Behavioural Brain Research 191 (2008) 104–110

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Effects of the genetic background on cognitive performances of TG2576 mice

Jean Michel Lassalle[a,b,*], Hélène Halley[a,b], Stéphanie Daumas[a,b,1], Laure Verret[a,b], Bernard Francés[a,b]

[a] Université de Toulouse, France
[b] CNRS, UMR 5169, Centre de Recherches sur la Cognition Animale, France

ARTICLE INFO

Article history:
Received 19 February 2008
Accepted 13 March 2008
Available online 21 March 2008

Keywords:
Tg2576
Transgenic mouse
Genetic background
Elevated plus maze
Morris navigation task
Contextual and cued fear conditioning

ABSTRACT

Animal models of genetic diseases obtained by transferring human mutated genes in the mouse are widely used in biomedical based research. They constitute efficient tools to study mechanisms underlying abnormal phenotypes. Unfortunately, the phenotype of the transgene is often obscured by the genetic background of the embryonic stem cells and that of the recipient strain used to create the transgenic line. It is also known, from the literature, that repeatedly backcrossing a transgenic strain to an inbred background may have unfavorable effects that can result in the loss of the transgenic line. In order to analyze the influences of the genetic background on the transgene expression, we studied the effects of the hAPPswe transgene involved in Alzheimer's Amyloid Pathology, in 3 genetic backgrounds differing by their genetic heterogeneity (homozygous vs heterozygous) and the strain of origin (C57BL6, CBA, B6SJL F1) after only one generation backcrossing. Three different behavioral paradigms were used to assess the psychological and cognitive phenotypic differences: elevated plus maze, morris navigation task and contextual fear conditioning. Our data indicate that the best solution to maintain the transgenic line is to backcross repeatedly the transgenic mice into the F1 hybrid cross that was used to create the transgenic strain, whereas phenotyping should be performed comparatively after only one generation backcrossing into various well chosen F1 or inbred backgrounds.

1. Introduction

In the last decade, animal models of genetic diseases were developed by transferring human mutated genes from families, in which the disease was inherited, to recipient mice [6,14,18,32]. One of the most successful transgenic murine models of Amyloid Pathology is the Tg(HuAPP695-SWE)2576 mouse developed by Hsiao et al. [16] and maintained by repeated backcrossing to B6 × SJL F1 hybrid. The behavioral, cognitive and neurophysiological phenotypes of this model have been extensively studied in this background [5,7,16,24,28,37]. However, the confusing influence of the genetic background on the expression of the transgene has been repeatedly pointed out in genetically modified mice [13,17,21,22,29–31] and has evolved into a widespread concern in mouse-based biomedical research [19,20,36].

In order to avoid confusing the phenotype of the transgene or the modified gene, and the phenotype of the lines they stem from [8,12,26,27], it has been proposed to backcross the transgenic line to an inbred strain [11,25]. Some authors [34,35] have proposed to follow the strategy that emerged as a consensus from the Branbury-Conference [2] that mutations should be maintained by 3 repeated backcrosses to at least 2 inbred strains while phenotypic characterization should be performed in F1 hybrids resulting from the cross between the 2 congenic lines. However, unexpectedly, repeated backcrosses of a transgene into an inbred background can have unfavorable effects such as inbreeding depression or increased sensitivity to aging, resulting in performance impairments that may preclude conclusive evidence of the deleterious effect of the transgene. It may also happen that backcrossing the transgene to a particular strain increases its toxicity, resulting in the loss of the transgenic line. That is what happened to the hAPP695 transgene which is lethal in inbred FVB/N [15] and B6 mice after only 3–4 backcrossing generations [4]. Thus, inserting the transgene in various genetic backgrounds would allow to study different modes of regulation of the transgene and its functions and help to reveal a phenotype that would have been obscured in a different background [4,23], improving the power of mutant models of human disorders [3,9]. Therefore, the aim of our study has been to analyze the effects of the genetic background on the expression of the hSWE

* Corresponding author at: Centre de Recherches sur la Cognition Animale, Université Paul Sabatier, Bât IVR3 b3, 118 route de Narbonne, 31062 Toulouse Cedex 4, France. Tel.: +33 561 556 170; fax: +33 561 556 154.
E-mail address: lassalle@cict.fr (J.M. Lassalle).
[1] Current address: Laboratory for Cognitive Neuroscience, Centre for Cognitive and Neural Systems, Division of Neuroscience, The University of Edinburgh, 1 George square, Edinburgh EH8 9JZ, UK.

0166-4328/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2008.03.017

APP695 transgene after only one generation backcrossing, in order to avoid increasing the lethality of the transgene to either the CBA/J or the C57BL/6J inbred backgrounds, or to the B6 × SJL F1 isogenic hybrid background.

Phenotypic differences between the backgrounds have been checked by comparing inbred C57BL/6, CBA and B6SJL F1 hybrid mice. Behavioral and cognitive performances of these mice have been assessed by comparing Tg+ and Tg− from the same litter, using three experimental paradigms. The elevated plus maze allowed to evaluate locomotor activity, anxiety reactions and disinhibition. The Morris navigation task was used to compare the acquisition, as well as short-term retention of a hippocampo-dependant spatial memory with distributed learning whereas the contextual fear conditioning measured the long-term retention of an episodic-like memory acquired during a single training session. We choose the B6SJL F1 and C5BL/6 backgrounds because of their ability to allow repeated backcrossing without deleterious consequences (B6SJL F1) and to increase the lethality of the APP transgene since the second or third backcross generation (C57BL/6), respectively. We also studied the CBA background because it is a non-albino strain that had never been used so far as a background strain for the APP transgene.

Our expertise in Tg2576 mouse breeding and the results of the present study confirm, as already established by Hsiao et al. [16], that the most suitable method to maintain the Tg2576 strain is to repeatedly backcross transgenic mice to F1 hybrids from the inbred strains that were used to create the transgenic construct. Meanwhile, the main outcome of this study is that backcrossing transgenic mice to various isogenic F1 hybrids and inbred strains for a single generation would constitute a cost effective and most likely an optimal strategy to detect most of the cognitive effects of a transgene in the mouse.

