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The tail suspension test: A new method for screening antidepressants in mice

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Abstract. A novel test procedure for antidepressants was designed in which a mouse is suspended by the tail from a lever, the movements of the animal being recorded. The total duration of the test (6 min) can be divided into periods of agitation and immobility. Several psychotropic drugs were studied: amphetamine, amitriptyline, atropine, desipramine, mianserin, nomifensine and viloxazine. Antidepressant drugs decrease the duration of immobility, as do psychostimulants and atropine. If coupled with measurement of locomotor activity in different conditions, the test can separate the locomotor stimulant doses from antidepressant doses. Diazepam increases the duration of immobility.

The main advantages of this procedure are (1) the use of a simple, objective test situation, (2) the concordance of the results with the validated "behavioral despair" test from Porsolt and, (3) the sensitivity to a wide range of drug doses.

Key words: Immobility test – Antidepressants – Screening method – Mice

Porsolt (1981) proposed a model for screening antidepressants in mice, called "behavioral despair". In this test, a mouse placed in water swims, apparently trying to escape; it then alternates swimming and immobility periods. Antidepressants (and some other drugs) reduce the immobility periods.

We report here the results of a new procedure, inspired by Porsolt's test situation, and based on a concept (the "searching-waiting strategy") which is described elsewhere (Stéru et al. 1982; Thierry et al. 1984).

From a theoretical point of view, this test supports the following hypothesis: a normal animal submitted to an insoluble, aversive situation alternates between two kinds of behaviors, agitation, and immobility. These can be named *searching-behavior* characterized by intense motor activity and expense of energy, and *waiting-behavior* with immobility and energy saving. The choice sequences between these kinds of behaviors can be named as the *searching-waiting strategy*. The following data support the assumption that antidepressant drugs modify the balance between these forms of behavior in the favour of searching.

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Materials and methods

The subjects were naive male NMRI mice (from Centre d'Elevage Roger Janvier, France), weight 22–24 g. The animals were housed in plastic cages in groups of ten per cage, at room temperature about 21 ± 1° C, and with free access to water and food. They were kept on an artificial 12 h/12 h day/night cycle.

The method is based on the observation that a mouse suspended by the tail shows alternate periods of agitation and immobility. For these experiments, the recording device was as follows: metallic gallows were connected to a nylon catheter (d = 1.5 mm, length = 350 mm) with a hook attached to its extremity. The distance between the floor of the device and the hook was 350 mm. The mouse was hung on the hook by an adhesive tape placed 20 mm from the extremity of its tail.

The mouse was 150 mm away from the nearest object and was both acoustically and visually isolated. The articulated stylus of the gallows was connected to a Marey capsula that transmitted any pressure difference to another capsula by a pneumatic connection. The receiver capsula was connected to a drawing stylus, marking on a cylinder covered with black smoke. The cylinder rotated at 2 cm/min regulated by an electric motor. This device provided an analogue record of the movements of the mouse. The device was set in order to ignore respiratory movements, and recorded only body movements.

On the recording (see sample on Fig. 1) it is easy to measure the length of immobility (flat recording), and to convert it to the duration of immobility. These measurements were always made under blind conditions.

The recording duration was 6 min.

Experimental procedure. Each group was composed of 10 or 20 mice. Control mice were given distilled water (D. W.) IP: 0.25 ml/20 g body weight, 30 min before test, except for controls for the amitriptyline group, which received D. W. 60 min before testing. All mice were isolated in plastic boxes (20 × 10 × 10 cm), between the injection and the test.

Treated mice where given the following drugs IP: *d*-amphetamine sulfate (Cooperative Pharmaceutique Française); amitriptyline hydrochloride (Roche); atropine sulfate (Sigma); desipramine hydrochloride (Ciba-Geigy); diazepam (Roche); imipramine hydrochloride (Ciba-Geigy); imipramine methiodide (Ciba-Geigy); mianserin hydrochloride (Organon); nomifensine maleate (Hoechst); viloxazine hydrochloride (I. C. I.-Pharma). The vehicle for

