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Neurobehavioral development of two mouse lines commonly used in transgenic studies

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Abstract

The present study was aimed at establishing the differences in the neurodevelopmental profile between two F2 lines derived from two F1 hybrid mouse strains (129 × C57BL/6 and C57BL/6 × SJL). The choice of the given strains was based on the frequent use of these mice in transgenic research. For the neurodevelopment phenotyping, we employed a test battery consisting of 23 somatometric, sensorial and motor tests. Significant variations between the strains were established in different functional domains. Some specific delays in the appearance of developmental landmarks were observed in F2 mice derived from crosses of F1 C57BL/6 × 129, whereas they acquired early developmental functions, such as the righting reflex, sooner than C57BL/6 × SJL-derived mice. C57BL/6 × 129 F2 offspring were spontaneously hypoactive, and their poorer motor performance was confirmed by low performance in the negative geotaxis test. However, there were no differences in the general psychomotor development as shown by the good performance in the homing test in both F2 lines. Both strains were susceptible to the handling procedures used, presenting a similar alteration in the response observed in the homing test as compared to nonhandled control mice. In conclusion, our work highlights the importance of the genetic background for transgenesis experiments and also the need for well-established testing protocols to obtain sufficient information at the first stage of behavioral screening of genetically modified mice.

Keywords: Neurodevelopment; Genetic background; Genetically modified mice

1. Introduction

The use of transgenic techniques to model human disease has led to major advances in our understanding of pathogenic mechanisms, but has also highlighted the limitations of conventional transgenic methodology for the production of accurate animal models and the difficulties associated with modeling human pathophysiology in mice. Important issues are the use of the same genetic background (Gerlai, 1996), as well as a good phenotypic characterization, based on standardized protocols (Crawley and Paylor, 1997; Crawley et al., 1997; Rogers et al., 1997) of the mouse strain used, since the impact of genetic manipulation might be influenced by this factor. However, it is tempting to compare the effects of genetic manipulations disregarding genetic background. Behavioral development is a continuous process starting during prenatal life and maturing after birth, and represents an important domain of phenotypic changes in genetically modified mice (Roubertoux et al., 1996). Adult phenotype can in fact be influenced by the deficit in one or more functional domains during neurodevelopment.

Many of the neurodevelopmental screening studies have been performed in inbred strains (Roubertoux et al., 1996; Le Roy et al., 1999; Anokhin et al., 1999). However, the analysis of transgenic and *knockout* mice is usually performed on an F1 hybrid genetic background or in F2 and F3 from C57BL6 × 129Sv crosses, which have also been shown to provide a suitable genetic background. Hybrid mice are produced by crossing mice of two different inbred strains. F1 hybrids are similar to inbred strains in that they are genetically and phenotypically uniform. The F2 generation, produced by matings of F1 × F1 mice, shows assortment

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between all the different loci of the parents. Advantages of using F1 hybrid mice include genetic and phenotypic uniformity and hybrid vigor. F2 offspring are often used as physiological controls for *knockout* mice that are maintained on a mixed C57Bl/6 × 129 (formerly B6129F1) background. A similar schedule is used for the generation of transgenic mice that are frequently maintained on a C57Bl/6 × SJL (B6SJLF1) background. Like the targeted mice, the genetic background composition of F2 mice varies among littermates because of gene segregation from the F1 hybrid parents. Although these F2 mice only provide an approximate genetic match to the B6129F1 background, they do contain exclusively genes derived from C57BL/6 or 129 genetic backgrounds. These strains do not differ in their sensitivity to pharmacological agents, but C57BL/6 mice differ substantially from the 129 strain on almost all measures of locomotor behavior (Paulus et al., 1999). On the other hand, 129 mice are more anxious on the elevated plus maze and open-field activity assays (Homanics et al., 1999; Paulus et al., 1999; Tarantino et al., 2000). Differences among strains and F1 hybrid lines derived from them have also been highlighted in different studies (Logue et al., 1997; Voikar et al., 2001), but neurobehavioral development has not been specifically and systematically explored in F1 hybrid mice or in their outcrosses producing F2 and F3 offspring, currently used in transgenesis experiments.

