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Impaired Motor Learning Performance in Cerebellar En-2 Mutant Mice

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Mice homozygous for a null mutation in their En-2 gene exhibit cerebellar neuroanatomical alterations including absence and misplacements of specific fissures and size reduction. The present study investigated cerebellar function by comparing the behavior of age-matched homozygous and heterozygous En-2 mutant and wild-type mice. Motor function of the mutants was found normal in several situations. Habituation to novelty in the open field was not significantly different in mutants. However, in a motor learning paradigm, the rotating rod, the performance of homozygous mutant mice improved significantly less than that of the heterozygous mice which were also significantly impaired compared to wild-type mice. Unlike other cerebellar mutants in which severe motor or sensory defects are obvious, the En-2 mouse model offers a unique tool to study the role of cerebellum in complex behavioral phenomena, including motor learning, without confounding effects.

Behavioral geneticists have been trying to understand the genetic bases of brain and behavior for a long time. The advent of recent transgenic methods has allowed investigators to study the effects of single genes (Smithies, 1993). Gene targeting makes it possible to produce single gene loss-of-function (null) mutations, thus opening up the possibility to dissect the genetic mechanisms underlying complex behavioral or neural phenotypes. In this article, we analyze the behavioral effects of a null mutation that disrupts the homeobox-containing gene, En-2, in the mouse and leads to altered cerebellar neuroanatomy.

Members of the *engrailed* (En) gene family encode evolutionarily highly conserved transcription factors that contain five similar protein domains including the homeodomain (Joyner, Kornberg, Coleman, Cox, & Martin, 1985; Logan et al., 1992). In Drosophila, the En gene functions in embryonic segmentation and later development of the nervous system (Garcia-Bellido & Santamaria, 1972; Kornberg, 1981; Lawrence & Johnson, 1984; Lawrence & Morata, 1976; Lawrence & Struhl, 1982). The murine engrailed genes, En-1 and En-2, are coexpressed in cells spanning the junction between mid- and hind-brain from the 8 somite stage (Davidson, Graham, Sime, & Hill, 1988; Davis & Joyner, 1988; Davis, Noble-Topham, Rossant, & Joyner, 1988; Njolstad & Fjose, 1988; Patel et al., 1989). In the adult mouse, En-1 and En-2 expression becomes limited to specific neuronal groups including motor nuclei in the pons region and cells in substantia nigra (Davis, et al., 1988; Davis & Joyner, 1988). In the cerebellum, En-2 alone is expressed in the granule and molecular cell layers (Joyner, Herrup, Auerbach, Davis, & Rossant, 1991).

To examine En-2 gene function, targeted mutations were made in the En-2 locus by homologous recombination in mouse embryonic stem cells (Joyner, Skarnes, & Rossant, 1989; Joyner et al., 1991; Millen, Wurst, Herrup, & Joyner, 1994). Mice homozygous for the En-2 mutations are viable and fertile. However, consistent with the cerebellum specific En-2 expression pattern during embryogenesis and adulthood, mutant mice homozygous for the disrupted En-2 gene exhibited a complex set of neuroanatomical alterations in their cerebellum (Joyner et al., 1991; Millen et al., 1994). The overall size of the cerebellum is reduced. Furthermore, abnormal folding pattern in the posterior vermis and the paraflocculus and fusion of folds in the hemispheres resulting in 3 instead of 4 lobules can be observed. The foliation defects became apparent shortly after birth whereas the size reduction could be observed as early as 15 days of embryogenesis and the mutant cerebellar primordia did not fuse at the midline until 17 days of embryogenesis. In addition, at embryonic stages, the colliculi were reduced in size.

The cerebellum plays a crucial role in coordinating and modulating the action of body muscles and makes the exhibi-

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tion of smooth and skilled movements possible (Altman, 1982; Lalonde & Botez, 1990). In behavioral neurology two major types of cerebellar symptoms can be distinguished: midline symptoms and lateral symptoms (Gilman, Bloedel, & Lechtenberg, 1981). Disruption of midline structures including the vermis, the floccunodular lobe, and the fastigial nucleus leads to abnormal motor and posture patterns. On the other hand, depending on the degree of abnormality, dysfunction of the lateral cerebellar zone, consisting of the cerebellar hemispheres and the interpositus and dentate nuclei can result in ataxia and tremor and an inability to alter fine repetitive movements. Animal behavioral studies have supported these findings and also confirmed that the cerebellum is involved not only in motor coordination per se but also in more complex behavioral phenomena, such as motor learning (for review see Lalonde & Botez, 1990). The cerebellum has also been shown to play some role in habituation (Leaton & Supple, 1991; Leaton, Cassella, & Borszcz, 1985; and also see Lalonde & Botez, 1990) and exploratory behavior (Lalonde, Botez, & Boivin, 1986).

