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THE AUTOMATED TAIL SUSPENSION TEST: A COMPUTERIZED DEVICE WHICH DIFFERENTIATES PSYCHOTROPIC DRUGS

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Abstract

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1. Mice when suspended by the tail will alternate between active attempts to escape and immobility. Immobility like that measured in the behavioral despair test is reduced by a wide variety of antidepressant agents.
2. The present paper describes a computerized version of this test (ITEMATIC-TST) which in addition to recording immobility measures the power of the movements.
3. Various tricyclic (amitriptyline, desipramine, imipramine), MAOI (clorgyline, moclobemide, nialamide, pargyline, toloxatone) and atypical antidepressants (bupropion, citalopram, indalpine, mianserin, nomifensine, viloxazine) were tested and compared with psychostimulants (d-amphetamine, caffeine), neuroleptics (chlorpromazine, haloperidol, sulpiride), anxiolytics (clobazam, diazepam) and agents acting on the cholinergic system (atropine, oxotremorine).
4. All antidrepressants decreased the duration of immobility and most increased the power of movements.
5. The psychostimulants also decreased immobility but only amphetamine increased the power of movements.
6. Neuroleptics increased immobility without affecting the power of movements, whereas anxiolytics increased immobility but decreased the power of movements.
7. Atropine had a profile similar to antidepressants whereas oxotremorine tended to have opposite effects.
8. The results suggest that the automated test system with its two parameters is not only sensitive to antidepressants but could also be useful for generating activity profiles for different kinds of psychotropic agent.

Keywords : antidepressants, immobility test, mice, psychotropic drugs, screening profile.

Abbreviations : monoamine oxidase (MAO), monoamine oxidase inhibitors (MAOI)

Introduction

Animals when placed in an aversive situation from which there is no escape will alternate between two behaviors : vigorous activity and immobility ("despair"). The immobile behavior induced in rodents by forcing them to swim in a restricted space has been proposed by one of us as a screening model for testing antidepressants and has been found sensitive to a wide variety of antidepressant agents including tricyclics, MAOI and atypical drugs such as mianserin, iprindole and viloxazine (Porsolt 1981). More recently, a similar phenomenon of immobility has been described in mice which are suspended by the tail (Stéru et al 1985a, Thierry et al 1984). Like behavioral despair, tail suspension-induced immobility is particularly sensitive to antidepressant treatments.

The original tail suspension method made use of a smoked drum to record periods of activity and immobility which were then scored manually. This procedure was very time consuming and laborious. The present paper describes the results obtained with a newly developed computerized device (ITEMATIC-TST) (Stéru et al 1985b ; Mico et al 1986) which in addition to recording the duration of immobility measures the power of the individual movements. This automatic apparatus not only provides an objective measure of behavioral changes occurring in this situation but also vastly increases the number of animals which can be tested by a single experimenter. Furthermore the measurement of two parameters, immobility and power of the movements, permits identification of different classes of psychotropic compound by means of their activity profiles in this primary screening test.

Material and Methods

Subjects

Naive male NMRI mice (from the Centre d'Elevage Roger Janvier, France) weighing 22 - 24 g were used. The animals were housed in plastic cages in groups of 10 per cage at a temperature of 21 $\pm$ 1° C with free access to water and food. An artificial non-reversed 12/12 h day/night cycle was imposed.

Drugs

The following drugs were investigated : amitriptyline hydrochloride (Roche), d-amphetamine sulphate (Coopérative Pharmaceutique Française), atropine sulphate (Sigma), bupropion hydrochloride (Wellcome), caffeine (Aldrich), chlorpromazine hydrochloride (Rhône Poulenc), citalopram hydrochloride (Lundbeck), clobazam (Diamant), clorgyline hydrochloride (May and Baker), desipramine hydrochloride (Ciba-Geigy), diazepam (Roche), haloperidol (Janssen), imipramine hydrochloride (Ciba-Geigy), imipramine methiodide (Ciba-Geigy), indalpine hydrochloride (Pharmuka), mianserin hydrochloride (Organon), moclobemide hydrochloride (Roche), nomifensine maleate (Hoechst), oxotremorine fumarate (Sigma), pargyline hydrochloride (Sigma), sulpiride (Delagrange), toloxatone (Delalande) and viloxazine hydrochloride (ICI).