2. Methods

2.1. Animals

Two hemizigous Tg2576 (HuAPP695swe in a C57BL6/SJL genetic background) male mice created by K. Hsiao (1995) and generously gifted by Mayo Foundation for Medical Education and Research to J.M. Lassalle, were obtained from Charles River Laboratories. The Tg strain has been maintained in the CRCA mouse breeding facility by backcrossing Tg males to B6SJL F1 females.

Seventy female mice, 17 ± 1-month-old were used in this study. This time point, where mice are older than those generally used in this kind of studies, was chosen to allow us to consider the effects of senescence. We used female mice because they were still living in social groups in good conditions, whereas most 17-month-old males could hardly be kept in groups because of their aggressiveness. Mice were issued from B6SJL Tg+ progenitor males of the 4th generation, crossed to (i) B6SJL F1/j F1 females, (ii) C57BL6/j (B6) inbred females and (iii) CBA/j (CBA) inbred females. Mice were genotyped using PCR. C57BL/6 and CBA inbred mice as well as B6SJL F1 hybrids were also included in the experimental design. It has been shown in our laboratory that by 17 months of age, Tg2576 mice of both sexes display widely spread β-amyloid deposits associated to cognitive impairments (unpublished data). Some albino mice issued from the backcross to B6SJL F1 have been excluded from the study.

All mice used in this study were bred and reared in the same conditions in our institute breeding facility. They were housed in groups of three to five per cage and kept on at 12-h light–dark cycle, with lights on at 8 a.m., with constant ambient temperature (21 ± 1 °C) and humidity (50 ± 10%). Food and water were available *ad libitum* throughout the experiments. The breeding facility for transgenic mice was authorized by the committee for genetic engineering of the French ministry for research (#4161, July 8, 2004). All procedures followed the guidelines of the European Communities Council Directive of November 24, 1986 (86/609/EEC). Animal samples sizes and their characteristics in the experimental design were as indicated in Table 1.

2.2. Apparatuses and training

In order to avoid interferences between the 3 behavioral measurements, testing sessions were distributed over several weeks.

2.2.1. Elevated plus maze (EPM)

The experimental device was made of yellow polyvinyl chloride (PVC). The four arms were 30 cm long and 10 cm wide, the two opposite closed arms being equipped with 20 cm high walls. The EPM was elevated 100 cm above the floor. It was surrounded by a white curtain without any conspicuous cue, at a distance of 80 cm. The first week of behavioral testing the mouse was dropped on the maze in the 10 cm^2 central zone, facing an open-arm and videotaped for a 10 min period. Mouse behavior was analyzed using an ethological keyboard that allowed recording the number of visits and the time spent in each arm and the central zone.

2.2.2. Morris navigation task (MNT)

This test was performed on the second week of experiments. The experimental conditions replicated those routinely and successfully used in our laboratory [1]. The circular swimming pool (120 cm in diameter and 30 cm in height) was made of yellow PVC, filled with water (24 ± 0.5 °C), made opaque with the Opacifier 631® to 9 cm below the edge of the wall. A circular goal platform (8 cm in diameter) was laid 0.5 cm under the surface of the water and 16 cm from the wall. The device was placed in a regular room with a temperature of 22 °C. Dropped into the water from a different quadrant on each trial, mice had to learn to navigate to the invisible platform using the spatial cues available on a white curtain surrounding the pool at about 1.5 meter distance. After a three trial pre-training session to find out the procedural components of the task, mice were given three consecutive trials a day for 4 days. Shortly after the third trial of the last session, mice were submitted to a probe test for short-term spatial memory. The platform was removed and the mouse, starting from the opposite quadrant, was allowed a 1-min search for the platform. The path was videotaped and a spatial bias index was computed as the difference between the number of times a 12-cm-diameter annulus surrounding the former location of the platform was crossed and the mean number of crossings of three annuli, symmetrically laid out in the quadrants where the platform had never been, divided by the total number of annulus crossings.

2.2.3. Contextual fear conditioning (CFC)

We performed this test two weeks after the end of MNT. Conditioning took place in a conditioning chamber that consisted of a rectangular PVC box (length 35 cm, width 20 cm, and height 25 cm) with three light-brown sides and a Plexiglas front wall. The floor was made of a grid with stainless-steel rods connected to a generator (Campden Instruments) delivering shocks of defined duration (2 s) and intensity (0.7 mA) through a shock-scrambler unit. A loudspeaker producing the tone (85 dB, 30 s) was fixed on the top of the conditioning chamber. Experiments were videotaped. Contextual memory was checked in the same experimental conditions as conditioning, whereas tone memory was assessed in a modified context as already described by Daumas et al. [10].

Conditioning consisted of a single training session with two trials. Mice were dropped individually into the conditioning chamber via the ceiling. After a 2-min exploration period, a sound (CS) was emitted for 30 s, and a foot-shock (US) was superposed to the tone during the last 2 s. After an inter-trial interval of 2 min, the paired CS–US was repeated, and 30 s after the second foot-shock, mice were gently removed from the chamber and returned to their home cage. Twenty-four hours

Table 1
Experimental design

Experimental group	Number	Genotype	Background	
• B6SJL F1	7	Isogenic control	B6SJL	Heterozygous F1
• B6SJL Tg+	11	Tg+	B6SJL	Heterozygous BC (1/2 B6, 1/2 SJL)
• B6SJL Tg−	11	Tg−	B6SJL	Heterozygous BC (1/2 B6, 1/2 SJL)
• B6	7	Isogenic control	B6	Homozygous
• B6 Tg+	7	Tg+	B6	Heterozygous BC (3/4 B6, 1/4 SJL)
• B6 Tg−	8	Tg−	B6	Heterozygous BC (3/4 B6, 1/4 SJL)
• CBA	6	Isogenic control	CBA	Homozygous
• CBA Tg+	7	Tg+	CBA	Heterozygous BC (1/2 CBA, 1/4 SJL, 1/4 B6)
• CBA Tg−	6	Tg−	CBA	Heterozygous BC (1/2 CBA, 1/4 SJL, 1/4 B6)
Total	70			

later, mice were individually checked for freezing to the context in the conditioning chamber for 4 min (contextual memory testing). Two hours later, they were tested for freezing in the modified context then, 2 min after their introduction in the modified chamber mice received a 2-min tone presentation during which they were also checked for freezing. Freezing was defined as the lack of movement beside respiration. It was scored every 5 s during conditioning and test sessions. The data were converted to the percentage of samples scored at freezing and calculated for the 4-min context test period and the 2-min tone test presentation.