Although En-2^{hd} mutant mice were reported not to exhibit any gross behavioral defects (Joyner et al., 1991), based on the En-2 expression pattern and on the observed cerebellar neuroanatomical alterations, one could predict the presence of some behavioral abnormalities associated with cerebellar dysfunction. In this article, our aim was to investigate such possible behavioral alterations. We analyzed the motor and posture patterns, coordination and balance, motor performance, muscle strength, habituation, and motor learning performance of homozygous mutant, heterozygous mutant, and age-matched wild-type mice. We show that mice homozygous for the En-2^{hd} mutation were significantly impaired in a motor learning paradigm compared to heterozygous mice, whose performance was also significantly below that of the wild-type mice. This finding is notable because neither the homozygous nor the heterozygous mice exhibited any detectable abnormality in their spontaneous motor or posture patterns or in other tests of motor function nor did they show muscle weakness. These results suggest that the En-2 null mutant mice will be a valuable tool with which one may study the role of cerebellum in more complex behavioral phenomena including motor learning without the confounding effects of motor or sensory defects that are usually seen in cerebellar mouse mutants or in mice of cerebellar lesion and ablation studies. Furthermore, our results showing impairment in the heterozygotes, in which no cerebellar neuroanatomy alterations are observable, suggest that En-2 may have a function, yet to be identified, not only in altering cerebellar neuroanatomy but also in other mechanisms undetected at the gross structural level. Finally, our article shows that the gene targeting approach can be highly successful in investigating the role of particular genes in brain function and behavior.

Method

Animals and Housing

Generation and maintenance of inbred 129/Sv En-2^{hd} mutant animals has been described elsewhere (Joyner et al., 1989; 1991; Millen et al., 1994). Heterozygous offspring were sibmated in order to produce homozygous mutant (En/En), heterozygous mutant (En/+) and wild-type (+/+) age-matched littermate mice. Age-matched mice from (En/+) × (En/+) or (En/+) × (En/En) crossings as well as (+/+) 129/Sv control were used in the experiments. All mice were bred and housed in the same room under identical conditions. Males and females were kept separately in groups of 3 to 4 in plastic cages (25 × 18 × 12 cm) on sawdust bedding at a temperature of 20 ± 1 °C and a relative humidity of 45%. Food and water were available ad libitum A 12-hr light–dark cycle was maintained with lights on at 7 a.m. and off at 7 p.m.

Apparatus and Testing Procedure

In all behavioral experiments the mice were tested singly between 10 a.m. to 5 p.m. The testing sequence of mice was randomized across genotypes and sexes. The genotypes were unknown to the observer at the time of behavioral recording. The following pieces of apparatus were used: open field, bar cross (Gerlai et al., 1993; Li et al., 1994), rotating rod (Pellegrino & Altman, 1979), bar hang, and tilting plane test (see e.g., Phillips & Crabbe, 1991). In the first set of experiments the same group of 12-week-old mice (for sample sizes see Table 1) was tested once first in the open field for 5 min and subsequently in the bar cross test for 3 min. In the second set of experiments a new group of 10-week-old mice (for sample sizes see Figure 1) were trained and tested on the rotating rod during a period of 8 days. The same group of mice was subsequently tested for habituation in the open field during a 6-min session and then on the bar hang apparatus. These mice were also tested for muscle strength more directly by measuring the weight they could pull. A separate group of mice (heterozygous and homozygous mutants) were compared in the tilting plane test.