Apparatus

The experiments were performed using the automated Tail Suspension apparatus ITEMATIC-TST. This new computerized device, developed and marketed by I.T.E.M-LABO (Paris), enables 6 mice to be tested simultaneously (Figure 1). Each mouse is suspended by the tail using adhesive tape to a hook connected to a strain gauge. The strain gauge picks up all movements of the mouse and transmits them to a central unit which digitalizes the signals. The signals are displayed visually using LEDs which permit on-line verification of the good functioning of each unit. Included in the central unit is a 9 level filtering device which can be set to the desired sensitivity to provide maximum discrimination of gross body movements from other micro-movements of the animal or its internal organs. The central processing unit calculates two parameters : the duration of immobility and the power of the movements. The duration of immobility is calculated from the cumulated time during which the animals movements do not exceed the threshold determined by the 9 level filtering device. The power of the movements is calculated from the total energy expended by the animal during the test as measured by the cumulated amplitudes of individual movements (arbitrary units) divided by the total time the animal is active. The central unitis connected to a microcomputer (Epson HX20) which provides on-line data collection and analysis including generation of the experimental schedule (randomisation), grouping of results, statistical analysis and graphical and numerical presentation.

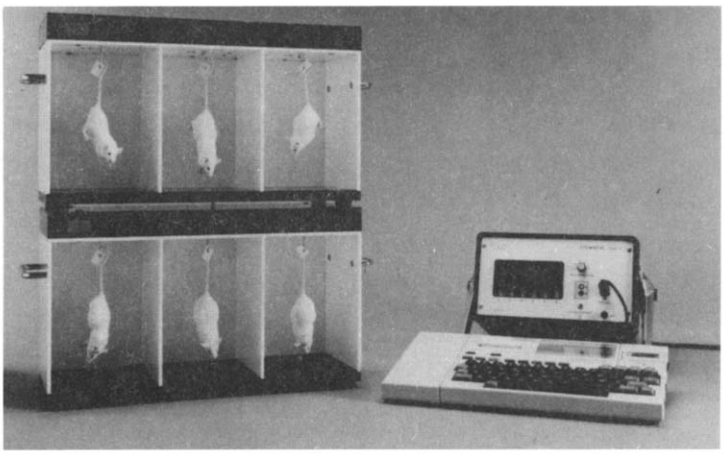

Fig. 1 - The ITEMATIC-TST with two suspension units superimposed (left), a central processing unit with LED displays (upper right) and an Epson HX20 microcomputer with extended memory (lower right).

Procedure

Animals were injected according to the experimental plan generated by the ITEMATIC-TST and were placed into individual plexiglass boxes (20 x 50 x 10 cm) between injection and the test. All drugs were injected i.p. 30 minutes before testing with the exception of amitriptyline, clorgyline, moclobemide, nialamide, pargyline and toloxatone where the injection time was 60 minutes. Moclobemide and toloxatone were administered p.o.. The test lasted 6 minutes and the measurements were performed for the total duration of the test. Soluble drugs were dissolved in distilled water and non-soluble drugs were dispersed in a suspension of acacia gum (5 %). Control animals received the vehicle. A concentration of 0.25 ml/20 g body weight was used throughout. Doses are expressed in terms of the salt. Ten animals were studied per group with the exception of mianserin and toloxatone where groups contained 20 animals.

Statistical Analyses

Differences between treated groups and their controls were analysed for statistical significance using Dunnett's t-test.

Results

Results in control animals

Control results were obtained in a total of 260 animals divided into 22 groups of 10 animals and 2 groups (the controls for mianserin and toloxatone) of 20 animals. The mean duration of immobility for all control groups combined was 89.1 seconds with individual control group means ranging from 56 to 116 seconds. About 75 % of the control means for immobility were obtained in the range 80 - 107 seconds. The mean power of the movements for the same control groups was 19.6 (arbitrary units) with individual control group means ranging from 13 to 32. Over 75 % of the control means for power of the movements were obtained in the range 13 to 24.

