensive posture, Es = escape, Al = alert posture, Tr = tail rattling, Ur = aggressive unrest, At = attack, Wa = walking across cage, Re = rearing.
Limits of confidence of means for $P = 0.05$ are given.

partner or leaving him were difficult to classify (they occurred in a context of sociable as well as of aggressive activities while after higher doses of amphetamine they represented a mere stereotype walking), length of a bout of resting varied greatly after drugs so that the incidence of rests was not a reliable indicator of the actual amount of resting, and finally, crouching and self-grooming occurred very infrequently and their incidence was not increased by any drug tested.

Rota-rod test

Mice were trained to maintain themselves on a slowly revolving rod (4 rev/minute). The surface of the rod (diameter 2 cm) was rough. Mice were tested 30 min after oral administration of drugs. The drug was considered effective when the animal fell from the rod more than once during a 1 min testing period. Mean effective doses and relative potencies of drugs were estimated according to the graphic probit method (Litchfield & Wilcoxon, 1949).

Results

Behaviour of non-aggressive isolates in control interactions

About 60% of isolates did not attack their partners in the control interaction. The non-aggressive isolates were classified in two groups (Figure 1): a large group of males exhibiting defence postures or escapes ('timid' mice representing 45% of all isolates, $n = 160$) and a small group showing a higher number of sociable activities ('sociable' mice, 15%, $n = 53$). The defensive-escape activity of the isolates was not a passive response to aggressive behaviour of their partners. Group-housed males interacting with timid isolates did not attack them nor did they show any apparent

