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Research report

Balance deficit enhances anxiety and balance training decreases anxiety in vestibular mutant mice

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HIGHLIGHTS

- Vestibular mutant mice were raised in either acrobatic or standard cages.
- Untrained vestibular mutant mice developed comorbid balance and anxiety disorders.
- Trained vestibular mutant mice showed recovered balance and anxiety scores.
- Results indicate a causal relationship between balance and anxiety disorders.
- Treatment of clinical anxiety may be helped by balance rehabilitation protocols.

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ABSTRACT

Treatment of anxiety disorders by either pharmacological or behavioral means is applied with the intention to directly target the limbic system or high brain centers that down-regulate limbic activity. In spite of intense and long treatment, remission is not achieved in many patients, suggesting that their pathophysiology is not addressed by either of the above treatments. An alternative pathophysiology may be a disordered vestibular system, which may be studied in the context of comorbidity of balance and anxiety disorders.

Here we studied whether mutant vestibular Headbanger (Hdb) mice demonstrate elevated anxiety and whether physical treatment of balance alleviates the behavioral symptoms of anxiety. Hdb and wildtype (Wt) mice were raised in either balance training or standard cages and were subjected repeatedly at 1–3 months of age to balance and anxiety-related tests. Results demonstrated progressive deterioration of balance performance and parallel elevation of anxiety in untrained Hdb as compared to untrained Wt mice. Training significantly improved balance performance of Hdb mice and in parallel, decreased the level of anxiety compared to untrained Hdb mice.

These findings confirm that vestibular pathophysiology may be causally related to development of anxiety and suggest that in some clinical cases of anxiety, the appropriate treatment is physical rehabilitation of balance.

1. Introduction

Anxiety disorders are among the most common mental disorders and strike all classes of the population. Monotherapy may consist of pharmacological treatment aiming at normalization of the limbic systems activity, abnormalities of which are believed to predispose a person to develop an anxiety disorder [13,17]. Combination therapy may consist of pharmacological treatment aided by either behavioral and/or cognitive treatments with the latter two treatments typically aiming at high brain centers that may down-regulate the limbic systems [2,11,33]. Surprisingly, although about 50–60% of patients may respond to treatment, clinical remission is typically achieved by only part of the responding patients [6,22]. The difficulty to reach remission suggests a pathophysiology that is not addressed by either of the above treatments.

The alternative pathophysiology predisposing to anxiety may be studied in patients presenting comorbidity of balance and

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anxiety disorders. Several anxiety disorders such as agoraphobia, panic attack and generalized anxiety are very often comorbid with impaired balance and with an acute or chronic vestibular dysfunction [1,14,25,4,5,20,30]. The causality factor in these forms of comorbidity is presently not always clear, particularly since compromised balance by itself is quite common in community surveys [36].

Animal models of comorbidity of balance and anxiety were reviewed by [23], stressing that comorbidity was demonstrated only in some models. Comorbidity was demonstrated in mouse strains with high levels of anxiety who also demonstrated poor balance skills [24]. We previously demonstrated a progressive deterioration of balance skills and a parallel enhancement of anxiety in Headbanger (Hdb) vestibular mutant mice [32]. These correlative findings provide no direct support for causal relations within the comorbidity. Nevertheless, we noted that Hdb mice present a disordered arrangement of the vestibular stereocilia already at P20 [31], which suggests that the disorder of balance is a causal factor for development of anxiety. A more convincing demonstration of this direction of causality may be achieved when physical training of balance, without any direct treatment of anxiety, will result in amelioration of anxiety symptoms. Confirmation of this hypothesis will support the 'generic' statement that anxiety may develop in the presence of a normative limbic system responding excessively during interaction with a deficient sensorimotor system. In clinical terms, the present study may demonstrate that some forms of anxiety may be treated by pure sensorimotor training. This may have a significant effect on clinical practice and increase the awareness that training may be advantageous not only to rehabilitation of balance but also to amelioration of anxiety in some individuals [21,35,36].

2. Methods

2.1. Mice

Vestibular mutant Headbanger (*Hdb*; 15/15 f/m) and C3HeB/FeJ wildtype (Wt; 15/15 f/m) mice were tested. The ENU-induced mutation was mapped to the region of the unconventional myosin gene myosin VIIa (*Myo7a*) and mutation screening revealed an $A > T$ transversion [31]. The *Hdb* mutation is manifested as progressive elongation of stereocilia of hair cells, documented as early as P20 in the utricle of the vestibular end-organs. The functional phenotype in adult mice is reflected in a progressive imbalance and decline in hearing of low pitch sounds. In our previous study, Hdb mice exhibited normal spontaneous behavior at 1 month of age and signs of balance impairment could be demonstrated only on structured balance tests. However, at age of 3 months, balance impairments became clearly manifested in spontaneous behavior with the mice exhibiting vertical and rotational movements of the head. All experimental protocols conformed to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University (P-04-005).