Neonatal handling is known to induce long-lasting changes in behavioral, neuroendocrine and neuromorphological features in rodents, and can also affect neurodevelopmental parameters (Mohammed et al., 1993; d'Amore et al., 1995; Baamonde et al., 1999). However, although there is a series of experiments performed on genetically selected rat lines (reviewed by Fernández-Teruel et al., 1997), at present there is no available information on the influence of genetic background on the positive or negative effect of environmental manipulation in mice.

Our goal was, therefore, to explore possible strain-related differences during the preweaning period in neurobehavioral development, and also to investigate the influence of postnatal handling on the F2 generation resulting from crosses of B6129F1 and B6SJLF1 hybrid mice. The selection of these strains was due to their frequent use to generate genetically modified mice.

2. Materials and method

2.1. Animals

C57BL/6 × 129Sv (B6129F1) and C57BL/6 × SJL (B6SJLF1) hybrid mice weighing 25–27 g were used. Mice from parental strains were obtained from CRIFFA (Barcelona, Spain). Animals were reared in the IRO animal facility during 2 months before the beginning of the experiments. Mice were maintained under standard rearing conditions of temperature (21 ± 1 °C) with a photoperiod of 12:12 h (lights on at 0730 h) and dust-free sawdust. Food pellets and water were available ad libitum. Breeding pairs were formed, and females visibly close to parturition were isolated. Litters were culled to no larger than eight pups. To avoid the effects of parity on behavioral ontogeny (Crusio and Schmitt, 1996) the first litter was discarded and the second litter was used for the experiment. Testing involved approximately equal numbers of female and male offspring from matings of F1 × F1 crosses. Litters from each genetic background were distributed between two separate experimental groups, one of them being submitted to the different experimental manipulations (handled group) and the second remaining undisturbed during the whole preweaning period. The latter group was only submitted to the homing test at postnatal day (PD) 14.

2.2. General procedure for postnatal observations

All the pregnant dams were allowed to deliver spontaneously. The day of delivery was designated as PD1 of age of the neonates (erroneous estimates on time of birth = ± 6 h). On delivery, the litter size of each dam was recorded and each pup was checked for gross abnormalities. The pups were individually marked with India ink on PD1 and were nursed by their natural dams until weaning. During the testing protocol whole litters were separated from the dams and maintained for 30 min in a warmed environment. To control for handling effects animals were separated into two different groups and we compared pups handled from PD1 to PD10 and pups handled from PD10 to PD21. Males and females were pooled to perform the neurodevelopmental screening, based on preliminary experiments demonstrating no significant sex effect on the measures. The ranges of ages at which responses were observed were defined from preliminary experiments so that the period of observation could be defined to reduce handling. All the measures were performed between 0730 a.m. and 1200 a.m. All experimental procedures were approved by the Animal Care Committee of the Institut Recerca Oncològica.

2.3. Assessment of body growth

The pups were weighed daily starting on PD1 to the nearest 0.01 g on a balance with automated compensation of movements during weighing. The length of the body from the tip of the nose to the base of the tail and the length of the tail were recorded daily.

2.4. Developmental landmarks

Developmental screening employed 118 animals. The unit of analysis was the day of attainment of the criterion for each landmark. A brief description of each measure follows:

1. *Pinna detachment*. Beginning on PD2, pups were inspected daily for the complete separation of the pinna

from the cranium. Prior to detachment, the distal portion of the pinnae folded over the auditory meatus and detachment was defined as the pinna being raised to a position of less than 90° of the final position.

2. *Incisor eruption*. Beginning on PD7, pups were inspected daily for the emergence of both lower and upper incisor from the gingiva.

3. *Eye opening*. Beginning on PD9, pups were inspected daily for the complete opening of both eyelids.

4. *Permeation of ear conduct*. Pups were inspected for permeation of the auditory conduct.

2.5. Neurobehavioral development

The neurobehavioral test used 35 males and females from eight different litters for each experimental condition. The test included sensorial and motor responses (Fox, 1965), with some modifications. These reflexologic and behavioral tests reflect the maturation of the central nervous system, are reactive to environmental and toxic conditions and their reliability is high.