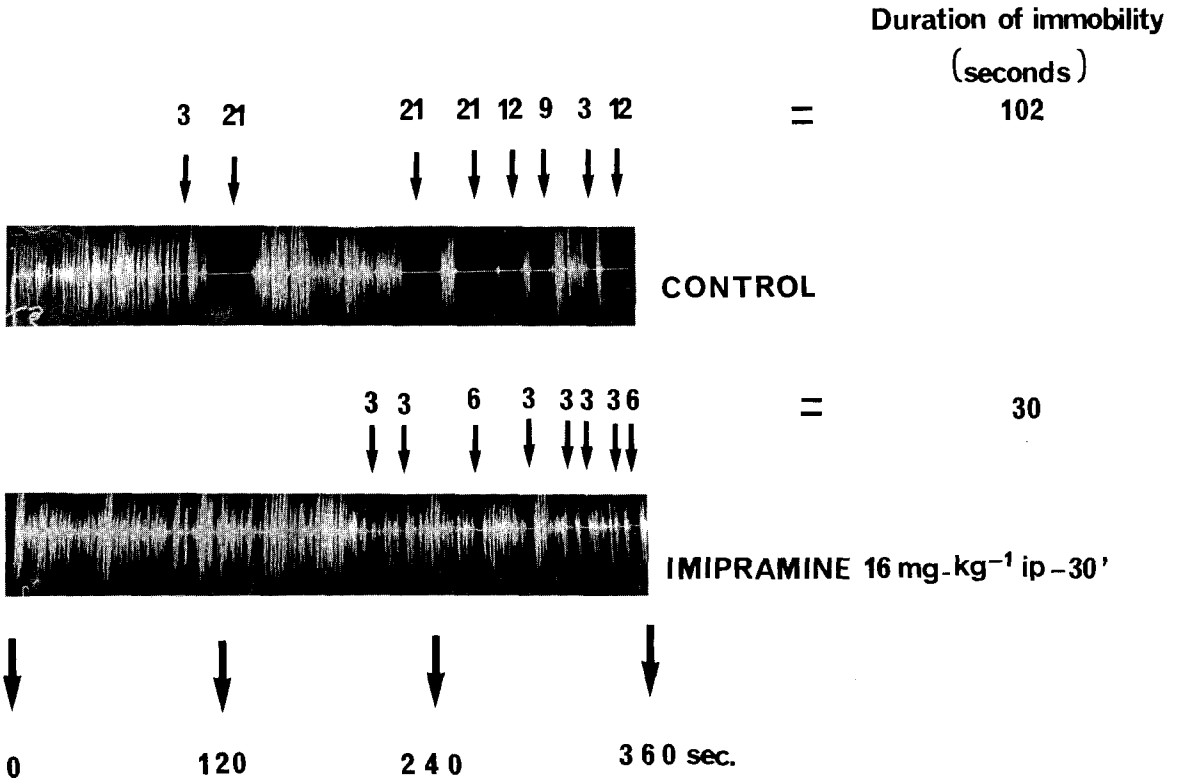

Fig. 1. Recording of a control and a 16 mg · kg^{-1} imipramine-treated mouse. The *arrows* indicate the immobility periods (s), the total immobility duration being summed on the right side

the injected drugs was distilled water or an acacia gum suspension for the non-soluble drugs.

Statistical procedures. Comparisons of the mean duration of immobility (in s) were performed using analysis of variance (ANOVA); those between the various treatment groups were performed using the Dunnett test.

Results

Behavior of control animals. Observation of the controls suggested that the mice suspended to the recording device by the tail made apparent escape efforts which could be classified into three types: (1) running movements, forward or backwards; (2) body torsion with attempts to catch the suspending bond; (3) body jerks.

After several attempts, the mice stopped moving and hung motionless. The agitation testing periods which continued to be performed were separated by longer or more frequent periods of immobility.

The pooled results obtained with 380 control mice, receiving D. W. 30 min before testing are shown in Fig. 2, which provides a frequency histogram of the distribution of the duration of immobility. During the different tests performed, 38 groups of 10 controls were studied. The mean duration of immobility was three times less than or equal to 60 s; seven times between 60 and 70 s, 16 times between 70 and 80 s, eight times between 80 and 90 s, three times between 100 and 110 s and once over 110 s.

Effects of drugs. Table 1 shows that imipramine, desipramine, and amitriptyline each produced a significant dose related reduction of immobility [$F(7,14) = 10.38$, $P < 0.001$ for imipramine; $F(8,12) = 6.69$, $P < 0.001$ for desipramine; $F(7,12) = 8.43$, $P < 0.001$ for amitriptyline].