2.3. Data analysis

The aim of this study being to analyze the effects of the genetic background on the expression of the hAPPswe transgene, we had to consider various factors and their interactions: the transgene (Tg+ vs Tg−), the origin of the background (B6SJL, B6 and CBA), and the genetic heterogeneity of the background (Homozygous for B6 and CBA, and partly heterozygous but isogenic for B6SJL F1 hybrids as well as the intercrosses, for B6SJL Tg and backcrosses, for B6 Tg and CBA Tg).

Statistical analyses have been performed using the MGLH model of Systat 10.2 [33]. Categorical variables have been created for effects coding as follows: (i) A composite variable called *Genotype* (9 levels: B6SJL, B6SJL Tg+, B6SJL Tg−, B6, B6 Tg+, B6 Tg−, CBA, CBA Tg+, CBA Tg−) allowed to test the significance of the overall variation; (ii) 2 analytical variables (excluding isogenic F1 and inbred mice) respectively called *Transgene* (2 levels: all Tg+ vs all Tg−) and *Background* (3 levels: B6SJL Tg+ and Tg−; B6 Tg+ and Tg−; CBA Tg+ and Tg) and (iii) 2 more analytical variables (excluding Tg+ mice) called *Iso vs Hetero* (2 levels: (B6SJL F1; B6 and CBA) vs (B6SJL Tg−, B6 Tg− and CBA Tg−) that accounts for the effect of the isogenization of the background genome or the effect of heterosis, and *NonTg Background* (3 levels: B6SJL F1 and B6SJL Tg−; B6 and B6 Tg−; CBA and CBA Tg−) that accounts for the effect of the alleles that can vary according to the strain of origin. Three separate ANOVAs have been performed to check for the effects of these different factors:

- ANOVA for Genotype (to check the overall variation).
- ANOVA for Transgene, Background and Transgene × Background interaction.
- ANOVA for Iso, NonTg Background and Iso × NonTg Background interaction.

Multiple R^2 are considered to estimate the proportion of the variation that is explained by experimental factors or categorical variables included in the model.

To satisfy the requirements for the use of ANOVA, the mean percentages of freezing scores (P) were transformed in $Q = \arcsin(\sqrt{P}/100)$ according to Daumas et al. [10]. Statistical analyses were performed on the Q variable.

3. Results

As mentioned in the introduction, backcrossing the transgene to a particular strain could increase its toxicity, resulting in the loss