2.2. Breeding

Mice were housed in a vivarium with ambient temperature kept at 23 °C under a reversed day/night cycle with all experimental manipulations taking place during the dark-wake phase of the cycle. Breeding involved 16 Wt females and 4 *Hdb* males that were rotated between four female cages for 8 days. Litters were born a maximum of two days apart within each cage. This breeding procedure was replicated successively three times resulting in a final sample of 60 mice.

2.3. Balance training

The training cages (60 × 30 × 20 cm) were designed to promote balance training. Their ceiling-grid motivated hanging and upside-down climbing, which is a major component of mouse behavior in a standard laboratory environment [29]. Cages were divided by vertical partitions to four equal compartments. Each of the compartments comprised of multiple balance challenging devices, e.g., a running wheel, narrow platforms, suspended narrow beams, canals, and vertical and horizontal ropes. Passage between the compartments on the floor level required walking on a narrow seesaw beam positioned across a small opening in one of the partitions; passage on the ceiling level required either jumping to the ceiling grid or vertical climbing over two partitions that were overlaid with a metallic grid. Regular mice food was accessible ad-lib through the ceiling grid, and a small portion of highly palatable food was delivered daily by three dispensers that were accessible only after managing the training devices. Animals used the devices vigorously from the day they were able to walk. The control cages of the same dimensions were designed to minimize balance training. Their solid ceiling prevented climbing and they lacked any partitions or training devices. Food in a similar quantity was distributed on the floor.

2.4. Balance tests

Balance was assessed by three behavioral tests in order to confirm balance impairment in *Hdb* mice.

2.4.1. Tail-hang test

Based on [32], mice were held by the distal end of the tail ~5 cm above a tabletop for 5 s. Normal response consists of the mice stretching their forelimbs toward the table surface, while vestibular mice tend to curl their trunk upward towards the tail. The behavior was scored on a scale from 0 to 4, where; 0 = stretching towards the floor with a maximum of a single curling up; 1 = stretching toward the floor and curling up twice; and so on; 4 = no stretching towards the floor and repeated curling up.

2.4.2. Elevated-platform test

Based on Eumorphia [16], standardized tests, mice were placed on a center of a plastic plate (5 × 5 cm) set 30 cm above the tabletop. Time until falling off the plate was recorded with a maximal score of 120 s.

2.4.3. Rotarod test

Based on [29], mice were placed on a rubber rod (5 cm in diameter), whose rotation accelerated linearly from 4 to 40 rpm over the 5 min period of the test. The latency to fall was recorded with maximal score of 300 s. Mice were tested three times under normal room illumination on day 1, with intervals of 10 and 20 min between the successive tests. This schedule was replicated under dim light conditions on day 2, to test whether the performance on the rotarod will be further compromised by restricted visual control.

2.5. Anxiety tests

Anxiety was assessed by two behavioral tests in order to test the beneficiary effect of balance training on anxiety.

2.5.1. Open-field test

Anxiety is manifested as avoidance of the open-field center and preferred occupancy of the home-base (HB) corner [12]. Based on [32], the open-field was a rectangular 120 × 120 × 25 cm arena with one corner lined with clean sawdust to promote its choice as a HB. Mice were placed in the HB corner facing the center of the

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open-field and were videotaped for 10 min using a ceiling camera. Videos were sampled off-line at 25 Hz and X–Y coordinates were extracted (Ethovision, Noldus v3) and analyzed by MATLAB program for the following dependent variables: (1) Cumulative time in the HB corner, defined as the square with highest cumulative time of occupancy after the field was divided to 6 × 6 squares each 20 × 20 cm; (2) time in a strip of 20 cm along the walls, but excluding the HB corner; (3) time in the center defined as an area of 40 × 40 cm in the center of the open-field; (5) traveled distance.

2.5.2. Elevate-plus-maze test

In the EPM test, anxiety is manifested as avoidance of the open-arms. Based on [32], the maze was set 45 cm above the floor and consisted of plus-shaped four-arms of 30 × 5 cm each, with a center square of 5 × 5 cm. The two open-arms were encircled by 0.25 cm high walls, whereas the two closed-arms were encircled by 15 cm high walls. Mice were placed at the distal end of a closed arm, facing the center of the maze, and were videotaped for 5 min using a ceiling camera. Videos were sampled off-line at 25 Hz (Ethovision, Noldus v3) and *X–Y* coordinates were extracted and analyzed by customized MATLAB program for the time in open-arms.