Tricyclic antidepressants

Amitriptyline, desipramine and imipramine all induced clear and dose-dependent decreases in the duration of immobility. They also tended to increase the power of the movements with statistically significant effects at least at one dose. A clear peak increase in the power of the movements was observed with amitriptyline at 8 mg/kg, followed at higher doses by a decline towards control levels. This decline is probably associated with the marked sedative/muscle relaxant effects observed at 16 and 32 mg/kg amitriptyline. In contrast to the tricyclic antidepressants imipramine-methiodode, a quaternary ammonium salt that crosses the blood brain barrier poorly, had no effect up to 16 mg/kg but tended to increase immobility and decrease power at 32 mg/kg. (Fig 2)

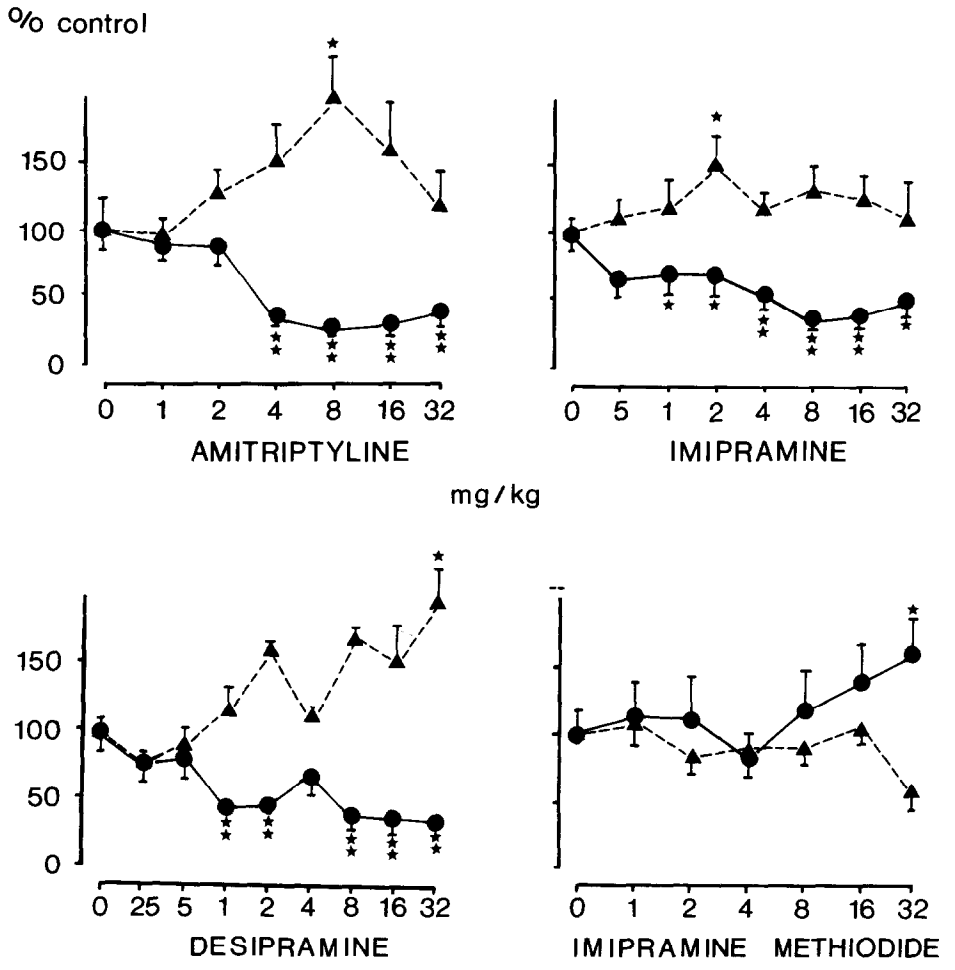

Fig. 2 - The effects of 3 tricyclic antidepressants (amitriptyline, desipramine and amipramine) and the quaternary ammonium salt imipramine methiodide on the duration of immobility (●——●) and the power of the movements (▲- - -▲) during a 6 minute test. Drug effects and the standard errors are expressed as percentages of control values. Drugs were administered i.p. 30 minutes before the test except for amitriptyline which was administered i.p. 60 minutes before the test. * $p < .05$; ** $p < .01$