1. *Surface righting response*. The pup was placed on its back and the latency to turn over to rest in the prone position with all four feet on the floor was recorded. The response was scored considering if the four paws were on the floor (3), if one or more paws remained beneath the body (2), if there were vigorous, but no efficacious attempts to right (1) and if there was no response (0). The day of positive response was recorded, and the response was considered positive when the animal reached a score of 3.

2. *Forepaw/hindpaw grasping*. It was considered positive when the pup flexed the paw to grasp an object that was gently stroking it. The day of appearance of the reflex was recorded.

3. *Cliff drop aversion*. The pup was placed on the edge of a cliff, the forepaws and the head over the edge. The response was positive if it turned and crawled away from the cliff.

4. *Forelimb/hindlimb placing*. When the dorsum of the paw or foot came in contact with the edge of an object, the hand or foot lifted and was placed on the object.

5. *Disappearance of rooting response*. After bilateral stimulation of the face, the pup crawled forwards, pushing the head in a rooting fashion.

6. *Disappearance of crossed extensor*. When pinched, the stimulated limb flexed while the opposite limb extended.

7. *Negative geotaxis test*. The pup was placed head downward on a 45° incline and the latency to turn 180° was recorded. In this test, a score of 0 was given in the absence of a turning response, (1) incomplete response, the pup turning about 90° but then freezes and, (2) complete response.

8. *Vibrissae placing*. The pup was suspended by the tail and lowered toward the tip of a cotton Q-Tip. At contact of the cotton with the vibrissae, the pup raised its head and performed a placing response.

9. *Tactile orientation*. The test assessed the head turning (orienting) response triggered by the application to one side of the perioral area of a cotton Q-Tip.

10. *Vertical climbing*. The pup was placed on a wire-mesh grid that was rotated to a vertical position. The angle at which it fell and the latency to climb in the vertical position of the grid were recorded.

11. *Preyer reflex/startle response*. We used a "custom-built clickbox," which generates a 20-kHz sound burst at an intensity of 90 dB SPL when held 30 cm directly above the mouse. The response of the pup as Preyer reflex, consisting of a moderately brisk flick of the pinna or startle response, was recorded.

12. *Suspension test*. The pups were hung on a wooden bar (4-mm diameter) by the hind legs and the latency to fall was measured. The traction capacity was also scored as (0) active grip with hind legs, (1) difficulty to grasp with hind legs, (2) unable to grasp with hind legs, (3) unable to lift hind legs.

13. *Visual placing response*. The day after eye opening, the pup was suspended by the tail and lowered towards the tip of a pencil without the vibrissae touching it. The response was positive if it extended the paws to touch it.

14. *Blast response*. Exaggerated jumping or running behavior in response to a gentle puff of air.

15. *Reaching response*. The animal is held by the tail above a flat surface and it is noted if the forepaws are stretched out to make contact with the surface.

2.6. Neuromotor development

On PD7, 10 and 14 the neuromotor development was assessed by means of the pivoting and walking tests.

1. *Pivoting locomotion*. The total number of degrees turned by the pup during a 60-s period was recorded. The test was performed on a flat surface covered with a green paper on which lines had been drawn to delineate four 90° quadrants. The number of degrees was scored only in completed 90° segments.

2. *Walking test*. The latency for a mouse to lift up on all four legs and walk a distance exceeding its body length was measured on a flat surface covered with a green paper.

2.7. Homing test

On PD14 individual pups were transferred to a cage containing 3/4 of new sawdust and 1/4 sawdust of the home litter ('goal arena'). The pups were placed at the opposite side of the goal arena, near to the wall. The time taken to reach the home litter sawdust was recorded (cut-off time 180 s).