The open-field apparatus, which evokes novelty-induced exploratory behavior (Crusio, Schwegler, & van Abeelen, 1989), was a plastic box (46 × 25 × 15 cm) whose bottom was covered with a sheet of paper with a 5 cm^2 grid pattern. In this situation and in the bar crossing test we recorded motor and posture patterns with a computer event recorder program developed by Gerlai (Gerlai & Hogan, 1992). This recording method is based on one of the fundamental tenets of ethology, which assumes that the apparently continuous stream of behavior can be broken down into mutually exclusive, distinct, successive motor-posture patterns that represent species specific units of behavior (Huntingford, 1984). The detailed definitions of the behavioral units of the mouse ethogram have been given elsewhere (Crusio & van Abeelen, 1986; Gerlai et al., 1993; van Abeelen, 1963). Briefly, the following behaviors were recorded and computed for cumulative frequency (number of times per test period) or relative duration (duration as a percentage of the test period): *leaning* (frequency), leaning against the wall with one or both forepaws while standing on the hindlegs; *long body* (duration), extending the forepaws and the frontal part of the body while anchoring the hindlegs, resulting in an elongated body shape; *defecation* (frequency), depositing fecal pellets; *urination* (frequency), urinating; *grooming* (duration), stereotypical face cleaning and fur licking movements; *locomotion score*, number of squares crossed on the floor grid (a cross was counted when the mouse entered a new square with both of its forepaws); *passivity* (duration), remaining motionless; *rearing* (frequency), standing upright on the hindlegs.

The bar cross apparatus was an elevated U-shaped platform with two wider (18 mm thick) bars and a connecting narrow (1 mm thick) challenge bar. In this situation finer motor coordination was tested for 3 min by recording the following spontaneously appearing motor-posture patterns: *sniff up* (frequency), pointing the nose upward with typical whisker movements; *sniff down* (frequency), holding the head below the level of the platform with the nose pointing downward with typical whisker movements; *turning* (frequency), turning 180 degrees on the wide bar; *slipping* (frequency), a sequence of events in which

Table 1
Motor and Posture Patterns in Wild-Type Mice and in Mice Heterozygous and Homozygous for the En-2^{hd} Mutation Measured in Open-Field and Bar-Crossing Tests

	Mice							
	Wild type (+/+; n = 9)		Heterozygous (En/+; n = 14)		Homozygous (En/En; n = 9)		ANOVA	
Test	M	SD	M	SD	M	SD	F	p
Open field								
Leaning$_d$	0.44	± 0.18	2.79	± 0.84	3.33	± 1.33	2.46	>.1
Long body$_d$	8.73	± 2.20	4.06	± 0.76	7.48	± 1.84	2.84	>.05
Defecation$_f$	1.11	± 0.31	2.36	± 0.37	3.00	± 0.47	**5.06**	**<.05**
Urination$_f$	0.11	± 0.11	0.21	± 0.11	0.22	± 0.15	0.22	>.8
Grooming$_d$	4.10	± 1.56	3.74	± 0.82	4.73	± 1.74	0.15	>.8
Locomotion$_s$	52.11	± 10.95	71.00	± 11.48	49.11	± 15.33	0.97	>.4
Passivity$_d$	65.66	± 4.82	51.78	± 5.45	60.93	± 7.49	1.49	>.3
Rearing$_f$	0.22	± 0.22	1.14	± 0.92	0.11	± 0.11	0.69	>.5
Bar cross								
Sniff up$_f$	2.22	± 0.76	1.79	± 0.50	3.33	± 0.99	1.22	>.3
Sniff down$_f$	10.78	± 2.48	13.43	± 1.34	13.89	± 2.81	0.58	>.5
Turning$_f$	0.22	± 0.22	0.43	± 0.25	0.78	± 0.43	0.73	>.4
Slipping$_f$	0.00	± 0.00	0.50	± 0.25	0.56	± 0.29	1.45	>.2
Locomotion$_d$	11.54	± 3.19	10.20	± 1.78	12.80	± 2.65	0.30	>.7
Passivity$_d$	61.74	± 9.62	59.44	± 4.54	54.90	± 8.64	0.20	>.8
Crossing attempt$_f$	2.22	± 0.88	2.71	± 1.05	2.44	± 1.26	0.05	>.9
Forced cross$_d$	10.33	± 2.28	23.57	± 5.55	14.14	± 2.09	2.48	>.1

Note. Significant genotype effect was found only in one parameter, defecation (boldface). Subscript d = duration relative to recording session (%); f = frequency; s = score; ANOVA = analysis of variance.

following a jerky movement, the mouse loses its balance, its hindlegs slip off the bar, and it hangs onto the bar with its forelegs; *locomotion* (duration), locomotor activity; *passivity* (duration), remaining motionless; *crossing attempt* (frequency), engaging the narrow bar with the two forepaws. After recording spontaneous behavioral elements during the 3-min observation period in the bar cross test mice were exposed to a *forced cross*. They were put onto the challenge bar and the duration to complete the crossing was measured. In order to eliminate possible olfactory cues left by previously tested mice, both the open field and the bar cross apparatus were thoroughly cleansed with an alcoholic germicidal deodorant spray and dried after each test session. In addition, in the open field, the sheet of paper with the square grid was changed between tests.