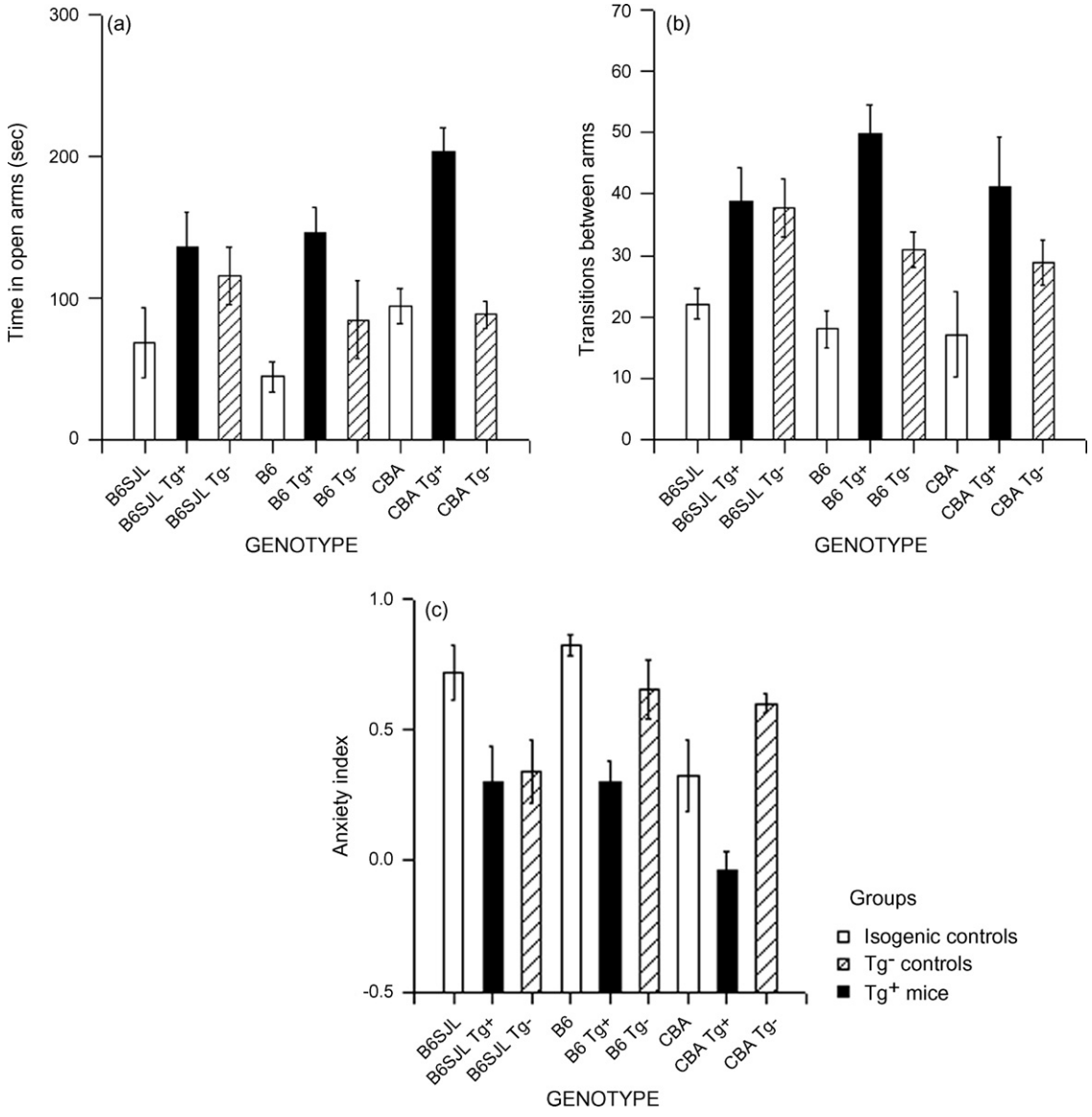

Fig. 1. Elevated plus maze. Panel (a): time spent in the open-arms during the 10 min session. Panel (b): number of transitions between the 4 arms. Panel (c): anxiety index. Measures are expressed as Mean (±SEM).

of the transgenic line. Accordingly, data records from our breeding facility show that mean litter size decreases from 6 to 4 then 0 pups during the 3 successive backcrossing generations to C57BL/6. On the other hand in the same conditions, mean litter sizes remain relatively stable when backcrossing to CBA (4.7, 5.3, 3.7 pups on average) for 3 generations or to B6SJL (10.3, 8.1, 5, 5.9, 7, 7 pups) for 6 generations.

3.1. Elevated plus maze (Fig. 1a–c)

As can be seen from Fig. 1a, the overall variation for time spent in the open-arms is significant (Genotype: $F(8, 58) = 4.700$, $P < 0.00$, $R^2 = 0.393$), the genotype factor explaining 39% of the variation. The effect of the transgene is also significant (Transgene: $F(1, 41) = 11.064$, $P = 0.002$, $R^2 = 0.261$). Tg+ mice spend more time in the open-arms than Tg− mice, although this difference is less pronounced in the B6SJL background in which Tg− mice also spend much time in the open-arms. Fig. 1b shows that the overall variation for activity is highly significant (Genotype: $F(8, 58) = 5.247$, $P < 0.001$, $R^2 = 0.420$). The genotype factor explains 42% of the total variance. There is a significant effect of the transgene ($F(1, 41) = 6.560$, $P = 0.014$, $R^2 = 0.191$) with no significant transgene × background interaction. Also Tg− control mice that have a large genetic heterogeneity are significantly more active than isogenic mice (Iso vs Hetero: $F(1, 38) = 15.826$, $P < 0.001$, $R^2 = 0.374$).

An anxiety index has been computed as: ((time in closed arms − time in open-arms)/(time in closed arms + time in open-arms)). The overall variation of the anxiety index over the 10 min of the test (Fig. 1c) is highly significant (Genotype: $F(8, 58) = 6.019$, $P < 0.001$, $R^2 = 0.454$). This model explains 45% of the total variance in the model. There is also a significant contribution of the transgene factor ($F(1, 41) = 14.135$, $P = 0.001$) which is completed by an interaction with the background (Transgene × Background: $F(2, 41) = 3.755$, $P = 0.032$), Tg+ mice showing lower anxiety scores than Tg− control mice, except in the B6SJL background. This model accounts for 33.9% of the total variance. A further analysis shows a significant variation in anxiety levels among the 3 NonTg background groups (NonTg Background: $F(2, 38) = 3.749$, $P = 0.033$) interacting with the iso vs hetero factor (Iso × NonTg Background: $F(2, 38) = 5.038$, $P = 0.011$) which also indicates that mice with an isogenic background are more anxious than those having an heterozygous background, except for CBA. These two factors together explain 35.7% of the variance in the model.

To summarize, the Tg+ mice spend more time in the open-arms and are more active than Tg− mice and than the isogenic controls, so that they seem to be less anxious (except in the B6SJL background). Interestingly, heterozygous mice prove more active than isogenic mice. Moreover mice with an isogenic background tend to be more anxious than those with a heterozygous one (except in the CBA background).

3.2. Morris navigation task

Escape latency (Fig. 2a–c)

Three ANOVAs with a repeated session factor were performed to analyze the data. The first ANOVA, using the genotype factor showed a significant between subjects effect (Genotype: $F(8, 60) = 9.992$, $P < 0.001$) indicating that the set of variables in the model influences escape latencies during the acquisition of the task. The within subjects analysis reveals that escape latencies improve across sessions (Session: $F(3, 180) = 8.026$, $P < 0.001$), although the whole set of variables merged in the genotype factor influence the evolution of performances across sessions interactively (Session × Genotype: $F(24, 180) = 2.144$, $P = 0.003$). The

