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# Behavioral and functional assessment of mice inner ear after chronic exposure to an ultrahigh $B_0$ field of 11.7 T or 17.2 T

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**Purpose:** Assess short-term and long-term effects of chronic exposure to an ultrahigh static magnetic ( $B_0$ ) field on mice inner ear in the context of MR safety of human scanning at 11.7 T.

**Methods:** Mice were chronically exposed to a  $B_0$  field of 11.7 T or 17.2 T during ten 2-h exposure sessions evenly distributed over a period of 5 weeks, resulting in a total of 20 h of exposure per mouse. During exposure sessions, mice were anesthetized and positioned either parallel or antiparallel to  $B_0$ . Before, during, and 2 weeks after the magnetic-field exposure period, mice performed behavioral tests (balance beam, rotarod, and swim tests) to evaluate their short-term and long-term motor coordination and balance. An auditory brainstem response (ABR) test was finally performed to assess the functional integrity of mice cochlea, 2 weeks after the last exposure.

**Results:** After awaking from anesthesia following  $B_0$  exposures at 11.7 T or 17.2 T, mice displayed a transient (<5 min) rotating behavior. The behavioral tests did not show any difference between the exposed and the control mice at any time point. Determination of ABR thresholds did not reveal an impairment of cochlea hair cells resulting from chronic  $B_0$  exposure.

**Conclusion:** Despite the transient disturbance of mice vestibular system observed immediately after  $B_0$  exposure, no short-term nor long-term alteration was detected with behavioral and ABR tests.

## KEYWORDS

auditory brainstem response, behavioral tests, ultrahigh field, vestibular system

## 1 | INTRODUCTION

Technical improvements allow a continuous increase of MR scanner static magnetic ( $B_0$ ) fields that is pushed by the intrinsic signal and BOLD contrast boost needed to reach higher spatial and/or temporal resolutions. The first

in vivo human MR images at 10.5 T were obtained in 2019<sup>1</sup> and are expected in 2023 with the Iseult 11.7T scanner.<sup>2</sup> Exposure to high  $B_0$  fields is associated with a transient disturbance of the vestibular system,<sup>3,4</sup> with subjects experiencing dizziness, vertigo, and/or nausea. These adverse sensory effects disappear a few minutes after entering or

exiting the scanner, and are more prominent at higher  $B_0$  fields.<sup>5–7</sup> In addition, physiological studies have measured a horizontal nystagmus in healthy subjects exposed to  $B_0$  fields ranging from 1.5 T to 7 T<sup>8,9</sup> that persisted during the whole exposure session and which direction was dependent on the subject's head position in the scanner. Rodents are also subject to a disturbance of the vestibular system in high  $B_0$  field scanners, as demonstrated by their transient (<5 min) rotating behavior after exposure.<sup>10</sup> The orientation of their tight circling trajectories is also dependent on their head orientation in the scanner: It is maximal for rodents exposed collinearly to  $B_0$ , and minimal for rodents exposed orthogonally to  $B_0$ .<sup>11</sup> These behavioral observations directly implicate an involvement of the vestibular system that was furthermore confirmed by the postmortem detection of neural activation in the vestibular nuclei of the brainstem.<sup>11</sup> The physiological mechanisms behind these vestibular perturbations are not yet fully understood because of the difficulty in studying *in vivo* the peripheral vestibular system. As a result, it is studied by indirect tests, such as behavioral tests or by postmortem analyses.

The vestibular system consists of the utricle, the saccule, and the semicircular canals, and is located inside the inner ear along with the cochlea. The vestibular system and the cochlea are filled with endolymph and are responsible for the detection of head motion and sound, respectively, through mechanotransduction. This process is ensured by the bending of the stereocilia (hair-like organelles) present on the apex of the hair cells. More specifically, in the vestibular system, a movement of the head induces a displacement of the endolymph that exerts a pressure on the gel-like matrix surrounding the stereocilia (e.g., the cupula in the semicircular canals), further leading to the generation of an action potential in the vestibular fibers. Several hypotheses were proposed to explain the vestibular stimulations in high  $B_0$  fields<sup>4,12,13</sup>: (i) diamagnetic forces causing a displacement of the otoliths (crystals located in the utricle and saccule), (ii) magneto-hydrodynamic effects inducing a displacement of the endolymph, or (iii) electromagnetic induction of a neural stimulation resulting from a magnetic field gradient. In 2011, Roberts et al. introduced a new hypothesis,<sup>9</sup> in which the  $B_0$  field interacts with the ionic currents present in the endolymph and generates an orthogonal Lorentz force strong enough to induce a pressure on the gel-like matrix surrounding the stereocilia, which results in a vestibular stimulation. According to this hypothesis, the Lorentz force is generated mostly in the endolymph of the utricle, which displaces the cupula of the lateral and superior semicircular canals in subjects with supine position in MRI scanners. This force persists during the whole  $B_0$  exposure and would therefore explain the continuous

nystagmus, as well as the head orientation-dependent and field strength-dependent vestibular stimulation observed in high  $B_0$  fields.