2.6. Procedure

Pregnant dames were assigned to one of 'balance training' or 'control' cages with 4 females per cage. Litters were born in the training or control cages and food and water were freely accessed close to the floor level until weaning at P21, when mothers were removed. At P28, litters were genotyped using the vestibular-sensitive swim test, sexed, culled and pups were redistributed among the 4 cages until the end of the study under the following constraints: pups were returned to the same functional cage; 5–6 pups per cage; f/m in separate cages; even distribution of Hdb/Wt per cage; and, litters of each mother distributed among the cages. The final sample consisted of 8 groups: genotype (Hdb/Wt), by sex (f/m), and by training (trained/control), with 7–8 mice at each group. Mice were taken to a quiet laboratory and balance and anxiety tests were applied repeatedly at the age of 1 month (P29-43), 2 months (P59-73) and 3 months (P89-119), with at least one day apart between the tests. Tail-hang test was applied at all 3 age times. Elevated-platform test was applied at 2 and 3 months of age; the 1 month test was omitted due to technical problems. Rotarod test was performed only at 3 months of age, to minimize the differential effects of rotarod practice on groups' performance. Open-field test was performed at 1 and 2 months of age; the 3 months test was omitted as the untrained Hdb mice were prone to circle violently at the center of the open-field upon their introduction to the open arena. Elevated-plus-maze was tested at all 3 age times.

2.7. Genotyping

At P28 mice were phenotyped using the vestibular-sensitive swim test. Based on Eumorphia convention, mice were held by the tail and released from a high of 5 cm into a container filled with water at 24–26 °C. Swimming was assessed for up to 10 s and scored as 'normal' that included immediate resurfacing after the dive followed by swimming along the periphery of the container, or 'abnormal' that included delay in resurfacing, swimming upside-down and swimming in tight circles. Mice with normal and abnormal swimming scores were defined as Wt and Hdb, respectively, and were assigned to the respective groups.

Genotyping at the end of the study, performed on 47 of 62 mice of the study, fully confirmed the swim-based Wt/Hdb segregation, as it did in our previous study [32]. Mice were anaesthetized and the distal 0.5 cm of the tail was cut. DNA was extracted and genotyping was performed to confirm the *Hdb*/Wt grouping of mice based initially on the swimming test. The genotyping assay consisted of PCR followed by either the restriction enzyme or direct sequencing methods to identify the *Myo7a Hdb* mutation [31].

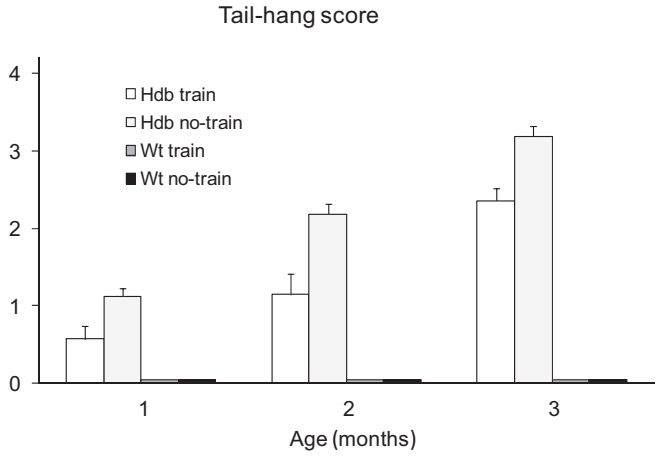

Fig. 1. Balance measured as performance on the tail-hang test (mean ± SEM), applied repeatedly in 1–3 months old Hdb and Wt mice raised in training or no-training cages. Performance was scored from '0' = normal stretching towards the table surface, up to '4' = abnormal repeated curling up towards the tail. The four groups included 14 to 16 mice each. All Wt mice scored '0' on the test. The Hdb mice scored progressively higher with age, but the rehabilitating effect of training on balance of the Hdb mice was confirmed by the trained mice scoring lower than the untrained mice at all age times (Kruskal–Wallis, $p < 0.01$, 0.001 and 0.001, respectively).

2.8. Data analysis

Parametric dependent variables were analyzed by repeated measures ANOVA with genotype (Hdb and Wt), sex (f and m) and training (trained and untrained mice) as between Ss variables. When appropriate the analysis also included age (1–3, or 1 and 2, or 2 and 3, months), time in test (10 min), and illumination (bright and dim), as within Ss variables. MANOVA was used when assumption of sphericity was violated in analysis of data of the open-field. Non-parametric depended variables were analyzed by Friedman and Kruskal–Wallis tests). Only statistically significant results are reported. The effect of the sex variable was typically very minor and infrequent and therefore was omitted in this report.

3. Results

3.1. Balance tests

3.1.1. Tail-hang test

Fig. 1 demonstrates the scores of the tail-hang test applied repeatedly to 1–3 months old mice of the four groups. All Wt mice scored '0' at all time-points reflecting normal stretching of their forelimbs toward the table, with this floor effect possibly masking the effect of training. In contrast, all Hdb mice showed some upward curling, scoring progressively higher with age (Friedman-test, $p < 0.001$). Nevertheless, the trained Hdb mice scored lower than untrained Hdb mice at all age times (Kruskal–Wallis tests; $p < 0.01$, $p < 0.001$, $p < 0.001$, for the three age times, respectively), confirming the beneficiary effect of training on performance of the Hdb mice along the first 3 months of their life.