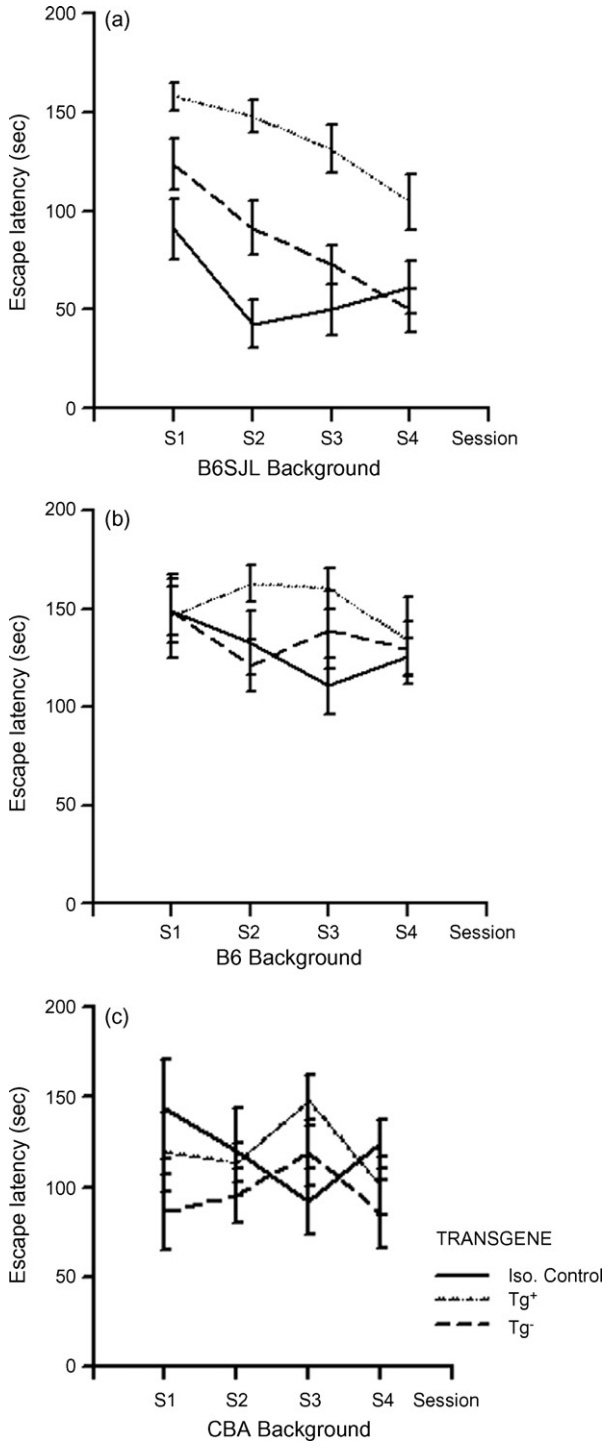

Fig. 2. Morris navigation task (escape latencies). Escape latencies, expressed as the sum of the four latencies (in seconds) within sessions (S1–S4), are plotted apart by background origin, in order to improve the clarity of the graph. Panel (a): B6SJL F1 background. Panel (b): C57BL/6 background. Panel (c): CBA background. Measures are expressed as Mean (±SEM).

second ANOVA shows that both the transgene (Transgene: $F(1, 43) = 18.591$, $P < 0.001$) and genetic background (Background: $F(2, 43) = 10.206$, $P < 0.001$) factors affect significantly global levels of performances and that both of them show a marginally significant interaction (Transgene × Background: $F(2, 43) = 2.443$, $P = 0.099$). It can be seen from Fig. 2 that Tg+ mice exhibit the longest latencies. Also, B6SJL Tg+ and Tg− mice exhibit escape latencies that are glob-

ally inferior to those of Tg+ B6 and Tg+ CBA and their Tg− controls. Moreover, although all mice improve significantly their escape latencies from session to session (within Session: $F(3, 129) = 7.410$, $P < 0.001$), Tg+ and Tg− B6SJL mice improve their escape performances more than Tg+ and Tg− mice with a B6 or CBA background (Session × Background: $F(6, 129) = 4.183$, $P = 0.001$). When Tg+ mice are discarded, the third ANOVA reveals that isogenic and heterozygous mice display performances that differ globally over the four sessions (NonTg Background: $F(2, 38) = 20.606$, $P < 0.001$). This effect is more obvious in B6SJL F1 mice, that show superior performances than in the B6 or CBA backgrounds, although the significance of the interaction with the background remains only marginally significant (Iso × NonTg Background: $F(2, 38) = 3.052$, $P = 0.059$). Likewise, if the within ANOVA shows that, globally, escape latencies decrease across training sessions (Session: $F(3, 114) = 6.969$, $P < 0.001$), performances improvements result from complex interactions with the level of heterozygosity of the background (Session × Iso: $F(3, 114) = 3.007$, $P = 0.033$) and the origin of strain of the background (Session × Iso × NonTg Background: $F(6, 114) = 3.007$, $P = 0.033$). This set of ANOVAs shows that the B6SJL background is the most appropriate to discriminate the effects of the transgene and those of the level of heterozygosity of the background on escape latencies data.

3.3. Spatial index (Fig. 3)

A spatial index has been computed as the number of crossings of the goal annulus minus the mean number of crossings of the 3 other annuli, divided by the total number of annulus crossings during the probe test session.

Shortly after the last training session, the overall variation of the short-term spatial memory index shown by Fig. 3 is highly significant (Genotype: $F(8, 60) = 5.412$, $P < 0.001$, $R^2 = 0.419$). The genotype factor accounts for 41.9% of this variation. The transgene has a significant effect (Transgene: $F(1, 43) = 22.228$, $P < 0.001$); Tg+ mice show values of the spatial index close to zero, B6SJL Tg+ mice showing a higher value. The genetic background modulates also the expression of the transgene (Background: $F(2, 43) = 3.526$, $P = 0.038$). They explain together 44.2% of the variation. The weakest difference between Tg+ and Tg− mice appears in the CBA background. The background effect is also apparent when considering only control Tg− and isogenic mice (NonTg Background: $F(2, 38) = 3.649$, $P = 0.036$ $R^2 = 0.224$). At this age, mice showing the weakest memory performance impairment are the B6SJL Tg− and B6SJL F1, thus providing the highest sensitivity to the potential effects of the transgene. Moreover, the isogenic vs heterozygous condition of the mice has no significant influence on spatial short-term memory performance.

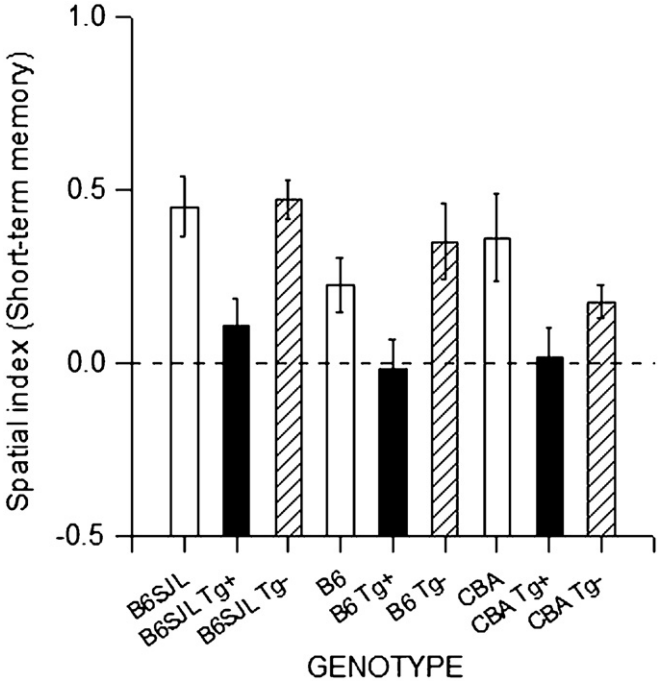

Fig. 3. Morris navigation task (short-term spatial memory). The spatial probe test has been performed on the same mice, shortly after the last training session. Measures are expressed as Mean (±SEM).