In 2021, a study reported long-term behavioral effects in mice chronically exposed to an ultrahigh  $B_0$  field of 16.4 T,<sup>14</sup> with a total exposure time of 24 h distributed over a period of 4 weeks (two sessions of 3 h per week). Six weeks after the last  $B_0$  exposure session, some mice exposed at 16.4 T still exhibited behavioral alterations, such as abnormal circling behavior in water maze, whereas mice undergoing the same experimental protocol at 10.5 T did not display any difference with the controls. These observations suggest that the 24-h exposure had generated long-term damage to the vestibular system in mice in these extreme conditions and raise important questions regarding MR safety at ultrahigh  $B_0$  fields. As such, the current study focuses on the detection of a potential impairment of mice inner ear during and/or after chronic exposure to an ultrahigh  $B_0$  field. Whereas in Tkáč et al.,<sup>14</sup> the mice could move to some extent in the holders in which they were exposed, in the current study they were anesthetized to keep their heads aligned with  $B_0$ , as during a human scan. Mice were exposed for a total of 20 h over a period of 5 weeks (two sessions of 2 h per week), with a group of mice exposed to a  $B_0$  field of 11.7 T, another group to 17.2 T, and a last group not exposed (but anesthetized) to serve as control. As an assessment of the mice vestibular system, behavioral tests were conducted longitudinally throughout the  $B_0$  exposure period and repeated 2 weeks after the last  $B_0$  exposure session. An auditory brainstem response (ABR) test was additionally performed at the end of the study, to detect any long-term impairment of the cochlea function. The ABR test provides insight into the health of the cochlea, which is also subject to Lorentz forces,<sup>3</sup> and therefore was used to evaluate their long-term consequences on cochlea hair cells.

## 2 | METHODS

### 2.1 | Animals

Experiments were conducted under the approval of the Ministère de l'Enseignement Supérieur et de la Recherche and the regional committee on animal ethics (CETEA/CEA/DSV IdF, protocol ID A22 062) on 12 male and 12 female C57BL/6 mice (Janvier, Saint Isle, France) aged 9 weeks at the beginning of the study. Mice were maintained under standard laboratory conditions with free access to food and water, 12-h light–dark cycles, and housed in cages of four animals. They were weighted regularly throughout the study.

## 2.2 | $B_0$ field exposure

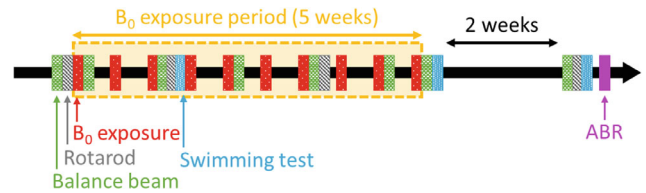
Mice were divided into three groups of  $N = 8$  animals (4 males and 4 females per group). The first group was used as a control (i.e., not exposed), the second group was exposed in an MRI preclinical scanner operating at 11.7 T (BioSpec; Bruker BioSpin, Ettlingen, Germany), and the third group in a scanner operating at 17.2 T (BioSpec).

Mice underwent 10  $B_0$  exposure sessions of 2 h each distributed evenly over a period of 5 weeks, resulting in a total of 20 h of exposure per mouse. No MR sequence (i.e., no RF waves nor gradients) was played during these sessions, so that mice were only exposed to the  $B_0$  field. During the  $B_0$  exposure sessions, mice were anesthetized with 1% isoflurane and placed in conical cylinders (50 mL volume and 30-mm-diameter Falcon tubes with holes in the front and the back for air flow), to restrain motion and ensure reproducible head position in the scanner across sessions. Mice of the control group underwent the same experimental conditions as exposed mice (anesthesia and placement in a conical cylinder). Mice exposed at 11.7 and 17.2 T were positioned horizontally at the center of the MRI preclinical scanners, half of them being oriented parallel to the  $B_0$  field and the other half being oriented antiparallel. Each mouse was systematically positioned in the same orientation across sessions.

## 2.3 | Behavioral tests

The set of behavioral tests performed to assess the mice vestibular system included balance beam, rotarod, and swimming tests. These tests were conducted longitudinally (i.e., before, during, and after the  $B_0$  exposure period). The chronology of the study is detailed in Figure 1, with the first day of the behavioral tests considered as Day 0. All behavioral tests were monitored by a camera and analyzed retrospectively.