3.1.2. Elevated-platform test

Fig. 2 demonstrates the time on an elevated-platform, tested repeatedly in 2 and 3 month old mice of the four groups. Wt groups, except for a single mouse, were able to stay safely on

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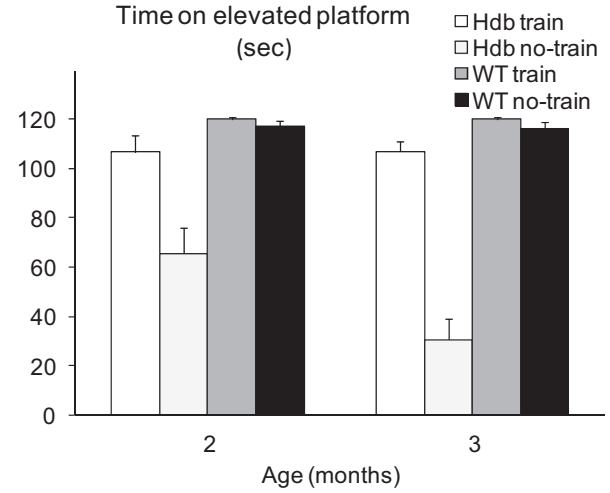

Fig. 2. Balance measured as time (max 120 s) on an elevated-platform until falling to the table (mean ± SEM), tested repeatedly in 2 and 3 months old Hdb and Wt mice raised in training or no-training cages. The 4 groups included 14 to 16 mice each. The rehabilitating effect of training on balance of the Hdb mice was confirmed by the trained Hdb mice staying on the platform as long as the Wt groups while the untrained Hdb mice stayed on the platform for shortest period at each age time (all comparisons; $p < 0.003$).

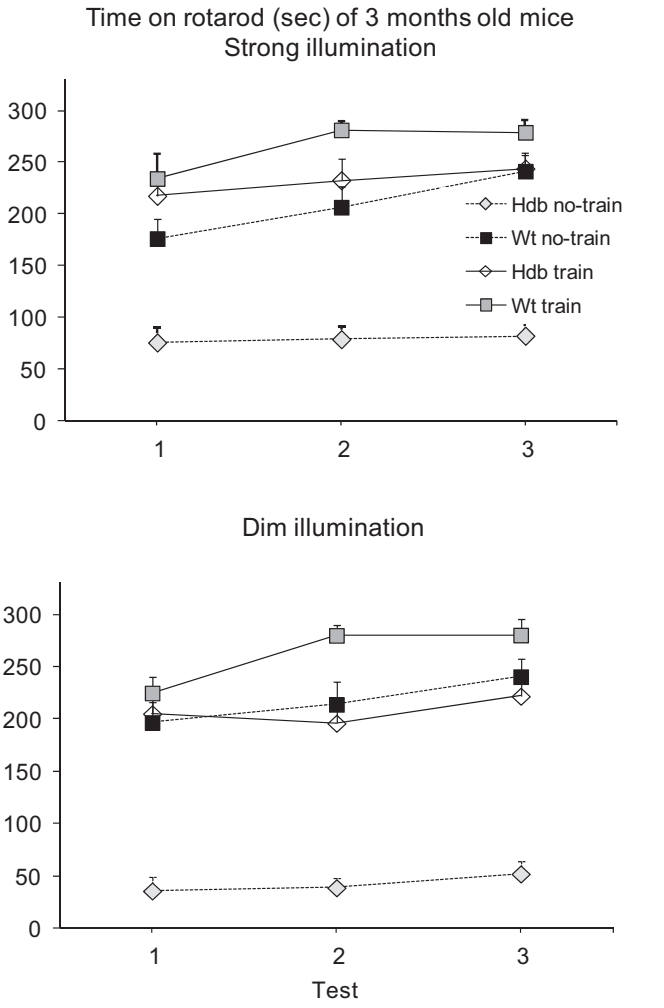

Fig. 3. Balance measured as time (max 300 s) running on a rotarod until falling to the table (mean ± SEM), tested in 3 month old Hdb and Wt mice raised in training or no-training cages. The test was applied over 2 days, 3 times/day interspaced by 10 and 20 min resting periods. The tests were applied under normal room illumination on the first day and under dim illumination on the second day. The 4 groups included 14 to 16 mice each. The rehabilitating effect of training on balance of the Hdb mice was confirmed by the trained Hdb mice staying on the rotarod as long as the Wt groups while the untrained Hdb mice stayed on the rotarod for shorter period compared to all other groups in all tests (all comparisons; $p < 0.001$).