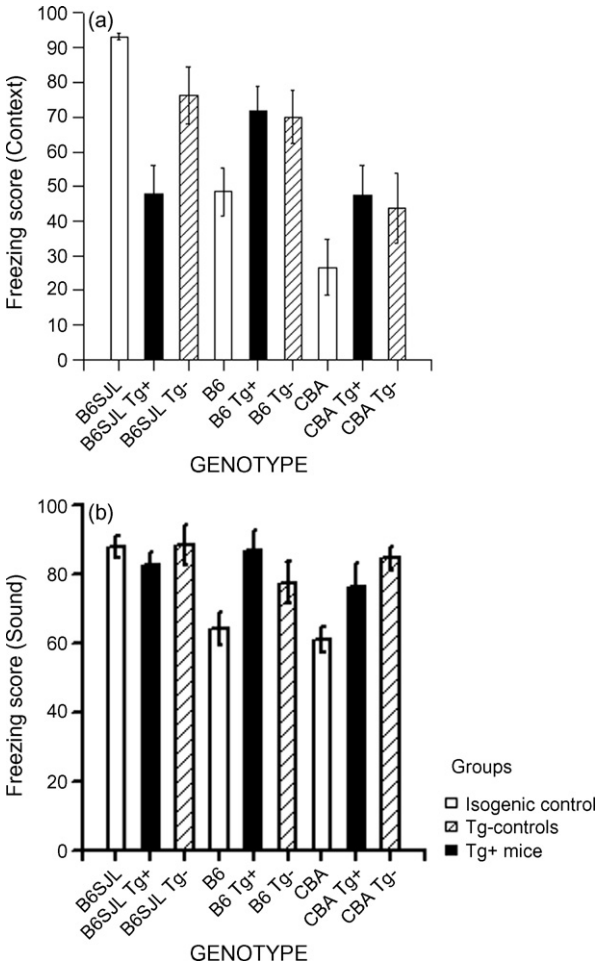

Fig. 4. Contextual fear conditioning. Results are presented as the percentage of time spent freezing during the context (Panel (a)) and tone presentations (Panel (b)). Measures are expressed as Mean (±SEM).

3.4. Contextual fear conditioning (Fig. 4a and b)

Fig. 4a shows a fairly large level of variation among groups, which is confirmed by the ANOVA on the genotype factor that explains 51.5% of the total variation (Genotype: $F(8, 57) = 7.571$, $P < 0.001$, $R^2 = 0.515$). The influence of the background is significant (Background: $F(2, 40) = 4.523$, $P = 0.017$, $R^2 = 0.297$). On the other hand, the effect of the transgene is somewhat paradoxical since it appears only as a marginally significant interaction with the genetic background (Transgene × Background: $F(2, 40) = 2.720$, $P = 0.078$), its effect being significant only in the B6SJL background (B6SJL Tg+ vs B6SJL Tg−: "t" = −2.585, df = 17, $P = 0.019$). Both of them account

for 29.7% of the variation. Actually, the main source of variance arises from the genetic background: the freezing level is very high in B6SJL controls (B6SJL F1 and B6SJL Tg−), intermediate in B6 controls (B6 and B6 Tg−) and rather low in CBA controls (CBA and CBA Tg−) (NonTg Backgrounds: $F(2, 36) = 25.433$, $P < 0.001$). This background effect on the level of expression of conditioned freezing interacts strongly with the level of genetic heterozygosity of the background (Iso × NonTgBcG: $F(2, 36) = 5.122$, $F = 0.011$). Altogether the two analytical variables explain 62.3% of the variation. Thus, it can be seen from Fig. 4a that B6SJL F1 hybrids show the highest level of freezing to the context compared to B6 and CBA inbred strains, which could be due to heterosis (B6SJL vs B6: "t" = 7.844, df = 12, $P < 0.000$; B6SJL vs CBA: "t" = 8.911, df = 11, $P < 0.000$). Also, the two backcrosses B6 Tg− and CBA Tg−, the background of which is partly heterozygous, present levels of conditioned freezing that tend to be higher than those of homozygous B6 and CBA strains.

Although 17-months-old, heterozygous mice and B6SJL F1 hybrids amazingly show high levels of episodic-like memory performance. In such a background, old transgenic mice are still unimpaired, except B6SJL Tg+ mice. Therefore, backcrossing to the B6SJL background is the best way to reveal short-term episodic-like memory impairments resulting from the effects of the HuApp 695-SWE transgene.

As can be seen from Fig. 4b, the level of freezing conditioned to the sound is rather high according to the age of mice. Nevertheless, the overall variation is significant (Genotype: $F(8, 57) = 3.901$, $P = 0.001$, $R^2 = 0.354$). Performances of Tg+ mice are not impaired and there is no effect of the background either on the expression of the transgene. On the other hand, both the effects of the isogenization of the background (Iso: $F(1, 36) = 10.582$, $P = 0.002$) and the effect of the strain of origin of the alleles (NonTg Backgrounds: $F(2, 36) = 8.933$, $P = 0.001$) are significant and explain together 45.6% of the overall variation. In fact, Fig. 4b clearly shows that these effects result from the lower level of freezing to the sound displayed by the two inbred strains, B6 and CBA compared to the B6SJL F1 Hybrids on the one hand, and to the heterozygous Tg+ and Tg− mice on the other hand. Thus inbreeding depression decreases single stimulus associative learning performances in aged B6 and CBA inbred strains.