2.8. Data analysis

Significance of the effects was assessed by a one-way ANOVA or multivariate analysis of variance (MANOVA)

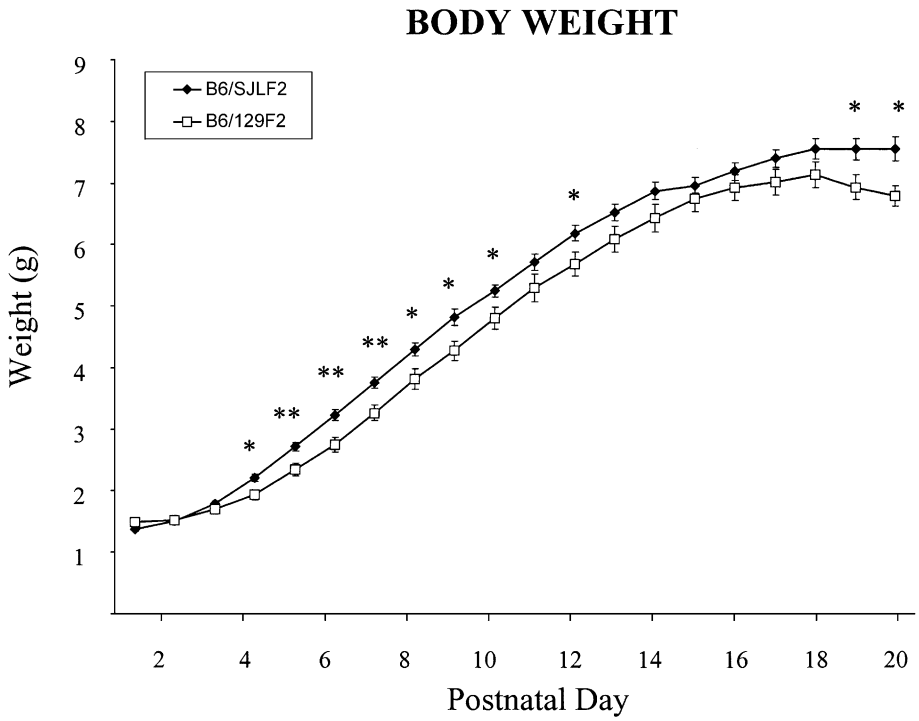

Fig. 1. Body weight curves from birth to weaning of the offspring from matings of B6129 F1 × F1 ($n = 20$) and B6SJL F1 × F1 ($n = 22$) crosses. Data are given as means ± S.E.M. * $P < .05$; ** $P < .01$.

with Bonferroni test for post hoc analyses. Student t test was used for comparisons between two groups. Repeated measures ANOVA was used for pairwise comparisons of two groups. Nonparametric data were analyzed with the chi-square test. Analysis was processed by using the SPSS program.

3. Results

3.1. Somatometry

The somatometric development was similar between F2 mice derived from B6129 and from B6SJL, as demonstrated

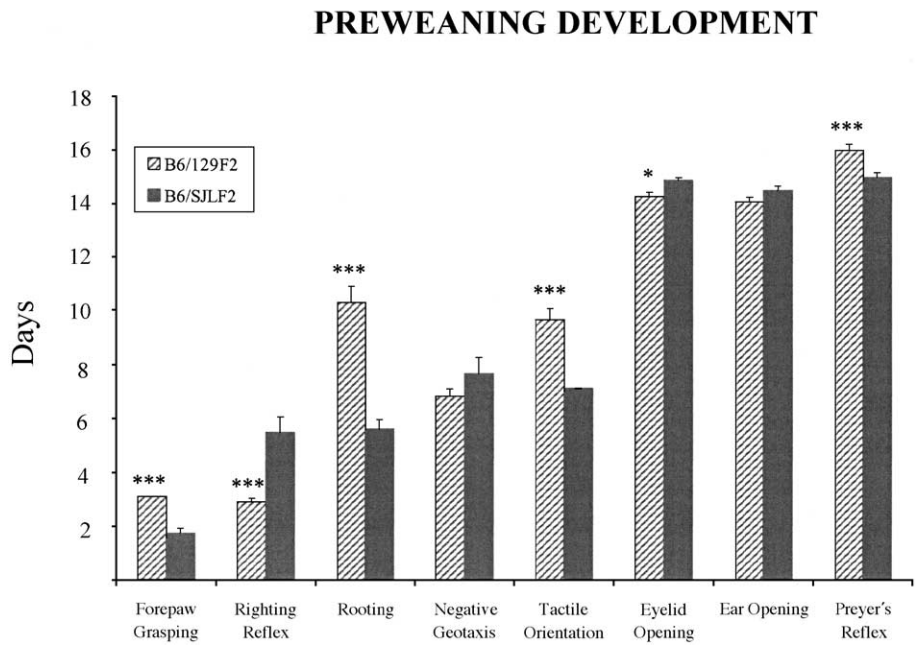

Fig. 2. Postnatal neurobehavioral development in the B6129F2 ($n = 20$) and B6SJLF2 ($n = 15$) crosses. Data are given as means ± S.E.M. * $P < .05$; *** $P < .001$; asterisks indicate significant between-group differences. Some of the tests lacking significant differences are not shown.