the platform for the maximal duration, while Hdb mice were likely to slip from the platform, resulting in a significant genotype effect [$F(1, 56) = 74.7$, $p < 0.001$]. Trained Hdb mice stayed longer on the platform, compared to untrained Hdb mice, resulting in a significant training effect [$F(1, 56) = 42.7$, $p < 0.0001$] and genotype by training interaction [$F(1, 56) = 34.1$, $p < 0.001$]. *T*-tests for independent measures confirmed the beneficiary effect of training on performance of the Hdb mice for each age (all comparisons; $p < 0.003$). Only untrained Hdb mice demonstrated age-related deterioration of performance, resulting in a significant genotype by age interaction [$F(1, 56) = 6.6$, $p < 0.05$], with *T*-tests for dependent measures confirming deterioration of performance across age only in untrained Hdb group ($p < 0.02$).

3.1.3. Rotarod test

Fig. 3 demonstrates the time on rotarod in 3 months old mice of the four groups, tested three times per day, first under strong and then under dim ambient illumination. In all tests, the Wt groups and the trained Hdb group were able to stay on the rotating rod much longer than the untrained Hdb group, resulting in a significant genotype by training interaction [$F(1, 56) = 68.0$, $p < 0.001$]. The Wt groups gained from repeated testing showing longer stay on the rod along the daily tests, while the Hdb groups showed no gain in performance along the tests, resulting in a significant genotype by tests interaction [$F(2, 112) = 4.5$, $p < 0.05$]. Finally, the performance of Wt groups was not affected by a change in ambient illumination while mice of Hdb groups stayed on the rod for shorter periods under the dim illumination, resulting in a significant genotype by illumination interaction [$F(1, 56) = 7.1$, $p < 0.01$].

3.2. Anxiety tests

3.2.1. Open-field test

Fig. 4 demonstrates the time spent in the center, in the strip along the walls, and in the home-base corner of the open-field along the 10 min of the test, which was applied repeatedly to 1 and 2 months old mice. At the two age times, mice showed a decrease in occupancy of the home-base corner along the 10 min of the test [$F(9, 48) = 6.3$, $p < 0.001$], which was associated with a time-wise increase in the occupancy of the strip along the walls and the center of the open-field. At the age of 1 month the groups did not differ in occupancy of the home-base. The effect of training on Hdb mice was visible at the age of 2 months, whereby the trained Hdb mice showed the shortest and the untrained Hdb mice showed the longest occupancy of the home-base corner, with the two Wt groups showing intermediate duration of occupancy, resulting in significant genotype by training by age interaction [$F(1, 56) = 6.6$, $p < 0.02$]. The shortest occupancy of the home-base by the trained Hdb mice was associated with the longest occupancy of the open-field center, while the untrained Hdb mice showed the shortest occupancy of the open-field center.

3.2.2. Elevated-plus-maze test

Fig. 5 demonstrates the % of time spent in the open-arms of the elevated-plus-maze tested repeatedly at 1–3 months old mice. Analysis demonstrated similar effects of training on Hdb mice along the repeated tests. Training increased the occupancy of the open-arms in both Hdb and Wt mice, but this effect was particularly strong among the Hdb groups, resulting in a significant training effect and genotype by training interaction [$F(1, 56) = 60.5$,

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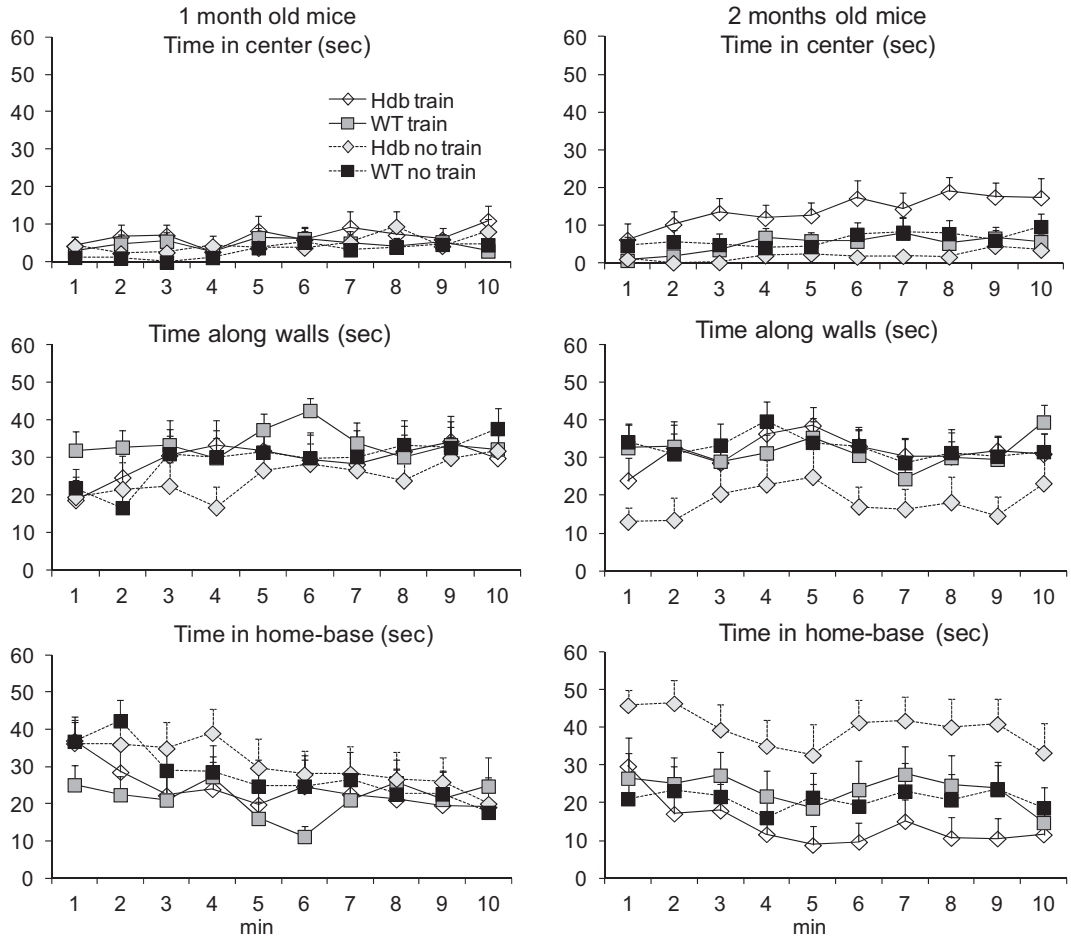