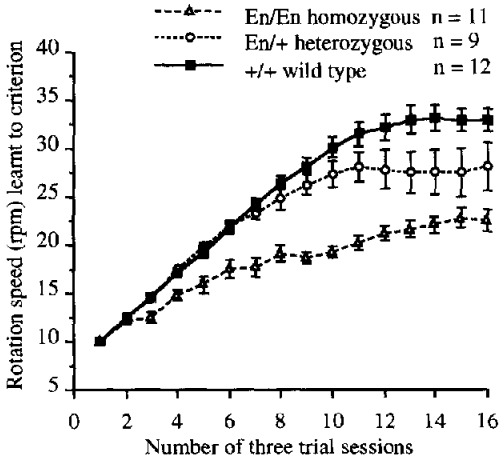

Figure 1. Performance of homozygous and heterozygous En-2^{hd} mutant and wild-type mice in the rotating rod motor-learning-paradigm during a 16-session (3 trials per session) training. The points represent the mean of the rotation speed learned to criterion. The error bars represent standard error. The sample sizes (n) are indicated. For training methods see the Method section. For the results of the statistical analyses see the Results section. Note that wild-type mice acquired the highest rotation speed, whereas the homozygous mutant mice acquired the lowest.

The rotating rod apparatus was made locally at Mount Sinai Hospital, Toronto, Canada, and used to measure the ability of mice to improve motor performance during repeated exposure to the apparatus. The rod, made of wood (3 cm diameter) was dusted with dynamac no-slip compound to enhance grip and painted with satin black enamel. The rod was directly driven by a stepper motor (12 V DC, 1.8° phase angle) whose revolution speed could be controlled with a variable speed motor driver. The rotation speed was measured by a tachometer. The rod was divided into five 4.5-cm-wide segments that were separated from each other by disks (15 cm diameter). The rod was mounted 20 cm above the floor of the apparatus case such that mice would drop into a bin positioned under the rod. This height was sufficient to discourage jumping off the rotating rod while not causing injury when the mice fell.

Before the first training session all mice (for sample sizes see Figure 1) were habituated to the apparatus by putting them on the rod rotating with 2.5 revolutions per min (rpm) and allowing them to stay on it for 2 min for three times. During training, the mice were to cope with an increasingly difficult motor task whose difficulty level was set according to the actual recent performance of each individual mouse. The training consisted of 16 sessions, 2 sessions a day with 90-min intersession interval, and three trials per session with 5 s intertrial interval. The maximum length of each trial was allowed to be 60 s, (i.e., the maximum cumulative training duration in a session was 180 s). The rotation speed of the rod was set at 10 rpm at the first trial for all mice. If the mice were able to stay on the rotating rod for a total cumulative duration of at least 60 s during a three-trial session, the rotation speed of the rod was increased by 2.5 rpm for the next session. If the mice

accumulated less than 60 s on-rod time during a three-trial session but more than 30 s, no speed increase was administered. If the mice could not accumulate 30 s on-rod time during a three-trial session for 2 consecutive sessions, the rotation speed was decreased by 2.5 rpm after 2 such sessions. The rotation speed the mice were able to master according to the definitions above (learning to criterion) was recorded and statistically analyzed.

The mice trained in the rotating rod paradigm were subsequently tested for ambulatory activity in the open field as described above and then in a bar-hanging test. In this latter test the mice were put on a wire bar (1.5 mm diameter) by making them grab the bar with their two forepaws. First the mice were habituated to the test procedure by allowing them to experience the task for 60 s. Subsequently two trials were conducted and the time the mice were able to spend hanging onto the bar was recorded. The maximum hang-on time allowed each time was 60 s. This test requires good coordination and muscle strength. In the data analysis the average duration recorded in the two trials was used.

