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Mouse lines differing in sensitivity to β-CCM differ in tasks used for testing antidepressants

Jean-Claude Do-Rego[a], Charles Suaudeau[a], Georges Chapouthier[b], Jean Costentin[a,*]

[a]IFRMP 23, Unité de Neuropsychopharmacologie Expérimentale, CNRS UMR 6036, UFR de Médecine et Pharmacie, 22 Boulevard Gambetta, 76183 Rouen Cedex, France

[b]UMR CNRS 7593, Hôpital Pitié-Salpêtrière, 91 Boulevard de l'Hôpital, 75634 Paris Cedex 13, France

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Abstract

Two lines of mice, previously selected for their sensitivity (BS) or their resistance (BR) to an anxiogenic benzodiazepine (BZ) receptor inverse agonist, methyl β-carboline-3-carboxylate (β-CCM), have recently been shown to present several differences in anxiety. In the present study, attempt was made to extend their behavioral profile in two situations classically used for testing antidepressant drugs. Reassessment of locomotor performance of these new populations confirmed that the motor activity of BR mice was lower than that of BS mice. In both the forced-swimming and the tail suspension tests, the immobility time of BS mice was significantly higher than that of BR mice. In the tail suspension test, two administrations of imipramine (30 mg/kg ip, 5 h and 30 min before testing) significantly reduced the immobility time of BS mice but not of BR mice. From these data, it appears that BS mice are more "depressed" than BR mice. Thus, these selectively bred lines may represent potentially useful animal models to investigate behavioral, neurochemical and neuroendocrine correlates of antidepressant action.

Keywords: Benzodiazepine receptor; Methyl β-carboline-3-carboxylate; Antidepressant; Imipramine; Forced-swimming test; Tail suspension test; Mouse

1. Introduction

Two new mouse lines have been selected for high sensitivity or resistance to the convulsive effects of methyl β-carboline-3-carboxylate (β-CCM), a benzodiazepine (BZ) receptor inverse agonist belonging to the β-carboline group (Chapouthier et al., 1998). β-CCM specifically binds to BZ sites of the GABA–BZ receptor complex and displays effects opposite to those of BZs. Whereas BZs have anticonvulsive, anxiolytic and amnestic effects, β-CCM, at low doses (0.2–0.3 mg/kg ip), enhances learning in a number of situations (Venault et al., 1986; Raffalli-Sébille et al., 1990); at moderate doses (1 mg/kg ip), it is anxiogenic (Prado de Carvalho et al., 1983); and at high doses (2–10 mg/kg ip), it induces seizures (Prado de Carvalho et al., 1984). For the selection of the two strains, an administration of 4 mg/kg β-CCM ip was used. Briefly, a heterogeneous but genetically controlled starting pool was obtained by intercrossing four sensitive (BALB/cBy, CBA/H, C3H/HeJ, DBA/2J) and four relatively resistant strains (C57BL/6J, C57BL/10J, XLII, NZB/B1NJ) (Chapouthier et al., 1998). Two lines were selected: one comprising pairs formed from subjects displaying the shortest convulsion latencies (β-CCM-sensitive group, BS) and another comprising pairs formed from subjects not convulsing at all within the 360 s of the test (β-CCM-resistant group, BR). During selection (six generations), inbreeding was avoided as much as possible. After selection, generations (G1–G14) of brother × sister mating were produced.

Spontaneous behavior of these two lines was then tested in several situations (Suaudeau et al., 2000). Behavior patterns were assessed in eight different behavioral tasks including: general locomotor activity, several tests classically used for measuring fear-motivated behaviors (open field, thigmotaxis, elevated plus-maze, light–dark discrimination, staircase), a test for measuring exploration (holeboard) and a test for measuring nociception (hot plate). In the absence of β-CCM, the results provided evidence for reduced motor activity and higher levels of anxiety in the BR line as compared to the BS line (Suaudeau et al., 2000). In another

* Corresponding author. Tel.: +33-2-35148593; fax: +33-2-35148603.
E-mail address: neuro.psyphar@univ-rouen.fr (J. Costentin).