Fig. 4. Anxiety measured as time (s) spent in the center, in a strip along the walls and in the home-base of the open field (mean ± SEM) along 10 min of the test applied repeatedly in 1 and 2 months old Hdb and Wt mice raised in training or no-training cages. The 4 groups included 14 to 16 mice each. At the age of 1 month the groups did not differ in their open-field behavior. The anxiolytic effect of training on Hdb mice was visible at the age of 2 months, whereby the trained Hdb mice showed the shortest and the untrained Hdb mice showed the longest occupancy of the home-base corner, resulting in significant genotype by training by age interaction [$F(1, 56) = 6.6$, $p < 0.02$].

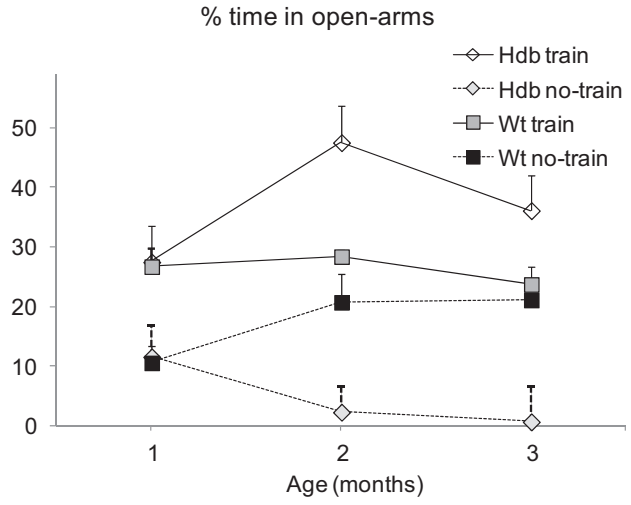

Fig. 5. Anxiety measured as % of time spent in the open-arms of the elevated-plus-maze (mean ± SEM) during 5 min sessions tested repeatedly in 1–3 months old Hdb and Wt mice raised in training or no-training cages. The 4 groups included 14 to 16 mice each. The anxiolytic effect of training on Hdb mice was visible at all age times with the trained Hdb mice showing highest, while the untrained Hdb mice showing lowest, occupancy of the open-arms, resulting in a significant training effect and genotype by training interaction [$F(1, 56) = 19.6$, $p < 0.001$, respectively].

$p < 0.001$, and, $F(1, 56) = 19.6$, $p < 0.001$, respectively]. In fact, at 3 months of age the beneficial effect of training was evident only among the Hdb groups, resulting in a significant genotype by training by age interaction [$F(2, 112) = 5.4$, $p < 0.01$].

4. Discussion

The present study aimed at demonstrating the comorbidity between the balance and anxiety disorders in the vestibular mutant Hdb mice and at testing the causal relations between the two disorders. To this end, Hdb and Wt mice were raised either in balance training or control cages and balance and anxiety tests were applied repeatedly along the first 3 months of life. Comorbidity was demonstrated in the untrained Hdb mice, who demonstrated progressive deterioration of balance and enhanced anxiety compared to Wt mice. A causal relationship was supported by the observation that improved balance in trained Hdb was also associated with an impressive amelioration of anxiety, compared to the level of anxiety in untrained Hdb mice and occasionally also compared to the anxiety in Wt mice.