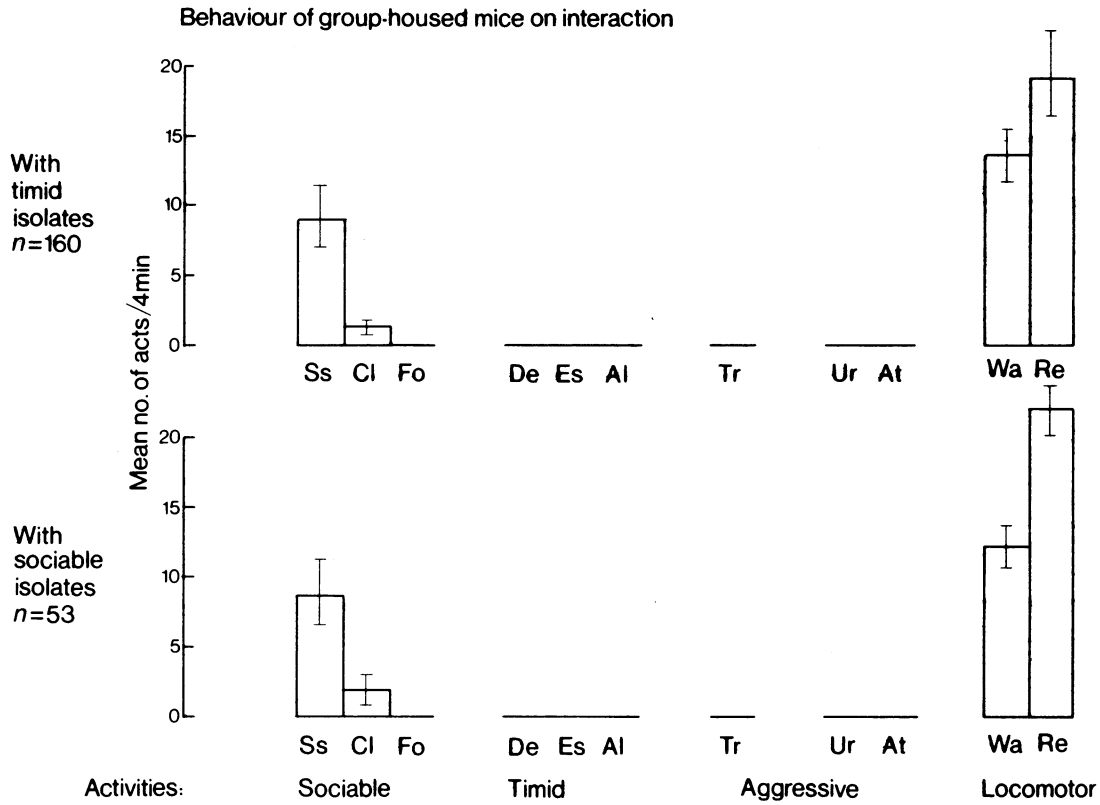

Figure 2 Behaviour of group-housed mice in the control paired interaction with timid or sociable singly-housed mice. Code for abbreviations is the same as in Figure 1. Limits of confidence of means for $P = 0.05$ are given.

aggressive activity (Figure 2). In fact, the behaviour of group-housed mice interacting with timid males did not differ from that of the group-housed mice interacting with sociable isolates. This suggests that the defensive-escape activity is provoked in isolates by the very presence of the strange partner and is therefore termed isolation-induced timidity.

The isolation-induced timidity was stable in repeated interactions: the correlation between the number of timid activities in the even and odd interactions in 47 isolates was highly significant (Figure 3).

The incidence of timid activities gradually decreased upon repeating interactions, particularly when the inter-trial intervals were as short as 2-4 days (Kršiak & Borgesová, unpublished results).

The rest of the isolates (about 40%, $n = 142$) which attacked their partners in the control interaction are not included in the present study.

Effect of drugs on behaviour of timid isolates

Diazepam (5 mg/kg, orally) and chlordiazepoxide (20 and 50 mg/kg) significantly lowered timid activities without reducing other components of behaviour (Figure 4). Diazepam seemed to stimulate rather than to inhibit the timid isolates: their sociability, walking across cage and total activity were significantly increased. Similarly, the timidity-reducing dose of barbitone (60 mg/kg) also increased sociability and total activity in the timid isolates (Figure 5). However, in contrast to diazepam, barbitone (60 mg/kg) stimulated aggressive activities. A lower dose of barbitone (20 mg/kg) significantly increased only sniffing and following partners.

Though the dose of chlorpromazine (7.5 mg/kg) inhibiting timidity did not significantly reduce another activity separately, a decrease was found when all activities were