Although the bar-hanging test clearly involves a factor associated with muscle strength, it also includes motor coordination. To measure muscle strength more directly we subjected the mice to a weight-pulling exercise. Mice were required to grab a metal bar (1.5 mm diameter, sand-papered for better grip) with their forepaws. The bar was attached to a scale with which the maximum weight the mice were able to pull was measured. Two sessions were carried out each containing five trials. The best result, that is the largest weight, the mice were able to achieve during each session was recorded and the two values were averaged for each mouse for statistical calculations.

Motor performance of a separate group of (heterozygous and homozygous mutant) mice was also compared in the tilting plane test. The mice were placed on a clear Plexiglas plane that was gradually tilted from 0° to 45° within 45 s. The position of the mice was perpendicular to the slope. The angle at which the mice fell off the plane was recorded. The test was repeated three times for each mouse.

Statistical Analysis

No sex differences or sex interaction effects were revealed in any of the tests (results not presented) therefore the data were pooled for sexes. The behaviors measured in the open field, bar cross, bar hang, and weight-pulling tests were analyzed by one-way analyses of variance (ANOVA) with factor genotype as the variable. In case of significant results the genotype groups were compared by the post hoc multiple comparison test Tukey's honestly significant difference (HSD). The results of the rotating rod training and of the tilting plane test were analyzed by a two-way repeated measure ANOVA with genotype and session as the repeated measure variable. The genotype groups were compared with post hoc Tukey's HSD tests by sessions. Because the variance homogeneity criterion of the parametric ANOVA was not fulfilled in all sessions in the rotating rod data set, logarithm scale transformation was applied to homogenize variances where necessary and the transformed data were used in the analysis. The habituation of the mice in the open field was also analyzed with a two-way repeated measure ANOVA with variables genotype and time (the repeated measure variable). For certain behavioral measures Pearson's product–moment correlation coefficients were also calculated.

Results

Open Field and Bar Cross

Analysis of the motor-posture patterns of the wild-type, heterozygous, and homozygous null mutant mice measured in the open field and bar cross apparatus (see Table 1) revealed no significant ($p > .05$) differences in all but one pattern: The frequency of defecation was significantly smaller ($p < .05$, post hoc Tukey's HSD test) in the wild-type mice compared to those of the heterozygous and homozygous mutant mice whose values were not significantly different from each other. These results indicate that neither the homozygous mutant nor the heterozygous mutant mice exhibited any gross motor-posture abnormalities, and also that their exploratory behavior in the novel situations and fine motor coordination were indistinguishable from those of the wild-type mice.

Rotating Rod Motor Learning Paradigm

The results obtained in the rotating rod motor learning test are summarized in Figure 1. ANOVAs revealed significant differences between genotype groups, Genotype $F(2, 29) = 23.95, p < .00001$, and indicated that all mice improved with training, Session $F(13, 377) = 418.79, p < .00001$, but also showed that this improvement was genotype dependent, Genotype × Session $F(26, 377) = 10.54, p < .00001$. Comparisons of the genotype groups by session (Tukey's HSD multiple comparison test) indicated that (a) during the first two sessions all mice were statistically indistinguishable ($p > .05$); (b) during Sessions 3 through 13 mutant homozygous mice were significantly impaired ($p < .03$) compared to heterozygous and wild-type, and the latter two were not significantly ($p > .05$) different up to Session 13; (c) during Sessions 14 through 16 mutant homozygous mice were significantly impaired ($p < .001$) compared to wild-type but were not different ($p > .05$) from heterozygotes; (d) during Sessions 13 through 15 heterozygotes were significantly ($p < .05$) impaired compared to wild-type mice. These results demonstrate that the performance of mice homozygous for the En-2^{hd} mutation was significantly impaired compared to those of the wild-type and heterozygous mice. Furthermore, because the performance of the heterozygous mice was in between those of the homozygous and wild-type, our findings suggest that the level of impairment is dependent upon the number of wild-type or mutant En-2 alleles present.