Monoamine Oxidase Inhibitors

Clorgyline, a specific inhibitor of type A MAO, caused clear and dose-dependent decreases in immobility without significant effects on the power of the movements up to the highest dose tested (16 mg/kg). Similar but non dose-dependent effects on immobility were observed with the two reversible type A MAOI moclobemide and toloxatone, again without any significant effects on the power of the movements. Pargyline, a specific inhibitor of type B MAO, had no consistent effect on either parameter up to 128 mg/kg but significantly decreased immobility and increased power at 256 mg/kg. Nialamide, a mixed MAOI, tended to decrease the duration of immobility at all doses tested ; this effect was not dose-dependent, however, and a significant reduction was only observed at the lowest dose (4 mg/kg). The power of the movements was not affected. (Fig 3)

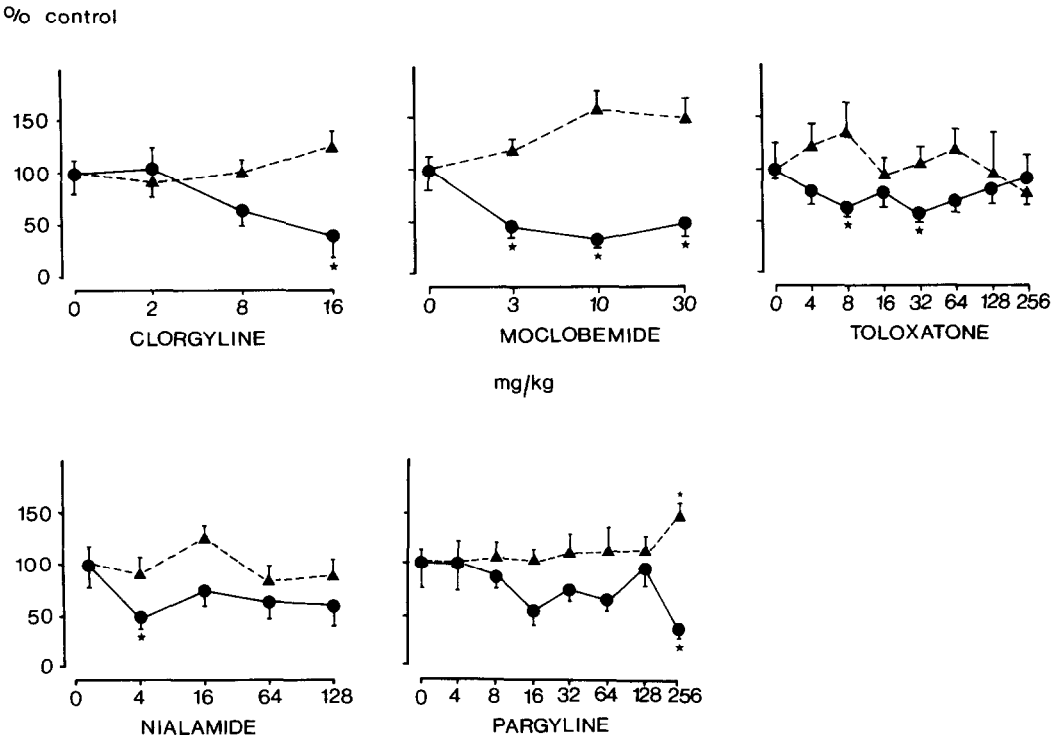

Fig. 3 - Effects of five MAO inhibitors (clorgyline, moclobemide nialamide, pargyline, toloxatone) on the duration of immobility (●—●) and the power of the movements (▲- -▲) during a 6 minute test. Drug effects and the standard errors are expressed as percentages of control values. Drugs were administered i.p. 60 minutes before the test except moclobemide and toloxatone which were administered p.o. 60 minutes before testing. * $p < .05$; ** $p < .01$