4. Discussion

The aims of this study were to analyze various aspects of the genetic background likely to interfere with the level of behavioral expression of the HuApp 695-SWE transgene in the Tg2576 aged mouse. As this transgene is directly involved in Alzheimer's type dementia, this study focused on behavioral paradigms that are sensitive to cognitive impairments. The experimental design involved different groups of animals that could be compared under different combinations. Thus, multifactorial analyses allowed comparing the effects of the transgene after only a single generation backcrossing in three different isogenic backgrounds. Comparisons of performances in non-transgenic mice enabled also to further estimate roles played by the structure of the genome (inbreeding depression vs heterosis) or the strain of origin of the alleles contributing to the phenotypic expression and their possible interactions with the transgene. Altogether, the results show that the composite genotype variable accounts for a large part in the variance of the various dependant variables in the three experimental paradigms (35.4–51.5%).

The analysis of transgene and background effects, that excludes isogenic mice, shows that alone or combined, they explain between 19.1% and 44.2% of the variation of the different measures. Their respective contributions vary according to the psychological or cognitive process under study. Raw measurements of activity and time spent in open-arms in the EPM are influenced only by the transgene whereas the transgene either alone or in interaction with the background, contributes to the variation of the estimated anxiety index. Likewise, escape latencies during the training sessions in the MNT and the spatial memory index are influenced by both the transgene and the genetic background that, this time, do not interact. Finally, the transgene has no main effect on contextual or sound conditioning memory, the background alone contributing to the variation of contextual memory. Nevertheless a marginally significant transgene by background interaction suggests that especially in the B6SJL background, the transgene may disturb contextual memory.

The experimental design allowed also to estimate the contribution of the level of isogenization of the background by comparing three isogenic groups (B6, CBA and B6SJL F1) to three non-isogenic groups (B6 Tg−, CBA Tg− and B6SJL Tg−) through the iso vs hetero variable. The nonTg background variable analyzed the effects of the origin of the alleles that contribute to the background. The strain of origin of the alleles is a source of variation that influences the anxiety in the EPM, escape latencies and the spatial index in the MNT and freezing responses to the context and to the sound in the CFC. The analysis of the iso vs hetero variable reveals that isogenic mice and especially inbred ones (B6 and CBA), are less active in the EPM and CFC and that they are more anxious and more impaired at contextual memory and show longer escape latencies in the MNT. For some measures, such as escape latencies and freezing to the context, an obvious heterosis effect can be observed in B6SJL F1 mice. Altogether the effects of isogeny and strain of origin of the alleles can account for a rather important part of the variation that ranges from 16.4% to 62.3%.

From these analyses, we can conclude that the phenotypic variation among the different groups of the experimental design is largely influenced by various factors pertaining to the background. Nevertheless, this design involving 3 different backgrounds allowed revealing distinctive phenotypic features of the HuAPP695-SWE Tg. Thus, at the psychological level, Tg+ mice turned out to be less anxious and more active both in the closed and open-arms with shorter latencies to enter open-arms. This indicates increased behavioral disinhibition as already observed by Ognibene et al. [28] which might result from a ventral-frontal cortex dysfunction. At the cognitive level, our results showed a short-term spatial memory deficit and a long-term episodic-like memory impairment that can be evidenced as soon as 24 h after learning and can be associated with a hippocampal dysfunction (see also [5,7,16,17]). On the other hand, cued fear conditioning is not disturbed (see also [7]), thus indicating that associative function of the amygdala is still working in 17-month-old Tg2576 mice.

However, the main outcome is that none of the three different backcrosses experienced in this study can be considered as optimal, since all the above described phenotypic features of the transgene could not be demonstrated in a single backcross. Thus, effects on anxiety and behavioral disinhibition (EPM) appear clearly in the CBA background, to a lesser extent in the B6 background and are hardly visible in the B6SJL background. On the other hand the more appropriate backgrounds for revealing spatial impairments (MNT) due to the transgene are the B6 and B6SJL backgrounds. Finally, episodic-like memory impairments (CFC) can be only brought to light in the B6SJL F1 background.

The main conclusion to be drawn from this study, therefore, is that there would be no obvious advantages to repeatedly backcrossing the transgene to an inbred strain, since this may result in a generalized inbreeding depression effect that increases sensitivity to aging, impairs behavioral performances and consequently increases the difficulty to reveal the deterioration due to the transgene. Moreover, some aspects of the transgenic phenotype can be definitely obscured in some background strains, due to a strain spe-

cific level of phenotypic expression. The best solution then would rather be to backcross the HuApp 695-SWE transgene to the B6SJL F1 hybrid, as recommended by K. Hsiao, to perpetuate the transgenic strain, while phenotyping the transgene should be performed by comparison of the first backcross generations to a set of inbred strains showing a diversity of well established behavioral profiles.

Acknowledgements

The authors are grateful to Karen K. Hsiao who provided the Tg 2576 mice with a MTA. This work was supported by CNRS, Université Paul Sabatier and grant # 03340 from ACI "Neurosciences intégratives et computationnelles".