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study, spontaneous aggressive behavior was assessed in the two lines. Results revealed that males of the BR line were less aggressive than males of the BS line (Guillot et al., 1999). Finally, the sensitivity of both lines to various physiological effects of other ligands of the $GABA_A$ receptor was studied in diazepam-induced anxiolysis (in an elevated plus-maze), diazepam-induced sedation (by recording the vigilance states) and picrotoxin- and pentylenetetrazol-induced seizures after intraperitoneal injections. Results provided evidence that the differential sensitivities of BS and BR lines to β-CCM could be extended to diazepam, picrotoxin and pentylenetetrazol, suggesting a genetic selection of a general sensitivity and resistance to several ligands of the $GABA_A$ receptor (Rinaldi et al., 2000). Thus, the two selected lines present several differences clearly related to emotionality or reactivity to anxiolytic/anxiogenic compounds.

The present work attempted to extend their behavioral profile in analyzing the reaction of these lines in two situations classically used for testing antidepressant compounds. After reassessing the locomotor performance of the new populations, animals were submitted to forced-swimming test and tail suspension test. In the forced-swimming test, developed by Porsolt et al. (1977), animals have to swim in a narrow cylinder from which they cannot escape: The immobility time is believed to be an index of "depressed state" often qualified as "behavioral despair". The tail suspension test (Steru et al., 1985, 1987) is a similar test where the immobility time and the power of the movements of mice suspended by the tail are recorded. In this second test, classically used for assessing the actions of antidepressants, the effect of antidepressant imipramine was assayed at 30 mg/kg ip, the dose commonly used in these tasks in mice (Porsolt et al., 1977; Steru et al., 1985; Van der Heyden et al., 1987; Vaugeois et al., 1996, 1997).

2. Materials and methods

2.1. Animals

Male and female mice of the two lines (generation G14), weighing 20–26 g, were used. Animals were given a rest period of 15 days to recover from transport. The animals were housed by 4 (males)–15 (females) in Makrolon cages ($38 \times 24 \times 18$ cm) with free access to tap water and food (UAR, France) in a ventilated room at a temperature of 21 ± 1 °C, under a 12-h light–dark cycle (light on between 07:00 and 19:00 h).

All the experiments were carried out between 09:00 and 17:00 h in testing rooms adjacent to the animal rooms. The procedures used in this study are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Different mice were used for the different tests.

2.2. Drug

Imipramine (Ciba Geigy, France) was dissolved in NaCl 0.9% and used in one experiment at the 30-mg/kg dose, which was determined as effective in NMRI (Porsolt et al., 1978; Van der Heyden et al., 1987) and CD1 (Porsolt et al., 1978; Vaugeois et al., 1996, 1997) mice in previous studies. Impramine or NaCl 0.9% was injected intraperitoneally 5 h and 30 min prior to testing in a volume of 0.2 ml/20 g body weight.

2.3. Locomotor activity

Locomotor activity was measured in a Digiscan actometer (Omnitech Electronics, Colombus, OH, USA), as described by Suaudeau et al. (2000). The animals were placed individually in $20 \times 20 \times 30$-cm compartments and put in dimly lit and quiet room. The recording apparatus was connected to a Compaq computer to process the data. Horizontal locomotor activity (the number of infrared beams crossed) and vertical locomotor activity (the number of infrared beams broken up to a height of 9 cm) were measured during a 60-min period.

2.4. Mouse forced-swimming test

During the 6 min of the forced-swimming test, the duration of immobility was measured as previously described by Porsolt et al. (1977), but using an apparatus modified as regard the diameter of the Plexiglas cylinder (14 cm instead of 10 cm) similarly as Semba and Takahashi (1988), since Sunal et al. (1994) have established that a cylinder with a higher diameter should decrease the false positive responses. The apparatus consisted of two Plexiglas cylinders (20 cm height, 14 cm internal diameter) placed side by side in a Makrolon cage ($38 \times 24 \times 18$ cm) filled with water (8 cm height) at 22 ± 1 °C. Two mice were tested simultaneously for a 6-min period inside vertical Plexiglas cylinders; a nontransparent screen placed between the two cylinders prevented mice from seeing each other. The total duration of immobility, after a delay of 2 min, was measured during a period of 4 min. Each mouse was considered to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water.