Atypical antidepressants

All the atypical antidepressants tested (bupropion, citalopram, indalpine, mianserin, nomifensine, viloxazine) caused significant decreases in the duration of immobility. These effects were dose-dependent except for mianserin where a biphasic effect was observed, first a decrease followed by a return towards control levels. This effect cannot be considered very robust as 20 animals were required to obtain statistically significant results. Bupropion, mianserin and viloxazine significantly increased the power of the movements with a similar but non-significant tendency with nomifensine. In contrast, citalopram and indalpine, two specific inhibitors of the uptake of serotonin, had no effects on the power of the movements except indalpine at the highest dose tested (64 mg/kg). (Fig 4)

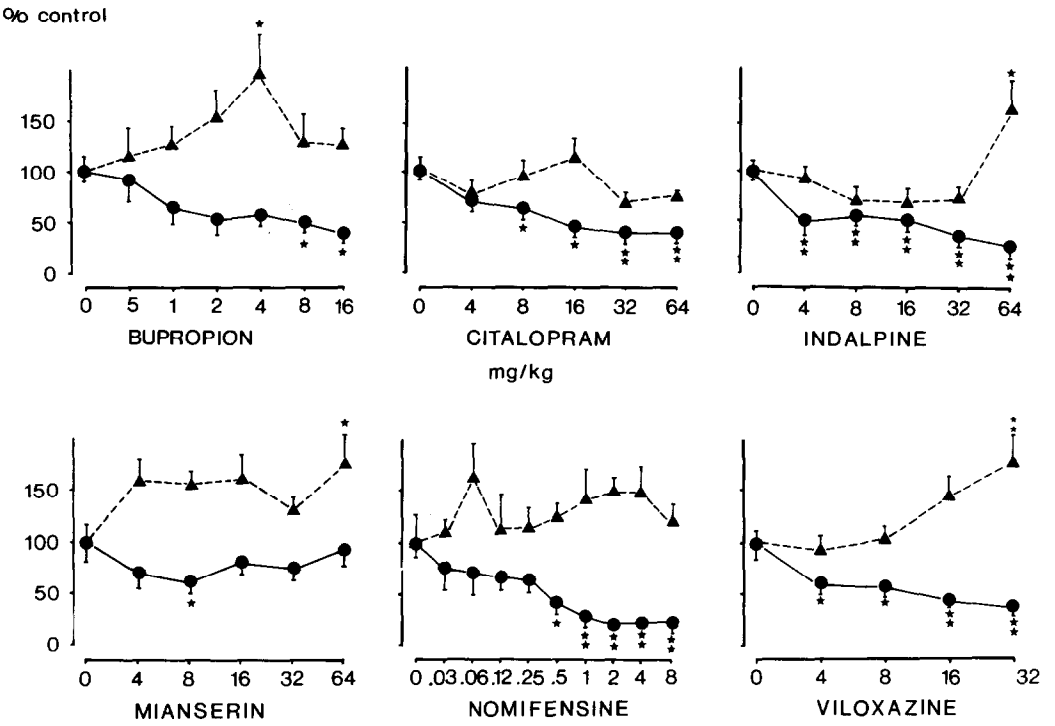

Fig. 4 - Effects of six atypical antidepressants (bupropion, citalopram, indalpine, mianserin, nomifensine, viloxazine) on the duration of immobility (●——●) and the power of the movements (▲- - -▲) during a 6 minute test. Drug effects and the standard errors are expressed as percentages of control values. Drugs were administered i.p. 30 minutes before the test. * $p < .05$; ** $p < .01$

Psychostimulants

Both amphetamine and caffeine caused significant decreases in the duration of immobility. These effects were largely dose-dependent except at the highest doses where a return to control values was observed. With amphetamine at 8 mg/kg, clear stereotypies were present. Caffeine had no effect on the power of the movements, whereas amphetamine increased the power of the movements up to doses inducing stereotypies. (Fig 5)