Habituation of Locomotory Activity in Open Field

A performance difference in a learning paradigm may be a result of a number of factors, one of which may be habituation to novelty. Differential ability to habituate to the training apparatus or procedure may lead to differences in the performance. In order to investigate habituation we subjected the mice of the three genotypes to an open-field test (Gerlai et al., 1993) subsequent to their training on the rotating rod and measured the change of their activity level during three consecutive 2-min intervals (Figure 2). Two-way ANOVAs indicated a significant time, $F(2, 58) = 37.80, p < .00001$, effect but no significant genotype, $F(2, 29) = 2.08, p > .05$, or Genotype × Time interaction, $F(4, 58) = 0.59, p > .05$, effects. However, one-way ANOVAs detected a genotype effect in the cases of Locomotion 2 and 3 with p values bordering significance level ($p = .052$ and .062 for Locomotion 2 and 3, respectively), suggesting the possibility of a genotype-dependent trend in our data.

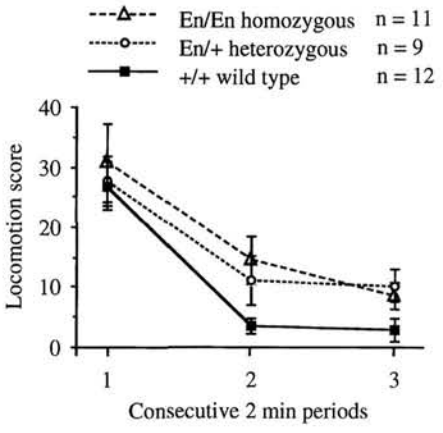

Figure 2. Habituation of locomotory activity in the homozygous, heterozygous En-2^{hd} mutant and wild-type mice in open field during three consecutive 2-min intervals. Points represent the mean of locomotion score. Error bars represent standard error. Sample sizes (n) are indicated. For recording methods see Method. For the results of the statistical analyses see the Results section. Note that the three genotypes of mice are not different in their locomotion scores, and all of them habituate to the open field.

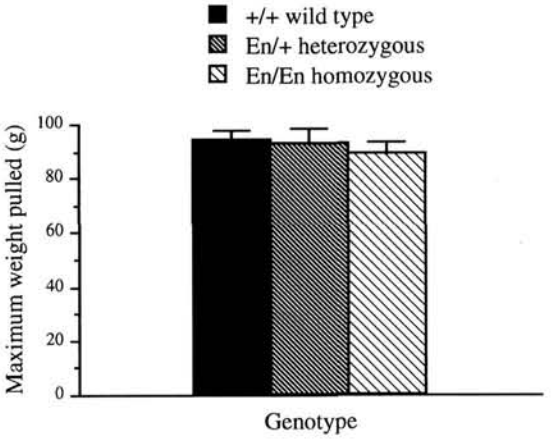

Figure 3. Grip strength (forepaw) as measured by the maximum weight (g) the mice were able to pull. The columns represent the mean of the performance of normal control (n = 11), En-2 heterozygous (n = 9) and En-2 homozygous (n = 11) mutant mice. Error bars represent standard error. Note that there is no significant difference between the mice of the three genotypes. For recording procedures see the Method section. For the results of statistical analysis see the Results section of this article.

In order to further investigate a possible effect of habituation on the rotating-rod–motor-learning performance we correlated a habituation measure with the rotation speed (rpm) mastered at the last (16th) training session. *Habituation* was defined as follows: habituation = Locomotion 1 − (Locomotion 2+ Locomotion 3), where Locomotion 1, 2, and 3 are the locomotion scores measured during the first, second, and third 2-min interval in the open field. The obtained correlation coefficient (r = .196) was not significant ($p > .05$) suggesting that the motor skill acquired by the mice in the motor learning paradigm and their ability to habituate to novelty are two unrelated behavioral measures. Further analysis of the data indicated no significant correlations between the performance at the 16th session and any of the locomotion scores in the open field (1st interval $r_1 = .036$; 2nd interval $r_2 = -.227$; 3rd interval $r_3 = -.085$; all p values are greater than .05). Suggesting that the activity level of the mice in the open field and the ability to master certain rotation speed during training on the rotating rod are also unrelated behavioral measures.