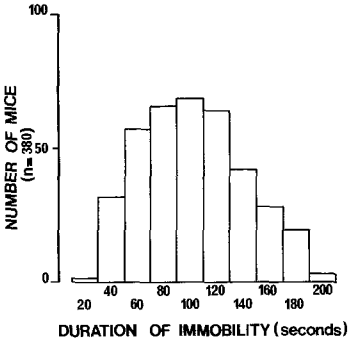

Fig. 2. Duration of immobility: frequency distribution of 38 groups of control mice

Imipramine methiodide, a quaternary ammonium that crosses the blood-brain barrier poorly, had no effect upon the duration of immobility, even at the highest dose; at this dose (32 mg/kg) three out of 10 mice died.

Table 2 shows that mianserin and viloxazine decreased the duration of immobility [$F(5,64) = 3.94$, $P < 0.001$ for mianserin; $F(4,45) = 10.11$, $P < 0.001$ for viloxazine]. However, for mianserin, there was a non linear dose-effect relationship. Nomifensine reduced immobility from a dose of 0.06 mg/kg [$F(9,23) = 20.12$, $P < 0.001$].

Table 3 shows that higher doses of atropine reduced immobility, as did amphetamine, at the higher doses. At lower doses, amphetamine increased immobility time, significantly at 0.25 mg/kg [$F(3,36) = 6.12$, $P < 0.001$ for atropine; $F(7,10) = 9.70$, $P < 0.001$ for amphetamine].

Table 1. Effect of tricyclic antidepressants and derivatives upon the duration of immobility. (Number of seconds during the 6 min duration of the test).

	0	0.125	0.5	1	2	4	8	16	32
Imipramine	102 ± 7.8		83.4 ± 14.4	70.2 ± 16.5	58.5 ± 7.9[b]	52.6 ± 10.9[b]	48.1 ± 9.8[b]	27.5 ± 6.5	19.6 ± 6[b]
Desipramine	94.0 ± 6.0	66.9 ± 16 NS	64.8 ± 15 NS	38.1 ± 10[b]	41.2 ± 8[b]	41.4 ± 12[b]	40.5 ± 11[b]	36.5 ± 10[b]	35.4 ± 7[b]
Amitriptyline	96.8 ± 7.0		59.0 ± 16 NS	44.4 ± 13[b]	57.0 ± 9.2[a]	59.7 ± 13 NS	44.7 ± 11[b]	26.5 ± 8[b]	32.7 ± 7[b]
Imipramine methiodide	80.7 ± 11			86.7 ± 14.0	64.8 ± 12.1	85.2 ± 10	68.7 ± 16	71.7 ± 12	104.7 ± 20 3 deaths

Drugs were injected IP 30 min before test expected for amitriptyline injected IP 60 min before test. NS Not significant, [a] $P < 0.05$, [b] $P < 0.01$ (Dunnett's test)

Table 2. Effect of atypical antidepressants upon the duration of immobility. (Number of seconds during the 6 min duration of the test)

	0	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64
Mianserin	86.2 ± 8.4								69.0 ± 12 NS	44.8 ± 6.8[b]	44.4 ± 6.7[a]	49.2 ± 11[a]	59.1 ± 7.7 NS
Viloxazine	96.6 ± 10								61.2 ± 16 NS	34.5 ± 9[b]	32.4 ± 8[b]	12.6 ± 4[b]	
Nomifensine	90.5 ± 6	102 ± 18	59.4 ± 9[a]	36.6 ± 6[b]	37.0 ± 8[b]	40.6 ± 6[b]	30.5 ± 6[b]	24.5 ± 4[b]	18.0 ± 5[b]	4.5 ± 2[b]			

Drugs were injected IP 30 min before test. NS Not significant, [a] $P < 0.05$, [b] $P < 0.01$ (Dunnett's test)

Table 3. Effect of different drugs upon the duration of immobility. (Number of seconds during the 6 min duration of the test)

	0	0.06	0.125	0.25	0.5	1	2	4	8	16
Dexamphetamine	97 ± 7	105.6 ± 17	117 ± 11	126 ± 8 NS	69.3 ± 9	72.3 ± 13	29.1 ± 12[b]	27.0 ± 7[b]		
Atropine	99.6 ± 11						84.6 ± 18	38.1 ± 6.6[b]		47.1 ± 8.4[b]
Diazepam	92.4 ± 11				105 ± 16		160 ± 17[b]		220 ± 19[b]	

Drugs were injected IP 30 min before test. NS Not significant, [a] $P < 0.05$, [b] $P < 0.01$ (Dunnett's test)