2.5. Tail suspension test

A computerized device (ITEMATIC-TST) developed by ITEM-Labo (Le Kremlin-Bicêtre, France) was used to measure the sum of periods of immobility (duration of immobility, in seconds) and the power of movements, which is calculated from the total energy expended by the animal during the test (as measured by the cumulated amplitudes of individual movements, in arbitrary units, divided by the total time the animal is active) (Steru

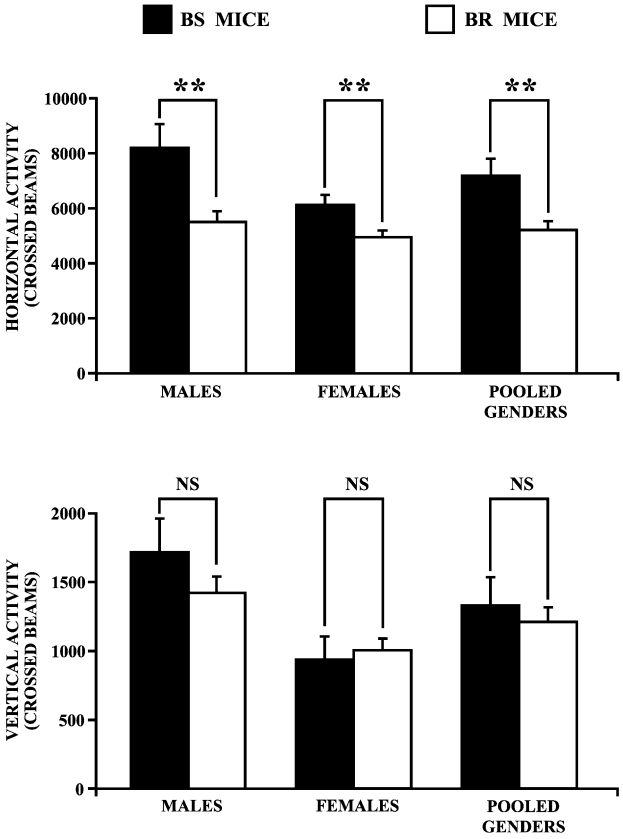

Fig. 1. Comparison in spontaneous motor activity of BS and BR mice. Horizontal and vertical activities, measured in automated activity cages in six successive 10-min periods, were expressed as a total number of horizontal or vertical light beams interrupted by the animal. Means ± S.E.M. from 8 males BS, 18 females BS, 24 males BR or 42 females BR mice; NS: $P > .05$, ** $P < .01$ (Student's t test).

et al., 1987; Van der Heyden et al., 1987). Mice were suspended by the tail, using adhesive Scotch tape, to a hook connected to a strain gauge that picked up all movements of the mouse and transmitted them to a central unit, which calculated the total duration of immobility and the power of movements during the 6 min of the test. Six animals were tested simultaneously.

2.6. Imipramine treatment

Mice were injected twice (5 h and 30 min before testing) either with NaCl 0.9% or imipramine (30 mg/kg ip) and submitted to the tail suspension test. The duration of immobility and the power of movements were measured during a 6 min period. Six animals were tested simultaneously.

2.7. Statistical analysis

Data are expressed as means ± S.E.M. Differences between groups were assessed by Student's t test. $P < .05$ was taken as the significant level of difference.

3. Results

3.1. Locomotor activity

The horizontal activity of BS mice was significantly higher than that of BR mice, either when data of both genders were pooled: 7179 ± 565 vs. 5228 ± 270 (means ± S.E.M. of BS and BR mice, respectively; $P < .01$) or when they were considered separately: in male mice 8211 ± 812 vs. 5505 ± 344 (means ± S.E.M. of BS and BR mice, respectively; $P < .01$); in female mice 6147 ± 318 vs. 4950 ± 197 (means ± S.E.M. of BS and BR mice, respectively; $P < .01$). On the contrary, their vertical activity did not differ significantly during the 60 min of testing, either when data of both genders were pooled: 1329 ± 196 vs. 1212 ± 93 (in BS and BR mice, respectively) or when they were considered separately: 1722 ± 232 vs. 1421 ± 109 (in BS and BR male mice, respectively) and 935 ± 159 vs. 1003 ± 76 (in BS and BR female mice, respectively) (Fig. 1).