Bar-Hanging, Weight-Pulling, and Tilting-Plane Tests

No significant genotype effects were revealed in any of these tests. The bar-hanging times (wild-type = 46.73 ± 2.76; heterozygous-mutant = 43.88 s ± 3.60; homozygous-mutant = 42.36 s ± 3.50) were not different between the genotype groups, ANOVA A, $F(2, 28) = 0.486$, $p > .05$, and no significant correlation was found between this measure and the top rotating rod revolution speed (Session 16) achieved (r = .175, $p > .05$) suggesting unaltered muscle strength and coordination abilities in the mutants. Results of the weight-pulling test (Figure 3) also confirmed the lack of muscle strength problems; ANOVA for genotype effect, $F(2, 27) = 0.35$, $p > .05$. The tilting plane test (Figure 4) also revealed no genotype differences and no trial effect (all $F < 1.18$ and p values $> .05$).

Discussion

Our results demonstrate that a null mutation in the En-2 gene in mice leads to a significant impairment in motor learning performance. Because the En-2^{hd} mutation disrupts cerebellar neuroanatomy, and because motor performance and motor learning depend on an intact cerebellum, one may hypothesize a direct link between the mutation and the observed motor-learning performance impairment. Although our data showing no significant abnormalities in the mutants in a number of tests (including the open-field, bar-cross, bar-

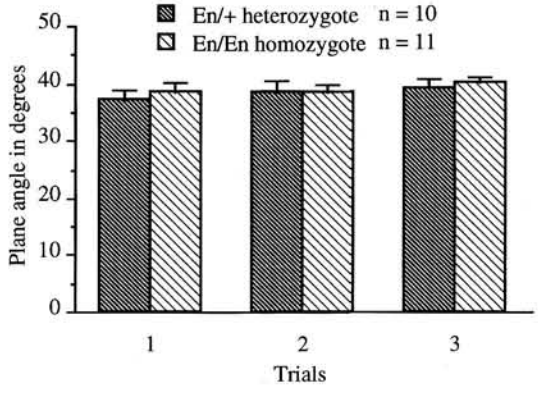

Figure 4. The tilting-plane test. The columns represent the mean of the angle (degrees) at which mice fell off the tilting plain. Error bars represent standard error. Sample sizes (n) are also shown. Note that heterozygous and homozygous mutants are not significantly different from each other and there is no difference between their performances measured at different trials. For recording methods see the Method section. For the details of statistical analysis see the Results section of this article.

hanging, weight-pulling, and tilting-plane tests) suggest that the impairment observed on the rotating rod may be due to a motor-learning deficit, we cannot completely rule out the effect of other factors, including subtle sensory or motor defects, altered habituation, or abnormal motivation, that could have remained undetected in the tests discussed above.

Nevertheless, the lack of gross abnormalities together with the observed impaired motor learning performance are highly significant findings in the light of other cerebellar mutant mouse models in which severe impairments are obvious. Using these models, it has been difficult to separate simple performance deficits from other behavioral abnormalities including motor learning impairment (see e.g., Lalonde & Botez, 1990). For instance, cerebellar mutant mice, including reeler, staggerer, and weaver mice, exhibited a severe *ataxia* (e.g. Goldowitz & Koch, 1986), a motor deficit that can seriously affect performance in a learning paradigm. Similarly, drug-induced neurotoxicity in the cerebellum led to strong ataxia (see e.g., Balaban, Fredericks, Wurpel, & Severs, 1988). A less severe motor deficit was produced by localized irradiation of the cerebellum in rats (Altman, 1987). Rats irradiated at birth or up to the age of 4 days exhibited intention tremor, mild ataxia, and inability to rear. However, rats that were irradiated between the age of 8–15 days did not show any tremor, ataxia, or inability to rear. These rats were also able to traverse a rotating rod for food reward similarly to normal control rats. Nevertheless, the irradiated rats did show a significantly elevated activity level upon exposure to the open field compared to their normal control counterparts (Altman, 1987). A finding implying that the open field is a sensitive test that can detect relatively mild motor defects. However, we did not observe any activity level change or altered motor or posture patterns, motor coordination, and muscle strength in the mutant mice compared to wild-type mice in the open field or in any other tests, some of which were specifically designed to detect finer alterations in motor performance (Gerlai et al., 1993; Li et al., 1994).