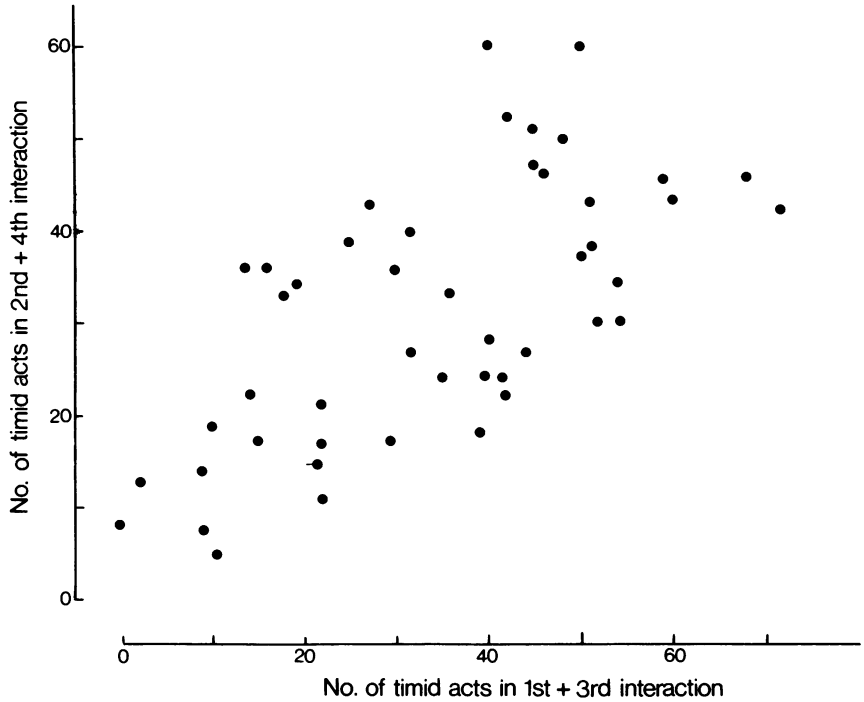

Figure 3 Correlation of occurrence of timid activities in the odd and even interactions in 47 timid singly-housed mice paired with non-aggressive group-housed mice. The interactions lasted 4 min and were repeated at weekly intervals. $r_S = 0.66, P < 0.001$.

combined, which suggests a general depressant effect of this dose of chlorpromazine (Figure 5). A lower dose of chlorpromazine (2.5 mg/kg) was ineffective.

Imipramine significantly reduced timid activities only in a high dose (80 mg/kg) which decreased walking across cage and rearing as well as all activities combined.

(+)-Amphetamine and LSD (Figure 6) did not reduce timid activities to any extent in any dose tested (0.25-4 mg/kg or 0.01 and 1 mg/kg respectively). On the contrary, 0.25 mg/kg of (+)-amphetamine significantly increased the number of alert postures and LSD (0.01 and 1 mg/kg) significantly stimulated tail-rattling. (+)-Amphetamine reduced sociable activities while LSD produced rather a general increase of all activities in the timid mice.

Inhibition of rota-rod activity

Mean effective doses of diazepam, chlordiazepoxide, barbitone, chlorpromazine and imipramine that induced falling off the rota-rod were lower in singly-housed mice than those in group-housed mice (Table 1). However, the ability to hold the rota-rod was smaller in isolates than in group-housed mice even when they had received no drugs: only 9 of 25 isolates remained on the rota-rod after 3 min in contrast to 19 of 24 group-housed males ($X^2 = 9.32$, $P < 0.005$). The untreated isolates seemed to be restless and frequently jumped from the rota-rod soon after being put on it.

Discussion

Isolation-induced timidity as the spontaneous tendency to withdraw from the non-aggressive partner does not seem to be a frequently described phenomenon in mice for several reasons. Another type of interaction (isolated versus isolated mice) seems to have been more commonly used, where defence and escape mostly represent passive responses to attacks of more aggressive partners