3.2. Mouse forced-swimming test

In the behavioral despair test, the immobility time of BS mice was significantly higher than that of BR mice, either when data of both genders were pooled: 188 ± 7 vs. 114 ± 9 s (means ± S.E.M. of BS and BR mice, respectively; $P < .001$) or when they were considered separately: in male mice 191 ± 11 vs. 125 ± 16 s (means ± S.E.M. of BS and BR mice, respectively; $P < .01$); in female mice 187 ± 10 vs. 107 ± 9 s (means ± S.E.M. of BS and BR mice, respectively; $P < .001$) (Fig. 2).

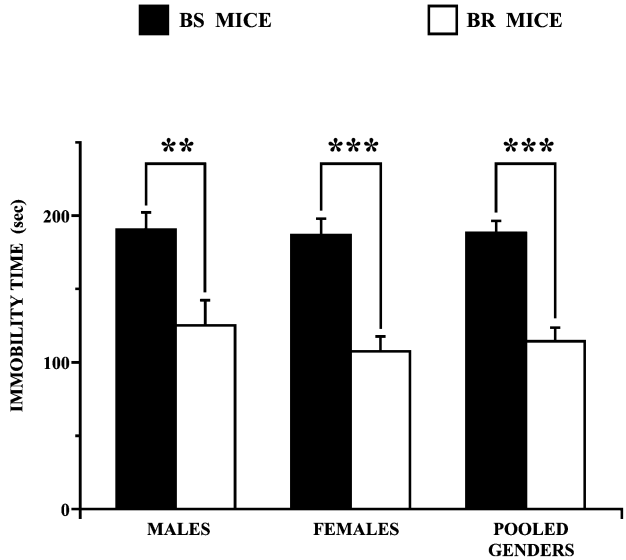

Fig. 2. Comparison in immobility times of BS and BR mice in the mouse forced-swimming test. The duration of immobility was measured during a period of 4 min, after a delay of 2 min. Means ± S.E.M. from 7 males BS, 11 females BS, 8 males BR or 12 females BR; ** $P < .01$, *** $P < .001$ (Student's t test).

3.3. Tail suspension test

In the tail suspension test, the immobility time of BS mice was significantly higher than that of BR mice, either when data of both genders were pooled: 180 ± 13 vs. 57 ± 7 s (means ± S.E.M. of BS and BR mice, respectively; $P < .001$) or when they were considered separately: in male mice 170 ± 14 vs. 55 ± 8 s (means ± S.E.M. of BS and BR mice, respectively; $P < .001$); in female mice 190 ± 12 vs. 60 ± 6 s (means ± S.E.M. of BS and BR mice, respectively; $P < .001$) (Fig. 3). In addition, the power of movements of BS mice: 8.8 ± 1.6 and 9.8 ± 1.6 (in male and female BS mice, respectively) was significantly lower ($P < .001$) than that of BR mice: 43.5 ± 5.2 and 40.8 ± 3.3 (in male and female BR mice, respectively) (Fig. 3).

3.4. Imipramine treatment

When BS mice were injected with imipramine, their immobility time (131 ± 13 s) was significantly reduced ($P < .001$), as compared to nontreated BS subjects (210 ± 8 s), but it remained significantly higher ($P < .001$) than that of BR mice: 70 ± 11 vs. 61 ± 10 s (means ± S.E.M. of nontreated and imipramine-treated BR mice, respectively). However, no significant difference was observed in power of movements: 14.3 ± 2.6 vs. 12.2 ± 1.1 (in nontreated and imipramine-treated BS mice, respectively). The immobility time and the power of movements (39.3 ± 5.5 vs. 49.2 ± 6.6, in nontreated and imipramine-treated, respectively) of BR mice were not significantly modified by imipramine treatment (Fig. 4).