by the parallel increase in body growth (weight curve), although from PD4 to PD10, there were significant differences between groups (Fig. 1), showing in B6129-derived pups a lower body weight. The number of pups per litter was culled to eight individuals, and no differences in the milk supply were observed by visual inspection of the abdomen of the pups. At PD18 again B6129-derived pups presented a significant reduction in growth, ($F(1,12) = 285$; $P < .001$, repeated measures ANOVA).

3.2. Reflexologic and neurobehavioral analysis

Reflexological tests and developmental landmarks are presented in Fig. 2. Forepaw grasping response appeared later in B6129 derived-pups (3.10 ± 0.001 in B6129F2 vs. 1.73 ± 0.02 in B6SJLF2; $t = 6.44$, $P < .001$), whereas forelimb placing did not differ between strains. Righting response emerged sooner in B6129F2 pups (0.84 ± 0.01 vs. 2.24 0.57; $t = 4.97$, $P < .001$). Rooting response disappeared later in the B6129F2 pups (10.31 ± 0.59 vs. 5.6 ± 0.35; $t = 6.84$, $P < .001$) and tactile orientation reflex also emerged later in this strain (9.65 ± 0.43 vs. 7.13 ± 0.002; $t = 4.97$, $P < .001$). Regarding the emergence of developmental landmarks, eyelid opening took place earlier in B6SJLF2, but the differences, although significant, were small (14.3 ± 0.15 vs. 15.0 ± 0.001; $t = 2.52$, $P < .05$). On the contrary, no differences were observed in incisor eruption and permeation of the auditive conduct between groups. However, the functional measure associated with ear opening, the Preyer's reflex, was significantly delayed in B6129F2 pups (16.0 ± 1.0 vs. 14.86 ± 0.17; $t = 3.18$, $P < .01$). No significant differences were observed in the other reflexes studied.

3.3. Neuromotor development

On PD7, 10 and 14, the neuromotor development was assessed by the pivoting and the walking tests. The pivoting locomotion task showed a clear hypoactive behavior in

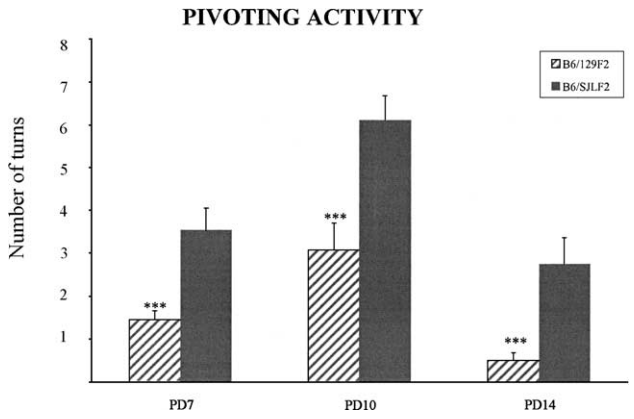

Fig. 3. Pivoting locomotion task. Number of pivoting in B6129F2 ($n = 26$) and B6SJLF2 ($n = 24$) crosses on PD7, 10 and 14. Data are given as means ± S.E.M. *** $P < .001$; asterisks indicate significant between-group differences.