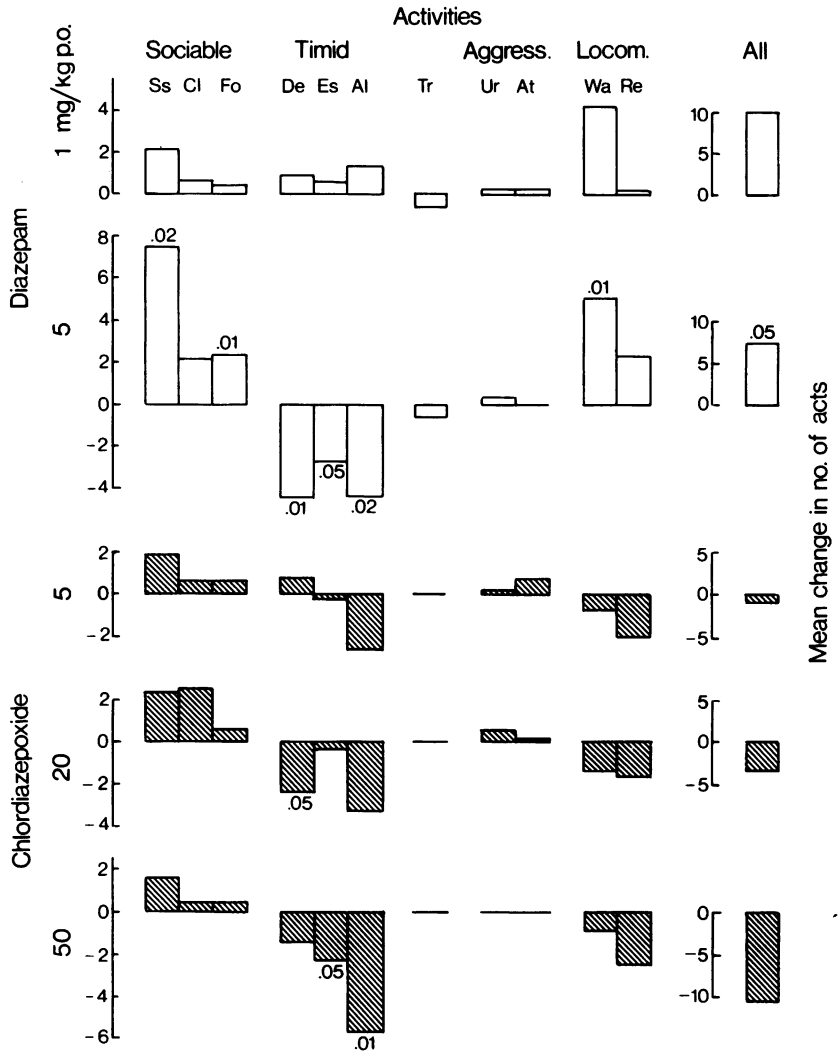

Figure 4 Behaviour of singly-housed timid mice given diazepam or chlordiazepoxide in paired interactions with non-aggressive group-housed mice. The ordinate scale shows the number of acts during 4 min expressed as the mean difference from activity in the control interaction (which did not differ significantly from that depicted in Figure 1). Effects of each dose represent mean results from 8 to 15 timid mice.

Table 1 Doses (mg/kg, orally) producing falling off the rota-rod

Drug	*Singly-housed mice* ED_{50} *95% confidence limits*	*Group-housed mice* ED_{50} *95% confidence limits*	P[+]
Diazepam	3.1 (2.0-4.8)	4.1 (2.7-6.4)	NS
Chlordiazepoxide	9.5 (6.6-13.8)	47.0 (33.1-66.7)	0.05
Barbitone	72.0 (48.0-108.0)	180.0 (86.7-195.0)	0.05
Chlorpromazine	5.0 (3.0-8.3)	8.8 (5.5-14.1)	NS
Imipramine	31.0 (23.1-41.5)	140.0 (101.5-193.2)	0.05

[+] Level of significance of the difference between the two ED_{50} values. NS = not significant.