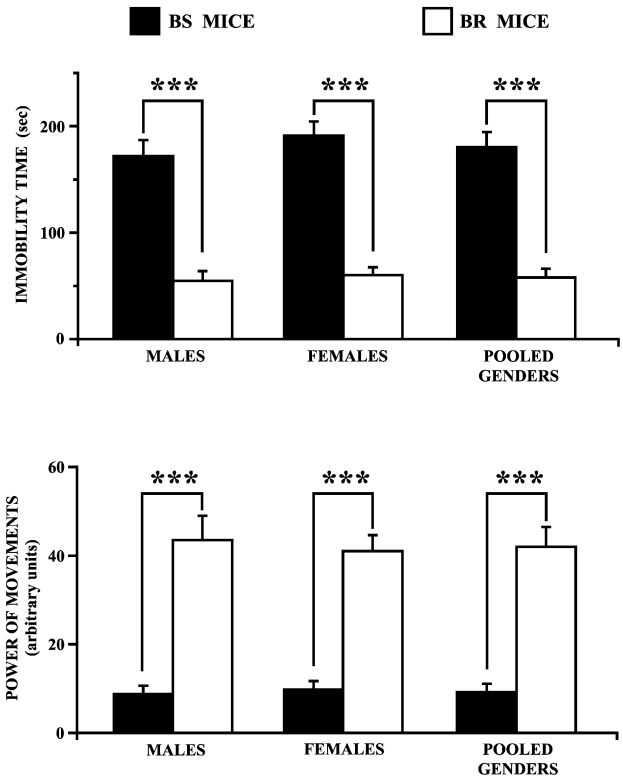

Fig. 3. Comparisons in the tail suspension test of immobility times and the power of movements of BS and BR mice. The duration of immobility and the power of movements were measured in a computerized device (ITEMATIC-TST) for 6 min. Means ± S.E.M. from 8 males BS, 18 females BS, 22 males BR or 42 females BR; *** $P < .001$ (Student's t test).

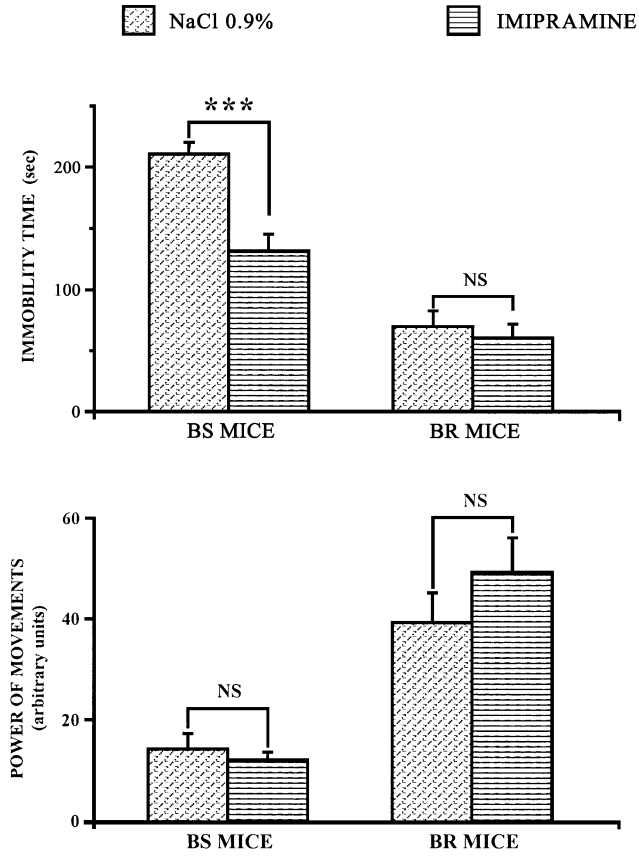

Fig. 4. Comparisons in the tail suspension test of the effects of an imipramine treatment in BS and BR mice. Mice were injected twice (5 h and 30 min before testing) either with NaCl 0.9% or imipramine (30 mg/kg ip) and submitted to the tail suspension test. The duration of immobility and the power of movements were measured during a 6-min period. Means ± S.E.M. from 12 mice per group; NS: $P > .05$, *** $P < .001$ (Student's t test).

4. Discussion and conclusion

The aim of these experiments was to analyze, in tasks classically used for testing antidepressant drugs, the behavior of the two lines selected for their sensitivity to a BZ receptor ligand (Chapouthier et al., 1998), known for their differences in anxiety measurements (Suaudeau et al., 2000) and in sensitivity or resistance to several ligands of the $GABA_A$ receptor (Rinaldi et al., 2000).