4.1.1. Balance in untrained Hdb mice

Vestibular Hdb mutants demonstrate a distinctive structural phenotype in the vestibular end-organs in the form of progressive elongation of stereocilia and a behavioral phenotype, taking

the form of head-banging and hyperactivity [31]. Informal observations revealed that the hyperactivity peaked with age to bouts of excessive circling, which led us to discontinue the testing when mice reached the age of 3 months and to verify that none of the tests was confounded by periods of circling. The balance-related tail-hang test was applied repeatedly at the age of 1–3 months and the elevated-platform test was applied repeatedly at the age of 2 and 3 months. The untrained Hdb mice demonstrated age-related progressive deterioration of balance in both tests, compared to Wt mice. The rotarod test was applied only at the age of 3 months and confirmed compromised balance in the untrained Hdb compared to Wt mice. The extent of the currently observed balance deficit was similar to that observed in our previous study in which mice were raised in cages equipped with ceiling-grid [32] that might have provide some basis for balance training [29]. Therefore, it seems that experiencing a single training device cannot compensate for the progressive deficit due to a dominant mutation in the Hdb mice. In conclusion, vestibular mutants raised in cages deprived of any facility for balance training demonstrated profound age-related progressive balance deterioration.

4.1.2. Anxiety in untrained Hdb mice

Enhanced anxiety in vestibular Hdb mice was demonstrated in a previous study [32] and was fully replicated in the present untrained Hdb mice. An anxiety-related open-field test was applied at the age of 1 and 2 months and demonstrated progressive exacerbation of anxiety in the form of an age-related extended occupancy of the home-base corner. An elevated-plus-maze test was applied at the age of 1–3 months with the untrained Hdb mice showing a nearly total avoidance of the open-arms from the very first testing. Thus, the floor effect most likely prevented demonstration of an age-related progressive exacerbation of anxiety. We suggest that these findings likely reflect elevated anxiety rather than reduced sensorimotor ability to visit the center of the open-field or the open arms. Indeed, in our previous studies, untrained Hdb demonstrated either equal or excessive locomotion, compared to that of wild-type controls, when the anxiogenic effects of the manipulation were decreased, such as reduced illumination of the arena or after a period of habituation to the arena [32]. These findings imply that it is the anxiety rather than the sensorimotor dysfunction that is constraining the untrained Hdb mice to the home-base or the closed-arms. Cumulatively, these findings demonstrate the developmental pattern of comorbidity of balance and anxiety disorders in untrained vestibular Hdb mice, where the age-related progressive deterioration of balance is associated with progressive exacerbation of anxiety.

4.1.3. The effect of training on balance

Informal observations of mice behavior in the training cages confirmed that both Wt and Hdb mice frequently interacted with all of the training devices and that they used the wall and ceiling grids and the seesaw platforms to travel between the four cage compartments. When highly palatable food was delivered, it was approached through balance challenging devices and quickly consumed. This form of continuous training had a remarkably beneficial effect on balance performance, with the trained Hdb mice significantly outperforming the untrained Hdb mice on all balance tests. The performance of the trained Hdb mice was indistinguishable from that of Wt mice on the elevated-platform at the age of 2 and 3 months and on the rotarod tests at the age of 3 months. The performance improved on the tail-hang test but was short of the level of Wt mice performance throughout the 3 months of testing. These findings demonstrate that experience gained in balance-promoting environment generalized to balance tests and the trained Hdb mice demonstrated significant rehabilitation of balance skills.

4.1.4. The effect of training on anxiety

Training led to an impressive amelioration of anxiety, with trained Hdb mice showing significantly less anxiety in comparison to the untrained Hdb mice on all tests of anxiety. At the age of 1 month, the behavioral profile of trained Hdb mice in the open-field was similar to that of Wt mice and at the age of 2 months they decreased the occupancy of the home-base corner and increased the occupancy of the center of the field above the level of Wt mice. In an elevated-plus-maze, the trained Hdb mice showed the longest occupancy of the open-arms throughout. These findings indicate that training of balance has impressive anxiolytic consequences, supporting the notion that anxiety in vestibular Hdb mice is causally related to balance deficits. Nevertheless, trained Hdb mice should also be subjected to anxiety tests that are not based on locomotion, to convincingly conclude that their improved performance on the locomotion-related anxiety tests reflects reduced anxiety and not post-training improved sensorimotor skills. Such an option emerged in our recent pilot in which we tested vestibular mice engineered to express the diphtheria toxin (DT) receptor on hair cells. Partial ablation of hair cells after the injection of the DT enhanced the autonomic reaction to a 2-min confinement stress manipulation [34]. Alleviation of the enhanced autonomic response by balance training may support the present conclusion of reduced anxiety in trained Hdb mice.