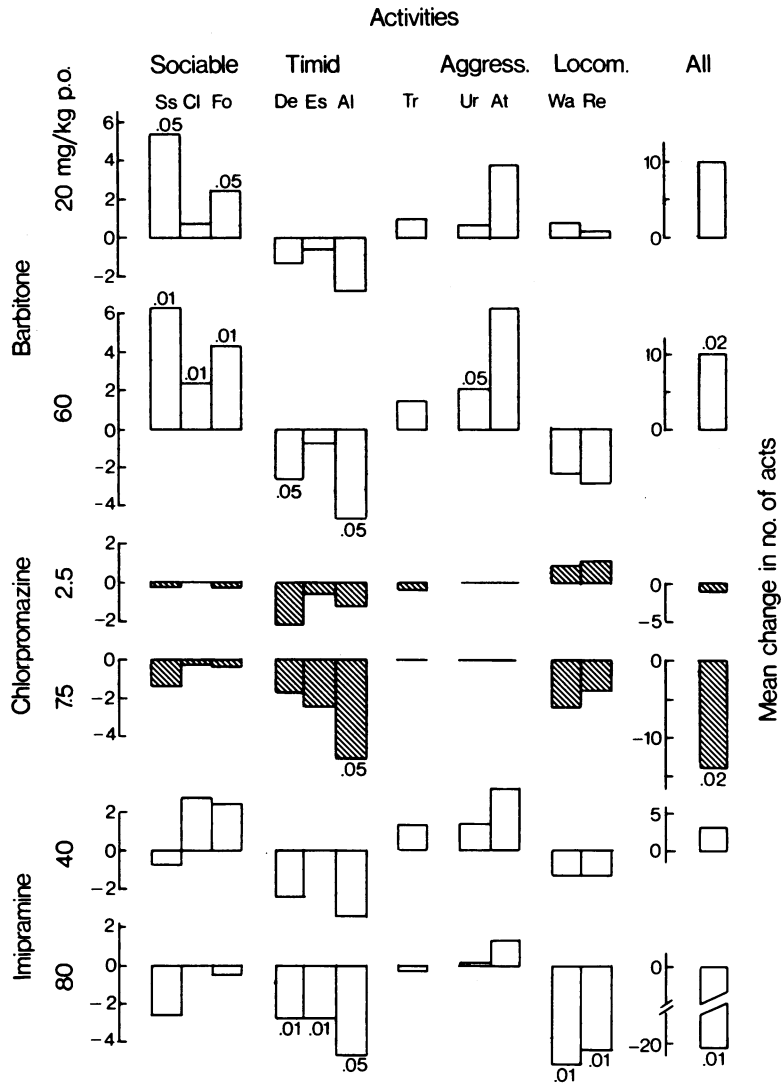

Figure 5 Behaviour of singly-housed timid mice given barbitone, chlorpromazine or imipramine in paired interactions with non-aggressive group-housed mice. Scale as for Figure 4. Effects of each dose represent mean results from 8 to 23 timid mice.

(e.g. Ginsburg & Allee, 1942: Valzelli, Giacalone & Garattini, 1967; Sofia, 1969). When the interaction of isolated versus group-housed mice was used, then in most cases only aggressive mice were selected (e.g. Yen, Stanger & Millman, 1959; Janssen *et al.*, 1960; DaVanzo, Daugherty & Kang, 1966). Nevertheless, Cairns & Nakelski (1971) observed that most isolated mice initially reacted by withdrawal, startle response or freezing to a mere approach or sniff by non-aggressive group-housed partners. These authors used C57 BL/10 mice, which suggests that the timid reaction is not limited to the strain used in the present study.

It is advisable to keep the aggressiveness and social activity of partners as low as possible, if the defensive-escape activity of singly-housed mice is to represent timidity rather than a passive submissive response. Since aggressiveness of male mice increases sharply as the number of males housed together decreases below ten (Welch & Welch, 1966), mice housed in groups of 20 were