Results on locomotor activity confirm the former data (Suaudeau et al., 2000) since BR mice were clearly less active than BS mice, with male BS mice significantly ($P < .01$) more active than female BS mice. As compared to BR mice, both male and female BS subjects showed a higher immobility time, without significant difference according to the gender, in the forced-swimming test and in the tail suspension test, as well as a reduced power of movements in the second test. On the contrary, the lack of difference observed between male and female mice in these tests is somewhat puzzling, more especially as it was also observed in immobility time. However, this situation is not exceptional because, according to Palanza (2001), most investigations of animal model of depression and behavioral responses to antidepressants have employed males, the few existing studies including female subjects demonstrate that males and females can respond either differently or even in opposite directions. One of the main concerns about the behavioral despair and the learned helplessness as animal models of depression is that while reported rates of depression are higher among women, female rats and mice seem to be less susceptible than males to the depression-like effects produced in these paradigms. For example, in the tail suspension test, the immobility time of females mice C57BL/6J and 129S6/SvEv strains is slightly higher or no different in that of males mice (Liu and Gershenfeld, 2001). Since BR mice display a lower locomotor activity than BS mice, the differences observed in these two tests cannot be explained by a difference in general activity. A classical interpretation of the first test would qualify BS mice as having a higher degree of "behavioral despair" (Porsolt et al., 1977). Other explanations cannot, however, be ruled out, such as different ways of adaptation to these stressful situations. If the action of the tested antidepressant, imipramine, is taken into account, another interpretation would be that BS mice are more "depressed" than BR mice. Indeed, in the present study, in the tail suspension test, the higher immobility time of BS mice could be partially reversed by the administration of the antidepressant imipramine (30 mg/kg ip). At this dose, prior dose response studies consistently demonstrated that imipramine produced, 30 min after its single intraperitoneal injection, a maximal antiimmobility effect in the tail suspension test without showing sedation (Steru et al., 1985, 1987; Van der Heyden et al., 1987; Teste et al., 1993; Liu and Gershenfeld, 2001). The question remains why the antidepressant treatment improves immobility time but not power of movements. Our results are in accordance with the original study by Steru et al. (1987), who did not observe any modification of the power of movements after a treatment with imipramine 30 mg/kg or two selective serotonin uptake inhibitors (citalopram and indalpine). However, a slight improvement of the power of movements has been occasionally observed for a peculiar dose of imipramine (2 mg/kg) or other tricyclic antidepressants (amitriptyline 8 mg/kg, desipramine 32 mg/kg) (Steru et al., 1987). In addition, studies using atypical antidepressants (bupropion, viloxazine, indalpine, mianserin) have sometimes detected in their dose–response curves a single dose that significantly increased the power of movements (Steru et al., 1987; Van der Heyden et al., 1987). Consequently, the absence of effect of imipramine (30 mg/kg ip) on the power of movements is, according to Steru et al. (1987), probably due to the potent serotonergic stimulating properties or lack of noradrenergic stimulating properties that distinguish this compound from the other antidepressants tested. Thus, it seems that the immobility criterion is always a much more sensitive measure than the power of movements. Finally, though it is obvious that animal models cannot perfectly mimic human pathologies (Thiébot, 1993), the analogy with "depressive state" can be drawn, as has been proposed in the past (Porsolt et al., 1977). If this analogy is extended, since it is often found in humans that anxiety is associated with depression in an "anxio-depressive syndrome" (Guelfi, 1993; Klein, 1993), it could have been expected that the most anxious of the two lines (BR) would also be the most immobile. The opposite, however, was observed: The less anxious line (BS) happened to be the most prone to immobility. This BS line is also known to be more aggressive than the BR line (Guillot et al., 1999).

In conclusion, the BS mice (sensitive to the convulsant effect of β-CCM), although less anxious and displaying a higher level of spontaneous locomotor activity than BR mice (insensitive to the convulsant effect of β-CCM), appear more helpless than the latter, which may be reversed by the imipramine treatment.

These data tend to show that complex behavioral reactions such as emotionality, aggression or depressed state cannot be considered as simple consequences of a unique mechanism. They can be dissociated in several traits, which can occasionally combine to give very different behavioral profiles. Thus, again, the establishment of an analogy between animal behavior and human pathological traits, such as, in this case, depression, should be done with caution. Results like the present ones clearly demonstrate the interest and the limitations of such analogies.

The observed differences, lying on the sensitivity to the convulsant effect of β-CCM, which interacts with BZ receptors, raises the hypothesis of an involvement of the GABA transmission in the resignation of one of these lines.

The mice of the BS line, which display the more marked immobility in the tail suspension test (resigned or depressed), could advantageously serve, in this test, as substitutes to NMRI mice selected by Steru et al. (1985) as displaying, among other strains of mice, the higher scores of immobility.

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