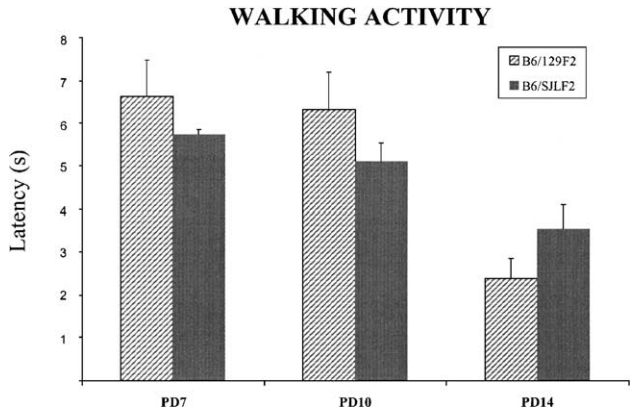

Fig. 4. Walking activity test. Latency to initiate walking (seconds) in B6129F2 ($n = 25$) and B6SJLF2 ($n = 15$) crosses on PD7, 10 and 14. Data are given as means ± S.E.M.

B6129F2- as compared to B6SJLF2-derived mice (Fig. 3), most important at PD14, where walking activity should be mature. A nonsignificant tendency for a delay was concomitantly found in walking activity (Fig. 4), as demonstrated by the differences in the percentage of reduction of the latency to walk shown by B6129F2 mice (PD7 vs. PD10, 4.61% of reduction, PD10 vs. PD14, 62.31% of reduction) compared to offspring from B6SJL F1 × F1 crosses (PD7 vs. PD10, 11.14% of reduction, PD10 vs. PD14, 33.55% of reduction).

3.4. Homing test

The homing of the pups to their nest did not differ significantly between both crosses as reflected by the latency to reach the familiar sawdust that reached similar values in B6129F2 and in B6SJLF2 mice (Fig. 5).

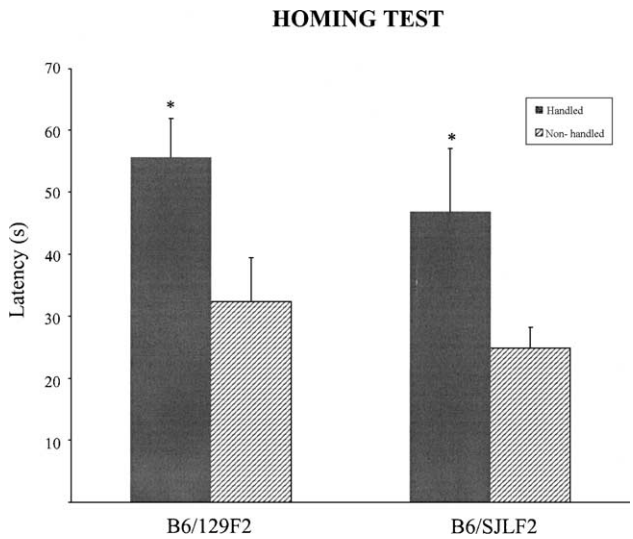

Fig. 5. Homing test. Latency to reach the home litter sawdust (seconds) in the handled and nonhandled B6129F2 ($n = 19$) and B6SJLF2 ($n = 15$) crosses on PD14. Data are given as means ± S.E.M.

3.5. Handling effects

Testing for handling effects showed no significant differences between the PD1–10 handled group and the PD10–19 handled pups in the F2 from B6129 F1 × F1 crosses and B6SJL F1 × F1 crosses in any behavioral or somatometric measures. However, handling produced significant effects in the performance of the homing test between handled and nonhandled groups of B6129- and B6SJL-derived pups ($t = 2.257$, $P < .05$ and $t = 2.061$, $P < .05$, respectively). In this general psychomotor development test, nonhandled animals showed a better performance that was significantly different from their respective, genetically similar and handled counterparts (Fig. 5).

4. Discussion

There is a tendency in the literature to compare the effects of silencing and/or overexpressing a particular gene, disregarding the influences of genetic background. Many behavioral analyses to discard the effects of genetic background have been conducted on inbred strains because of the more homogeneous genetic background, but comparisons in genetically modified mice are usually performed on F1 hybrid mice or on F2 and F3 generations resulting from 129/Sv × C57BL/6 crosses.