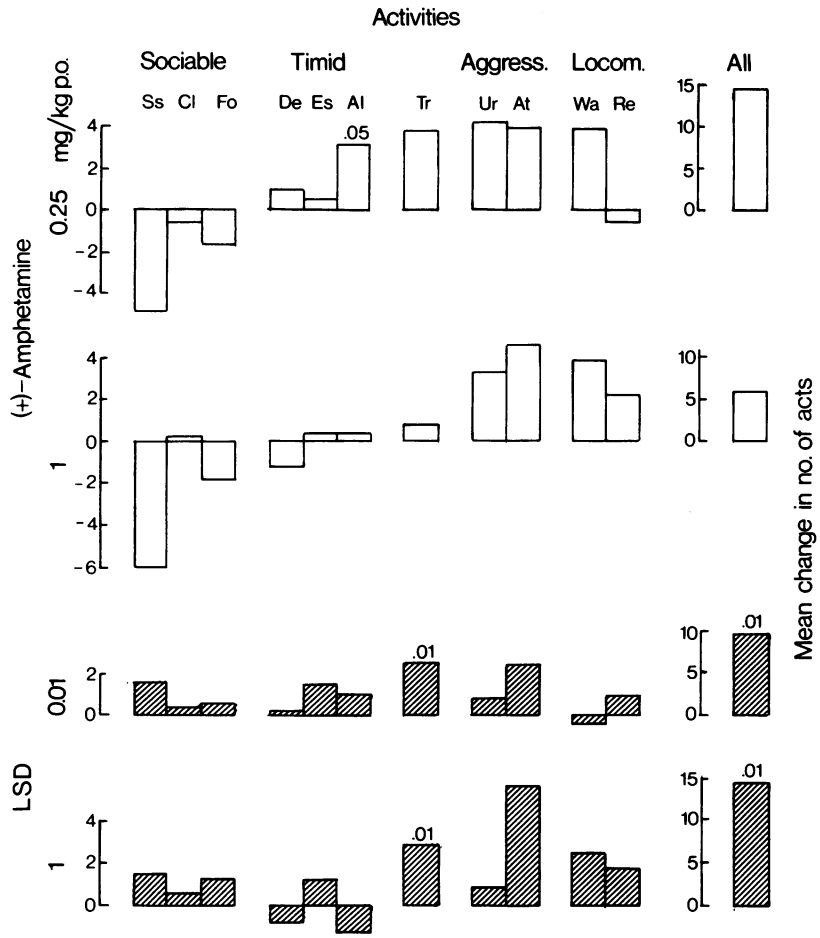

Figure 6 Behaviour of singly-housed timid mice given (+)-amphetamine or LSD in paired interactions with non-aggressive group-housed mice. Scale as for Figure 4. Effects of each dose represent mean results from 10 to 19 timid mice.

Table 2 Summary of experimental therapy of isolation-induced timidity in mice

Drug	*Lowest dose inhibiting timidity (LDIT) (mg/kg, p.o.)*	*'Adverse effects'*			LD_{50} *(mg/kg, p.o.)*	$LD_{50}/LDIT$
		'General sedation'	*'Muscular weakness'*	*Other*		
Diazepam	5.0	no	no	no	720[+]	144
Chlordiazepoxide	20.0	no	no	no	620[+]	31
Chlorpromazine	7.5	yes	no	no	319[++]	43
Barbitone	60.0	no	no	incr. aggress.	600[++]	10
Imipramine	80.0	yes	yes	no	400[++]	5

(+)-Amphetamine increases timidity; LSD increases timidity.
'General sedation' = a significant decrease of all behavioural activities combined. 'Muscular weakness' = a systematic decrease of upright- or walk-type movements.
[+] Randall, Scheckel & Banziger, 1965; [++] Barnes & Eltherington, 1965.

chosen as partners of isolates. Furthermore, group-housed mice were not given opportunities to adapt themselves in the testing cage, so that their social activities appeared to be substituted by a strong tendency to explore the cage. As a result, group-housed mice never attacked timid isolates throughout the whole experiment.

Table 2 summarizes the evaluation of experimental therapy of isolation-induced timidity in mice. When the therapeutic efficacy is judged according to intensity of 'adverse' effects produced by the lowest 'therapeutic' dose in timid isolates, then diazepam and chlordiazepoxide appear to be 'drugs of choice' for the treatment of the mouse timidity (no adverse effects). Chlorpromazine and barbitone seem to be less suitable drugs because of sedative effects of chlorpromazine or increased aggressiveness after barbitone. Imipramine can be classified as quite unsuitable because of the marked toxicity of its effective dose, and amphetamine and LSD seem to be 'contraindicated' in the timid mice, as they tended to increase timidity. Accordingly, therapeutic efficacy of these drugs in the isolation-induced timidity in mice seems to be in good agreement with their effects in relieving anxiety in man.