4.1.5. Theoretical implications

Present findings may be anchored in the two stage theory of learning [27], which we extended to the three stage theory of learning [15]. Briefly, an encounter with a challenging situation triggers at the first stage, an unconditioned fear response and initiates fast acquisition of conditioned fear responses. These fear responses prevail throughout the second stage, which is typically longer and during which time the subject is slowly acquiring some adaptive motor responses that may specifically address the particular environmental challenge. In the third stage, the subject is already equipped with the specific skills to address the environmental challenge, and this adaptive state turns the conditioned fear responses into a redundant one and therefore promotes their extinction [26]. Inherent to this approach is the understanding that amelioration of anxiety was a causal consequence of acquisition of the adaptive motor response, rather than any direct manipulation of the anxiety itself. When the three stage theory of learning is applied to understand the comorbidity between balance and anxiety disorders, we foresee some major variations in the normal cascade of events: a patient with peripheral vestibular pathology reacts in the first stage with unconditioned fear and acquires conditioned fears and avoidance to the environment and stimuli that challenged his/her balance; due to his/her vestibular pathology, the patient has difficulty in learning the adaptive balance regaining responses in the second stage of learning; the unfortunate result is that the third stage of learning is either delayed or not reached at all, and anxiety turns to a chronic state. Treating this form of anxiety would require intervention directed at the balance system.

4.1.6. Implications for psychiatric research

Early students of biological psychiatry, while facing the multifaceted expressions of anxiety, nevertheless settled on co-localizing the variety of anxiety disorders selectively in the dysfunctional brain's limbic system. The translational implications included development and massive treatment by potent anxiolytic

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drugs, which leave to this day a high rate of unresponsive patients [6]. Further conceptual advances emerged through the realization that on top of the multifaceted anxiety, it may also have variable neuronal origins. Specifically, imaging technology enabled simultaneous monitoring of the limbic and cortical activity, demonstrating that anxiety may be the consequence of the prefrontal cortex's difficulty in inhibiting limbic output[3]. The translational implications of these findings led to behavioral and cognitive 'anxiolytic' therapies aimed at control of the limbic output through neocortical activation. Unfortunately, these therapies also leave a high rate of unresponsive patients [22]. The present study represents further extension of the multi-domain approach toward psychiatric disorders, by concentrating on disorders comorbid with the psychiatric category in question. Specifically, we demonstrated that comorbidity of balance and anxiety disorders may have a causal relationship. This implies that some forms of clinical anxiety may be the consequence of a central or peripheral balance disorder. While pharmacological or cognitive treatment may be ineffective in these individuals, treatment of the affected vestibular system or other somatosensory systems, which compensate for the vestibular loss, may provide improvement [7]. Apparently, these forms of anxiety may further blur the distinction between psychiatry and neurology in terms of diagnosis and responsibility for treatment.

4.1.7. Clinical implications

There are several similarities between the behavior of the Hdb mice and patients with impaired vestibular function and imbalance. Anxiety is very frequent in patients with acute and chronic vestibular disorders [14,20,30]. For instance, patients with acute vestibular neuritis, demonstrate severe anxiety that is greater than anxiety experienced by patients with other more disabling neurological condition such as a significant cerebrovascular accident not affecting the vestibular system [30]. With time, there is a recovery or compensation of the vestibular function but in many cases, patients develop a form of somatoform vertigo and dizziness described as phobic postural vertigo [9]. These patients demonstrate dizziness and subjective disturbance of balance in spaces such as bridges, crowed environments like shopping malls or supermarkets, which are accompanied by anxiety and avoidance of these challenging situations [19]. In line with the present findings, anxiety of these patients may be treated by balance treatment programs.

However, one of the caveats of this approach is that it is not clear whether the conventional balance treatment programs are actually targeting the vestibular system. Naïve rats exposed to acrobatic training similar to the training in the present study, demonstrated synaptogenesis in the paramedian lobule of the cerebellum [8]. In the clinical setting, vestibular rehabilitation leads to recovery of balance [18,28]. But the rehabilitation of balance after peripheral vestibular loss consists of exercise protocols aiming to compensate for the vestibular failure through enhancement of other balance-related systems, such as proprioception and vision [10,21]. These protocols generally combined with cognitive behavioral therapy, desensitization techniques and sporting activities are also used for the treatment of patients with phobic postural vertigo [19]. Similarly, we report here that the treated Hdb mice showed an impressive improvement of balance skills. However, currently there is no support for the claim that the behavioral training had any corrective effect on the structural or physiological phenotype of the vestibular end-organs. In fact, a few trained Hdb mice subjected to the vestibular-specific swim test at the age of 3 months showed chaotic underwater swimming, which required their immediate rescue from the water, and prevented us from applying this test to the rest of the sample. This shows that balance training had a minor, if any, effect on the vestibular system itself and the rehabilitated balance performance is most likely traced to the somatosensory and visual channels. This topic is currently being addressed in our follow-up studies.

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