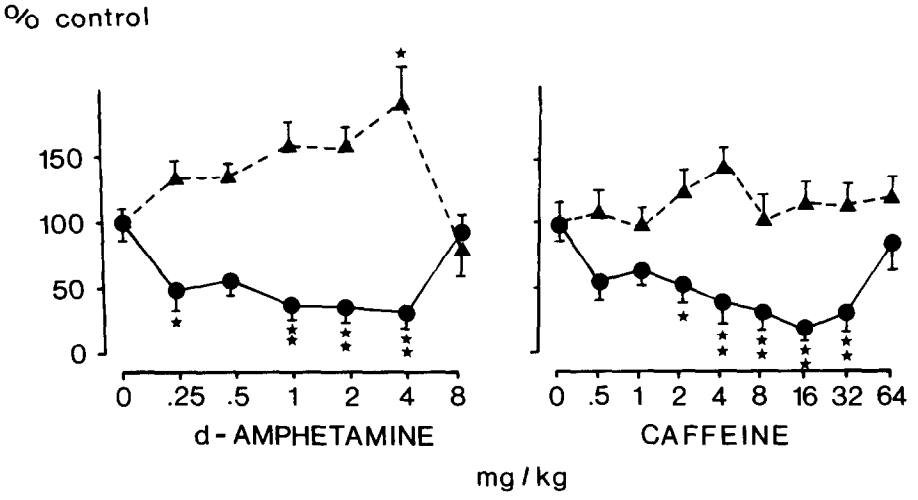

Fig. 5 - Effects of two psychostimulants (d-amphetamine, caffeine) on the duration of immobility (●——●) and the power of the movements (▲- - -▲) during a 6 minute test. Drug effects and the standard errors are expressed as percentages of control values. Drugs were administered i.p. 30 minutes before the test. * $p < .05$; ** $p < .01$

Neuroleptics and Minor Tranquilizers

In contrast to the antidepressants and psychostimulants, all three neuroleptics tested, chlorpromazine, haloperidol and sulpiride, dose-dependently increased the duration of immobility but had no significant effects on the power of the movements in the dose ranges tested. Similar increases in the duration of immobility were observed with the two minor tranquilizers tested (diazepam, clobazam) but these compounds, in contrast to the neuroleptics, caused significant dose-dependent decreases in the power of the movements. The decreases in the power of the movements at the higher doses were associated with clear signs of myorelaxation. (Fig 6)

Drugs Acting on the Cholinergic System

The cholinergic receptor blocker atropine significantly decreased the duration of immobility with a similar effect being observed at all doses tested (2 - 16 mg/kg). Atropine tended to increase the power of the movements but a significant effect was only observed at the lowest dose (2 mg/kg) with a return towards control values at higher doses. Oxotremorine, a cholinergic receptor agonist, had little effect on the parameters measured apart from a small but significant decrease in the power of the movements at 1 mg/kg accompanied at this one dose by a non-significant increase in the duration of immobility. (Fig 7)