When the therapeutic efficacy is judged by comparing lowest doses inhibiting timidity with some more commonly used measure of 'neurotoxicity', for instance falling off the rota-rod (e.g. Sofia, 1969; Valzelli, 1973), none of the tested drugs would reduce timidity at less than the 'neurotoxic' dose (Table 1). Mice rear on hind legs when displaying defensive postures and they run away when escaping. The reduced number of defensive postures and escapes after diazepam does not seem to be due to reduced ability to raise the front part of the body or to walk, as rearing and walking across cage were not reduced but increased in the diazepam-treated mice. Moreover, 5 mg/kg of diazepam stimulated sociability which represents a well-coordinated and directed activity. Therefore, the diazepam-reduced timidity does not seem to be due to a neuromuscular impairment as the low ED_{50} of diazepam for falling off the rota-rod might indicate.

Validity of the rota-rod test for assessing selectivity of behavioural effects of drugs seems to be questionable. If some animals spend less time on the rota-rod it does not invariably mean that these animals are less capable of a particular behavioural act (e.g. to escape, to attack, etc.). The untreated singly-housed mice were less able to maintain themselves on the rota-rod than the group-housed animals, yet, they exhibited complex motor activities on social interaction. Some benzodiazepines stimulate spontaneous locomotion in mice in doses that are many times higher than those producing falling off the rota-rod (Gluckman, 1971). The reduced ability to hold the rota-rod does not seem to reflect a unitary 'neurotoxic' change, since apart from ataxia or myorelaxation, it might be caused by an increased restlessness, e.g. by increased spontaneous jumping off the rota-rod. Furthermore, differences in housing conditions of animals used for the rota-rod and a behavioural test can significantly influence the value of a 'neurotoxic' dose derived from the rota-rod test, as showed by the present and Valzelli's (1973) results. Thus, the rota-rod test should be used with caution as the criterion for judging specificity of behavioural effects of drugs. Comparison of the changes in the behavioural activity in question (e.g., defensive upright posture) with other activities involving a similar type of movement but occurring in another behavioural context (e.g. exploratory rearing) in the same animal and setting (a procedure originally used by Silverman, 1965), seems to provide a more relevant as well as a more economic measure of selectivity of behavioural effects of drugs.

The incidence of 'adverse' effects seems to correlate rather with the ratio between LD_{50} and the lowest dose inhibiting timidity (LDIT): 'adverse' effects were less frequent or absent when the LD_{50} was more than ten times higher than LDIT. Diazepam had the largest 'safety margin'; its LDIT was 144 times smaller than its LD_{50} (Table 2).

Recording of non-timid activities in timid isolates can be helpful not only for assessment of the selectivity of timidity-reducing effects of drugs, as discussed above, but also for detection of aggression-evoking and sociability-increasing effects of drugs. Apart from barbitone, which significantly stimulated aggressive unrest in the present study, ethyl alcohol also evoked tail-rattling, aggressive unrest and attacks in timid mice (Kršiak, unpublished observation). The aggression-stimulating effects of drugs have so far been studied largely in aggressive animals (Kršiak, 1974). It may also be relevant to ascertain which drugs can facilitate aggressive behaviour in animals which usually do not respond aggressively to aversive stimulation. Timid singly-housed mice which do not usually attack their non-aggressive partners, yet appear to be potential attackers, seem to represent a convenient measure for detection of aggression-evoking activity of drugs.

Diazepam and barbitone increased sociability in timid mice whereas chlorpromazine and amphetamine did not. These findings are in agreement with effects of drugs on sociability in other species. Amylobarbitone increased social investigation and aggression in rats (Silverman, 1966a).

Chlordiazepoxide stimulated social sniffing in golden hamsters (Poole, 1973). On the other hand, both chlorpromazine and amphetamine increased escape from the partner in rats (Silverman, 1966b). Amphetamine decreased mutual grooming or sniffing in monkeys (Kjellberg & Randrup, 1973) and in rats (Schiørring & Randrup, 1971; Syme & Syme, 1973). Little is known as to what kind of psychotropic effect in man is predicted by increased sociability in animals. Nevertheless, the recording of sociable activities in timid mice might help to differentiate effects of sedatives relieving anxiety from those of neuroleptics.

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