Table 4. Effect of desipramine (16 mg/kg) in chronic administration (twice a day, for 7 days). (Number of seconds during the 6 min duration of the test)

Chronic treatment (twice a day, 7 days)	Acute treatment (inj. 30 min before test)	Duration of immobility (s ± SEM)
D. W.	D. W.	70.2 ± 15
Desipramine	Desipramine	13.3 ± 2.2[b]
Desipramine	D. W.	101 ± 16
D. W.	Desipramine	28.0 ± 7.3[a]
No treatment	No treatment	64.0 ± 15

D. W. = distilled water, [a] $P < 0.05$, (Dunnett's test), [b] $P < 0.01$

Diazepam increased the duration of immobility at 0.5, 2, and 8 mg/kg [$F(3,46) = 14.50$, $P < 0.001$].

Effect of 7 days' administration of desipramine. When desipramine was injected twice a day for 7 days, there was no significant decrease in immobility when the last injection was given 12 h before the test. When there was one more injection 30 min before the test, the decrease in immobility was greater (but not significantly) than when there was only an acute treatment with the same dose of desipramine. It appears that the chronic treatment with desipramine does not diminish the acute effect of the drug on this test (and perhaps increases it).

Discussion

Action of antidepressants. All of the above experiments showed that every antidepressant studied decreased immobility, in terms of duration. However, this test does not measure merely locomotor stimulation, as can be seen from two arguments:

1) The sedative antidepressants (such as amitriptyline and mianserin) decrease immobility at doses previously shown to be sedative for locomotor activity (Porsolt et al. 1978).

2) The locomotor stimulatory antidepressants, such as nomifensine, decrease the duration of immobility at doses

that are clearly smaller than the locomotor stimulant doses (Hoffman 1973). It can be concluded that the tail-suspension test can dissociate the locomotor stimulant from the antidepressant effects of antidepressants, when the latter occurs at lower doses. Clear dose-effect relationships were found with amitriptyline, desipramine, imipramine, nomifensine, viloxazine, and, in the opposite direction, with diazepam.

One could emphasize that in a really satisfactory model of depression, acute administration of drugs should not be active, as far as repeated treatment administrations are necessary in human patients. We cannot explain why acute drug effects are observed.

The lack of effect of imipramine methiodide suggests that the action of the antidepressants on this test is mediated by a central mechanism.

No linear dose-effect relationship was found with two drugs, mianserin and amphetamine. For mianserin, the locomotor sedative effect may mask the reduction of immobility. For amphetamine, the dose-effect curve seems to be biphasic. It is known (Simon 1970) that amphetamine at low doses decreases locomotor activity and may have an anxiogenic effect, which accounts for the enhanced, "freezing"-like immobilisation of mice in the tail-suspension test. Another possible explanation is that low doses of amphetamine decrease catecholamine availability in brain (Huang and Maas 1981), and the tail suspension test may interfere with catecholamines, in the sense that raised levels of catecholamines lead, to a decrease in tail suspension induced immobility. Further experiments will be designed to verify this.

The reduction in immobility time does not seem to be specific to clinically active antidepressants: atropine and amphetamine also reduce immobility in the test. However, both drugs have been described as having a clear stimulant effect in animals, and a potential antidepressant effect in man. Indeed for both drugs, Porsolt (1981) reported similar results using his test.

Chronic (twice a day for 7 days) administration was not very effective in modifying behavior on this test when the last injection was given 12 h before the test. One possible explanation of the difference between the effects of chronic administration in man and rat can be accounted for by pharmacokinetics.

Half-life is about 70 min, in brain and blood in mice, which is very different from that in humans (12–77 h) (Diquet et al. submitted). However, it should be noted that if acute administration is preceded by 2 weeks' administration of the same drug, the acute effect is not diminished and even increased – although not significantly – unlike in other tests. These preliminary results are in good agreement with the results reported by Porsolt with his test (1981).

Two main differences between the Tail Suspension Test and Porsolt's test can be listed. First, the immersion, which is necessary to produce the "behavioral despair", induces a deep hypothermia in mice (personal unpublished observations for mice; for rats, Porsolt et al. 1979); this is avoided in the Tail Suspension Test. A second point is the recording of an objective measure in the Tail Suspension Test, which might be more precise than the human observation on which is based the appreciation of immobility in the "behavioral despair" test. The third difference between the two tests is that the Tail Suspension Test is more sensitive to lower doses of drug and provides, as might be suggested in some cases by these preliminary results, a clearer dose-effect relationship.

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