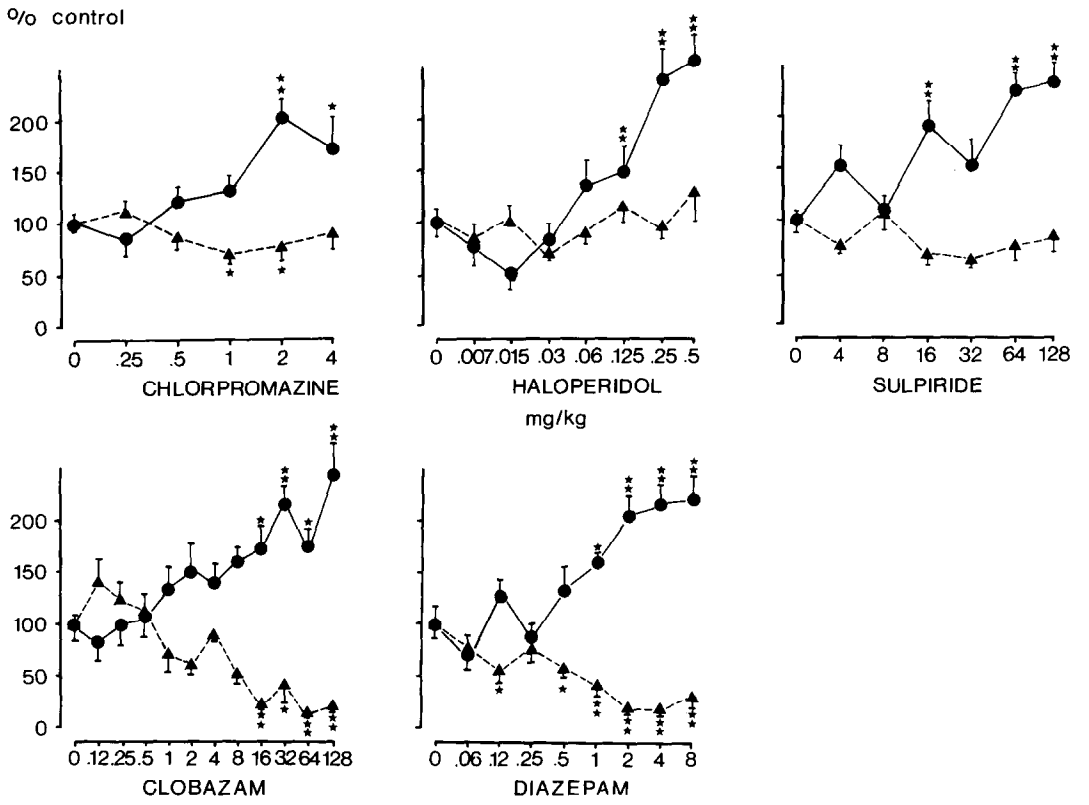

Fig. 6 - Effects of three neuroleptics (chlorpromazine, haloperidol, sulpiride) and two minor tranquilizers (clobazam, diazepam) on the duration of immobility (●——●) and the power of the movements (▲– – –▲) during a 6 minute test. Drug effects and the standard errors are expressed as percentages of control values. Drugs were administered i.p. 30 minutes before the test. * $p < .05$; ** $p < .01$

Discussion

Effectiveness of antidepressants

The results of these experiments have shown that a wide variety of antidepressants with differing mechanisms of action decrease the duration of immobility in the Tail Suspension Test as measured automatically using the ITEMATIC-TST. Three typical tricyclic compounds (amitriptyline, desipramine, imipramine) were clearly active whereas the quaternary ammonium salt of imipramine,

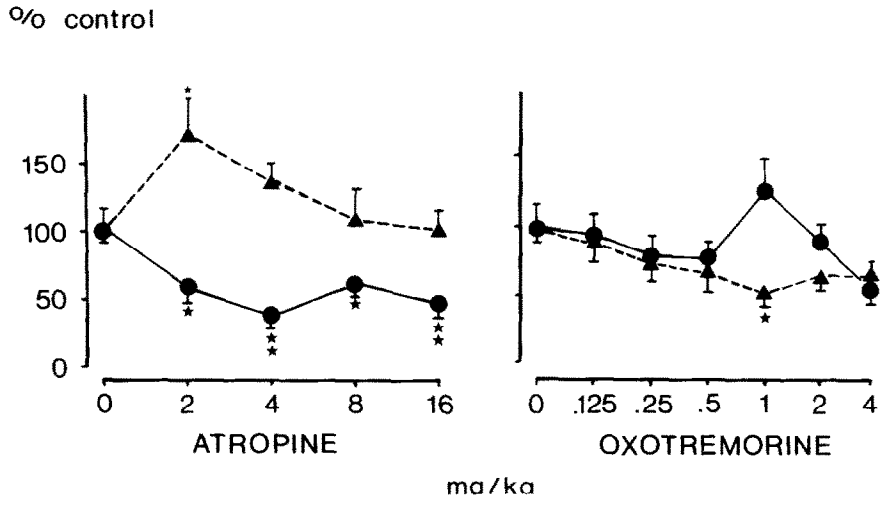

Fig. 7 - Effects of a cholinergic antagonist (atropine) and agonist (oxotremorine) on the duration of immobility (●——●) and the power of the movements (▲ — ▲) during a 6 minute test. Drug effects and the standard errors are expressed as percentages of control values. Drugs were administered i.p. 30 minutes before the test. * $p < .05$; ** $p < .01$