We compared the neurobehavioral development of the F2 generations resulting from crosses of two hybrid strains frequently used in transgenesis experiments, B6129 and B6SJL, and analyzed the effects of postnatal handling on the homing test in both. There are few reports on potential differences in neurodevelopmental aspects of mice from inbred strains. Comparisons between C57BL/6 and 129Sv inbred strains have revealed heterocrony in the development of a number of specific functional systems (Anokhin et al., 1999). However, no data exist regarding neurodevelopment of F2 offspring derived from both lines, although studies of genetically modified murine models are usually performed on this segregating genetic background. Our experiments revealed that F2 from B6129 hybrid mice presented a general delay in neurobehavioral development as compared with the F2 generation from B6SJLF1 crosses, with this retardation particularly involving more measures that reflect the maturation of specific functional domains.

Regarding somatic growth, there was a subtle reduction in the somatic development of B6129F2 pups, as shown by a reduction in body weight, but the differences only reached statistical significance during a restricted period of development. Comparison between B6129 and B6SJLF2 mice revealed a shift to the right of the body weight curve for B6129. B6129F2 mice presented a persistence of archaic responses, as revealed by the longer persistence of the rooting response. However, the appearance of developmental landmarks such as eye opening was slightly retarded in the B6SJLF2 mice. The persistence of archaic reflexes and the appearance of developmental landmarks are used as indications of the general maturation of the nervous system. Regarding the neurobehavioral data, the only parameter that presented a significantly earlier appearance in B6129 F2 mice was the righting response. This response requires the integrity of muscular and motor function, but is also dependent on the adequate acquisition of symmetrical coordination between the left and right sides of the body. It has been demonstrated that, generally, the parental 129Sv strain presents a faster maturation of early neonatal responses, such as the righting test, whereas C57BL/6 mice are more efficient in tasks performed at later stages of development (Anokhin et al., 1999). On the other hand, retardation in forepaw grasping, a measure that reflects the development of fine motricity without alterations in hindlimb placing, was observed in mice bearing the 129 genetic background, as well as retardation in the tactile orientation test and the appearance of the Preyer's reflex, which indicate tactile and auditory sensitivity.

Measures reflecting cranio-caudal maturation such as the latency to initiate walking, or other measures also dependent on colliculi maturation such as negative geotaxis, were not significantly different in both crosses. Regarding the neuromotor development, there were no significant differences between strains in the latency to walk at PD7, PD10 or PD14. B6129F2 mice presented a significant hypoactivity in the pivoting test that was maintained during the entire testing period. The 129 strain has been widely recognized to be hypoactive with respect to other strains such as C57BL/6 or FVB (Voikar et al., 2001; Paulus et al., 1999; Balogh et al., 1999) and to isogenic strains such as their outcrossed F1 offspring (Voikar et al., 2001). Locomotor activity has been used as a critical assay to establish the phenotypes for various genetic manipulations of mice. It is arguable that a delay in maturation of locomotor activity and the hypoactivity observed in the pivoting test might also influence adult locomotor behavior.

Although delays in different motor and sensory tests existed for the B6129 cross, this retardation did not result in a corresponding delay in a comprehensive psychomotor development test, the homing test. The homing test did reveal the negative effects of 2 weeks of handling on psychomotor development, but this affected both lines equally, as a similar increase in the latency to reach the goal arena was observed in both handled groups versus their nonhandled counterparts. These results might seem to contradict previous reports describing the positive effects of handling on neurodevelopment (Mohammed et al., 1993; d'Amore et al., 1995). It has been suggested, however, that handling reduces emotionality so it may be that the 'less fearful', handled mice in the present study were less motivated to escape to the home litter area than their 'more fearful', nonhandled counterparts were.

Our results demonstrate differences in neurobehavioral development between two F2 lines derived from two F1 hybrid mouse strains (129 × C57BL/6 and C57BL/6J ×

SJL), frequently used for the generation of transgenic murine models. Some specific delays were found in B6129F2, whereas they acquired early developmental functions, such as the righting reflex, sooner than B6SJLF2 mice. B6129F2 mice were spontaneously hypoactive but there were no differences in the general psychomotor development as shown by the good performance in the homing test in both F2 lines. Both strains were susceptible to the handling procedures used, presenting a similar retardation in the homing test as compared to nonhandled control mice. In conclusion, our work sheds light on the possible mechanism by which the phenotypic impact of a mutation can be influenced by the genetic background and suggests that the comparison between B6129F2 and B6SJLF2 might be feasible for some neurodevelopmental aspects.

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