Factors other than sensory or motor defects could also contribute to an impaired learning performance. Decreased ability to habituate to novelty may be such a factor. It might interfere with the learning performance during training that involves application of an apparatus and handling procedure novel to the mice. A trend, although not statistically significant, is apparent in our data suggesting the possibility of a mild alteration in habituation of the mutants in the open field: Mutant mice appear to habituate more slowly (i.e., decrease their locomotion less quickly with time). Sensitivity to novel stimuli may be one reason that could lead to inability to habituate. One behavioral parameter, defecation frequency, might indicate the presence of such sensitivity in the mutant mice showing an increase in the mutants compared to wild-type. Defecation in open field has been used as a measure of emotionality evoked by the novelty (see, e.g., Eysenck & Broadhurst, 1964). However, the validity of this measure has been criticized. Archer (1973) regarded it as oversimplified and explained that defecation can depend upon several factors, some of which may be entirely unrelated to emotionality. Nevertheless, we cannot rule out the possibility that the mutant mice are somewhat more sensitive to novelty and habituate slightly more slowly, a defect that could potentially affect their performance on a motor learning paradigm.

Muscle weakness could also compromise performance on a motor learning test. This explanation, however, is not likely because a relatively decreased muscle strength in the mutant mice might have led to a decreased overall activity level in the open field test that was carried out after the rotating rod training. However, no such decrease was observed and there was no correlation between the level of locomotory activity in the open field and the mastered rotation speed in the motor learning task. These findings were confirmed by the bar-hanging test and the weight-pulling exercise in which both the mutant and wild-type mice performed equally well.

Finally, although motivation per se is difficult to test, our data do not support its role in the differences in the motor learning performance observed between the mutant and normal mice. First, the open-field tests suggested that the motivation to exhibit activity upon exposure to novel situations was not different between the mutant and wild-type mice. Second, the lack of difference between the mutant and wild-type mice during the first two sessions of the motor learning training indicates that all mice were motivated to try and able to solve the rotating rod task. In summary, our results clearly indicate that the En-2^{hd} mutant mice lacked any gross abnormalities; however, they showed a significant motor-learning performance deficit.

Although the cerebellum has been implicated in motor learning performance (see, e.g., Ghez, 1991), the exact neuroanatomical structures and pathways involved are debated (for a review see Llinas & Welsh, 1993). For the En-2 mutants a specific set of alterations has been characterized (Joyner et al., 1991; Millen et al., 1994). In the En-2^{hd} homozygous mutant mice the folding pattern in the posterior cerebellum was abnormal, most notably in the posterior vermis (realignment of the eighth fold with the ninth instead of with the sixth and seventh), in the hemispheres (number of lobules reduced from 4 to 3) and also in the paraflocculus (size reduction and abnormal folding). In contrast, the cytoarchitecture of the cerebellum is normal. At the microscopic level the dendritic arborization of Purkinje cells by silver staining (Millen, unpublished observation) appears unaltered. Furthermore, the folding pattern of the anterior part of the cerebellum also is relatively normal. Although each folium may act as an independent processing module carrying out integrative operations on a patterned assortment of afferent inputs unique to each folium (Welker, 1990), altered foliation observed in the posterior cerebellar structures may result in specific behavioral abnormalities such as impaired motor-learning performance.

Alternatively, the observed motor-learning performance deficit may be due to a cerebellar defect other than abnormal foliation. The results of the rotating rod training show a gene copy-number dependent effect, the mutant homozygous mice being the most impaired, and the heterozygous mice, although significantly worse than wild-type in the later sessions, less impaired. This copy-number effect is curious as no cerebellar neuroanatomy alterations were reported for the heterozygotes (Joyner et al., 1991; Millen et al., 1994). This finding implies that the observed behavioral abnormality may be, at least in part, a result of a factor other than altered gross cerebellar

neuroanatomy. One such factor may be cerebellar neurophysiology, for instance altered long-term depression, a synaptic phenomenon observed in the cerebellum (see e.g., Aiba et al., 1994) that, together with long-term potentiation, is implicated in certain forms of learning (Artola & Singer, 1993).

Proving the role of the cerebellum in complex behavioral phenomena including motor learning has been difficult because experimentally induced cerebellar damage leads to gross motor or sensory defects that confound learning tasks (for review, see Lalonde & Botez, 1990). The En-2 mouse model offers a unique tool without such confounding effects, allowing the investigator to study more precisely the role of cerebellum in learning and other more complex behavioral phenomena. Furthermore, this mouse model demonstrates that single-gene manipulations can lead to specific brain and behavior changes that will further our understanding of how genetic information translates into behavior.

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