imipramine methiodide, which passes the blood brain barrier only poorly, had little effect in the same dose range. This suggests that the effects observed with amitriptyline, desipramine and imipramine were central in origin. Of interest also were the results obtained with the MAOI. Clorgyline, moclobemide and toloxatone, specific inhibitors of type A MAO (Neff and Fuentes 1976), were clearly active whereas pargyline, a specific inhibitor of type B MAO at low doses (Neff and Fuentes 1976), was only active at a very high and probably non-specific dose with intermediate results being obtained with the mixed type MAOI nialamide. Further compounds would need to be tested before concluding that the Tail Suspension Test is sensitive only to type A MAOI but it can be noted that clorgyline, moclobemide and toloxatone are clinically active as antidepressants (Murphy et al 1979, Dencker and Nagy 1983, Casacchia et al 1984) whereas the antidepressant effects of pargyline are at best limited (Murphy et al 1979). In this respect, the results obtained with atypical antidepressants are pertinent because these compounds, in particular mianserin, while possessing demonstrated clinical efficacy, are not readily detected using classical pharmacological tests for antidepressant activity (Porsolt 1981).

Comparison with the behavioral despair test

The results obtained in the present experiments confirm those obtained in an earlier version of the Tail Suspension Test where individual results were analysed manually from smoked drum recordings of the animals' activity (Steru et al 1985a). Furthermore the findings with the Tail Suspension Test are largely consistent with those reported for the "behavioral despair" test where immobility is induced in rodents by forcing them to swim in water from which they cannot escape (Porsolt 1981).

In both tests immobility is decreased by antidepressants, psychostimulants and anticholinergics and increased by tranquilizing agents in particular neuroleptics. One important pharmacological difference is that antidepressants which selectively inhibit the uptake of serotonin (eg citalopram, indalpine) are clearly active in the Tail Suspension Test but not in the "behavioral despair" test. A further difference is that antidepressants are active in the Tail Suspension Test at doses considerably lower than those found active in the "behavioral despair" test. For example, imipramine significantly reduces suspension-induced immobility from 1 mg/kg i.p. (present experiments, Steru et al 1985a) whereas about 30 mg/kg is required to significantly reduce immobility induced by forced swimming (Porsolt et al 1977). The reasons for these differences are not clear but may be related to the marked hypothermia occurring during forced swimming (Porsolt et al 1979) or the presumably greater physiological stress induced in these conditions (Thierry et al 1986). Whatever the reasons, the fact that the Tail Suspension Test is sensitive to selective serotonin uptake blockers and in general to lower doses of test compounds represents an important advantage for this procedure as a primary behavioral screening test for antidepressants.

Utility of the power of movements parameter

A further factor of potential interest is the possibility, provided by the apparatus, of measuring the power of the movements emitted by the animal during the test. Although the available data are preliminary, there appear to be interesting differences between the compounds in their effects on this parameter. For example, while tricyclic antidepressants and most of the atypical agents tend to increase the power of the movements, the two serotonin uptake inhibitors had no effect, as was also the case with the MAOI. Although confirmatory data are required it is tempting to speculate that the absence of effect on this parameter might be due to the potent serotonergic stimulating properties or lack of noradrenergic stimulating properties which distinguish these compounds from the other antidepressants tested. Furthermore, while both minor and major tranquilizers increased the duration of immobility, they could be clearly distinguished in terms of their effects on the power of the movements. The decrease of power observed with diazepam and clobazam might well reflect the muscle relaxing activity of these benzodiazepines (Randall and Kappell 1973).

Ethical considerations

A final factor which cannot be ignored concerns the ethics of behavioral models, particularly those proposed for the testing of antidepressant drugs where by definition the animal is not rendered happy. The experimenter's aim in this kind of research should be to reduce the animal's discomfort to a minimum which is still compatible with the research goal. We have analyzed this aspect in detail elsewhere (Thierry et al 1986) and concluded that the Tail Suspension Test procedure appears to cause a minimum of suffering to the experimental animals and in any case considerably less than that induced in the traditional "behavioral despair" test (Porsolt 1981) or in "learned helplessness" paradigms (Sherman et al 1982) or even in the several variants of the reserpine test where the animals can remain under the influence of reserpine for several hours.

Conclusions

The automated version of the Tail Suspension Test described in the present paper appears to constitute a rapid, objective, sensitive and ethically acceptable screening procedure for detecting

antidepressant activity. There appear to be few false negatives and the availability of two behavioral measures, immobility and the power of the movements, suggest that this automated version of the test could also be useful for determining activity profiles for different kinds of psychotropic agents.

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