References

[1] Amson R, Lassalle J-M, Halley H, Prieur S, Lethrosne F, Roperch J-P, et al. Behavioral alterations associated with apoptosis and down-regulation of presenilin-1 in the brains of p-53 deficient mice. Proc Nat Acad Sci USA 2000;97:5346–50.
[2] Branbury-Conference. Mutant mice and neuroscience: recommendations concerning genetic background. Neuron 1997;19:755–9.
[3] Brennan FX, Albeck DS, Paylor R. Fmr1 knockout mice are impaired in a leverpress escape/avoidance task. Genes Brain Behav 2006;5:467–71.
[4] Carlson GA, Borchelt DR, Dake A, Turner S, DanielsonV, Coffin JD, et al. Genetic modification of the phenotypes produced by amyloid precursor protein overexpression in transgenic mice. Human Mol Genet 1997;6:1951–9.
[5] Chapman PF, White GL, Jones MW, Cooper-Blacketer D, Marshall VJ, Irizzary M, et al. Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. Nature 1999;3:271–6.
[6] Codita A, Winblad B, Mohammed AH. Of mice and men: more neurobiology in dementia. Curr Opin Psychiatry 2006;1:555–63.
[7] Corcoran K, Lu Y, Turner RS, Maren S. Overexpression of hAPPswe rewarded alternation and contextual fear conditioning in a transgenic mouse model of Alzheimer's disease. Learn Mem 2002;9:243–52.
[8] Crawley JN. What's wrong with my mouse? Behavioral phenotyping of transgenic and knockout mice. New York: Wiley; 2000.
[9] Crusio WE. Gene-targeting studies: new methods, old problems. Trends Neurosci 1996;19:186–7.
[10] Daumas S, Halley H, Lassalle JM. Disruption of hippocampal CA3 network: effects on episodic-like memory processing in C57BL/6J mice. Eur J Neurosci 2004;20:597–600.
[11] Gerlai R. Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? Trends Neurosci 1996;19:177–81.
[12] Gerlai R. Gene targeting: technical confounds and potential solutions in behavioral brain research. Behav Brain Res 2001;125:13–21.
[13] Hoover-Plow J, Shchurin A, Hart E, Sha J, Hill AE, Singer JB, et al. Genetic background determines response to homeostasis and thrombosis. BMC Blood Dis 2006;6:6. Open Access article from : http://www.biomedcentral.com.
[14] Hsiao Ashe K. Learning and memory in transgenic mice modeling Alzheimer's disease. Learn Mem 2001;8:301–8.
[15] Hsiao KK, Borchelt DR, Olson K, Johannsdottir R, Kitt C, Yunis W, et al. Age-related CNS disorder and early death in transgenic FVB/N mice overexpressing Alzheimer amyloid precursor proteins. Neuron 1995;15:1203–18.
[16] Hsiao KK, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, et al. Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. Science 1996;274:99–102.
[17] King DL, Arendash GW, Crawford F, Sterk T, Menendez J, Mullan MJ. Progressive and gender-dependent cognitive impairment in the APPsw transgenic mouse model of Alzheimer's disease. Behav Brain Res 1999;103:145–62.
[18] Kobayashi DT, Chen KS. Behavioral phenotypes of amyloid-based genetically modified mouse models of Alzheimer's disease. Genes Brain Behav 2005;3:173–96.
[19] Linder CC. The influence of genetic background on spontaneous and genetically engineered mouse models of complex diseases. Lab Anim 2001;30:34–9.
[20] Linder CC. Genetic variables that influence phenotype. ILAR J 2006;47:132–40.
[21] Lipp HP, Wolfer DP. Genetic background problems in the analysis of cognitive and neuronal changes in genetically modified mice. Clin Neurosci Res 2003;3:223–31.
[22] Lloret A, Dragileva E, Teed A, Espinola J, Fossale E, Tammy Gillis T, et al. Genetic background modifies nuclear mutant huntingtin accumulation and HD CAG repeat instability in Huntington's disease knock-in mice. Hum Mol Gen 2006;15:2015–24.
[23] Margara F, Müller U, Li Z-W, Lipp HP, Weissmann C, Stagljar M, et al. Genetic background changes the pattern of forebrain commissure defects in transgenic mice underexpressing the beta-amyloid-precursor protein. PNAS 1999;96:4656–61.
[24] Middei S, Daniele S, Caprioli A, Ghirardi O, Ammassari-Teule M. Progressive cognitive decline in a transgenic mouse model of Alzheimer's disease overexpressing mutant hAPPswe. Genes Brain Behav 2006;5:249–56.
[25] Müller U. Ten years of genetic targeting: targeted mouse mutant from vectors design to phenotype analysis. Mech Dev 1990;82:3–21.
[26] Nguyen PV, Abel T, Kandel ER, Bourtchouladze R. Strain-dependent differences in LTP and hippocampus-dependent memory in inbred mice. Learn Mem 2000;7:170–9.
[27] Nguyen PV, Gerlai R. Behavioural and physiological characterization of inbred mouse strains: prospects for elucidating the molecular mechanisms of mammalian learning and memory. Genes Brain Behav 2002;1:72–81.
[28] Ognibene E, Middei S, Daniele S, Adriani W, Ghirardi O, Caprioli A, et al. Aspects of spatial memory and behavioral disinhibition in Tg2576 transgenic mice as a model of Alzheimer's disease. Behav Brain Res 2005;156:225–32.
[29] Schalkwyk LC, Fernandes C, Nash MW, Kurrikoff K, Vasar E, Kõks S. Interpretation of knockout experiments: the congenic footprint. Genes Brain Behav 2007;6:299–303.
[30] Shoemaker AR, Moser AR, Midgley CA, Clipton L, Newton MA, Dove WF. A resistant genetic background leading to incomplete penetrance of intestinal neoplasia and reduced loss of heterozygosity in Apc^{Min}/+ mice. PNAS 1998;95:10826–31.
[31] Threadgill DW, Yee D, Matin A, Nadeau JH. Magnusson T Genealogy of the 129 inbred strains: 129/SvJ is a contamined inbred strain. Mam Gen 1997;8:390–3.
[32] Van Dam D, De Deyn PP. Drug discovery in dementia: the role of rodent models. Nat Rev 2006;5:956–70.
[33] Wilkinson L. SYSTAT: The System for Statistics. Evanston, IL: Systat Inc; 1990.
[34] Wolfer DP, Lipp HP. Dissecting the behavior of transgenic mice: is it the mutation, the genetic background or the environment? Exp Physiol 2000;85:627–34.
[35] Wolfer DP, Crusio W, Lipp HP. Knockout mice: simple solutions to the problems of genetic background and flanking genes. TINS 2000;7:336–40.
[36] Yoshiki A, Morikawi K. Mouse phenome research: implications of genetic background. ILAR J 2006;2:94–102.
[37] Zhou F, He X, Iwakura Y, Horai R, Stuart JM. Arthritis in mice that are deficient in interleukin-1 receptor antagonist is dependent on genetic background. Arthritis Rheum 2005;52:3731–8.