The balance beam test consisted of having the mouse walk over a thin beam to reach a dark box, positioned at the end of the beam, where it could hide. The beams were 1 m long and horizontally positioned, 50 cm above the floor, and the escape box was  $20 \times 20 \text{ cm}^2$ . Before  $B_0$  exposure, mice were trained during two consecutive days, with two runs per day, using a 15.8-mm-wide beam with a square section. Two different beams were used on testing days: one 15.4-mm-wide beam with a round section and one 10.7-mm-wide beam with a square section. The mice performed two runs on each beam per balance beam session, with 10 min to rest between the runs. A total of eight sessions was performed (Figure 1): one before the  $B_0$  exposure period, then one session per week during the  $B_0$  exposure period; the tests were performed 1 day following



**FIGURE 1** Experimental protocol for the three groups of mice (controls, exposed at 11.7 T and at 17.2 T). Mice underwent 10  $B_0$  field exposure sessions of 2 h evenly distributed over a period of 5 weeks. They performed behavioral tests (balance beam, rotarod, and swimming tests) before, during, and 2 weeks after the  $B_0$  exposure period, to detect any potential short-term or long-term impairment in their motor coordination and vestibular function resulting from  $B_0$  exposure. Auditory brainstem response (ABR) tests were performed additionally at the end of the study to assess the functional integrity of mice cochlea, and more precisely the effect of Lorentz forces on cochlea hair cells.

a  $B_0$  exposure, and the last session 2 weeks after the last  $B_0$  exposure. The latency to cross the beam and the number of foot-slips were manually recorded for each run and served as indicators of mice balance and motor coordination.

For the rotarod test, the mouse had to walk on a rotating rod. The rotarod device (Rota Rod Touch, Panlab, Spain) was made of a 3-cm-diameter cylinder that was set to rotate at a speed linearly increasing from 5 to 50 rotations per minute over a period of 5 min. Before the experiments, mice were trained during two consecutive days with four runs per day. On testing days, the animals underwent four runs per rotarod session. Four rotarod sessions were performed (Figure 1): one before the  $B_0$  exposure period, one after the beginning of the  $B_0$  exposure period, one at the middle of the  $B_0$  exposure period, and the last rotarod session 2 weeks after the last  $B_0$  exposure. Rotarod tests were performed consecutively on the same days as the balance beam sessions, after a few hours of rest. The latency to fall off the rod was automatically recorded by the rotarod device, as an indicator of mice motor coordination.

For the swimming test, the mouse was put at a starting point in a rectangular water tank (Figure 5A,  $72 \times 50 \text{ cm}^2$  tank, 10 cm deep, water temperature of  $23^\circ\text{C}$ ) and had to swim to a visible platform (13-cm-diameter cylinder) located at the other side of the tank (distance between the starting point and the platform = 50 cm). The mice underwent two runs per swimming session, with 15 min to rest between the runs. Three swimming sessions were performed (Figure 1): one at the middle and one at the end of the  $B_0$  exposure period (the tests being performed 2 days following a  $B_0$  exposure), and the last session 2 weeks after the last  $B_0$  exposure. Swimming trajectories were monitored using a dedicated tracking software (*Smart 3.0*, Panlab) that allowed extracting the swim distance to the platform. Potential rotations of the mice occurring during

**TABLE 1** Results of the two-way ANOVAs performed for the behavioral and ABR tests, to compare the results of the three groups of mice. Significance threshold was set to  $p < 0.05$ .

Nature of the test	Tested Parameter	Two-Way ANOVA First Variable $p$ -Value	Two-way ANOVA Second Variable $p$ -Value	Interaction Term
Balance beam	Latency to cross (round beam)	Time; 0.02	$B_0$ field; 0.69	0.35
	Number of foot-slips (round beam)	Time; < 0.001	$B_0$ field; 0.27	0.62
	Latency to cross (square beam)	Time; < 0.001	$B_0$ field; 0.70	0.77
	Number of foot-slips (square beam)	Time; 0.04	$B_0$ field; 0.94	0.56
Rotarod	Latency to fall	Time; 0.04	$B_0$ field; 0.99	0.77
Swimming test	Swimming distance	Time; < 0.001	$B_0$ field; 0.86	0.11
	Number of rotations	Time; 0.02	$B_0$ field; 0.46	0.65
ABR test	ABR threshold	Sound frequency; < 0.001	$B_0$ field; < 0.001	0.99

swimming (i.e., 360° turns), which could reveal alterations of the vestibular system, were manually counted. Swim distance and the number of rotations served as indicators of mice motor coordination and balance.

## 2.4 | ABR tests

ABR tests were performed after the end of all behavioral tests (i.e., 3 weeks after the last  $B_0$  exposure; see Figure 1). The ABR test provides insight into the health of the cochlea by measuring the lowest sound pressure level (SPL), which generates an evoked potential in the brainstem when presenting an auditory stimulus played at a given frequency. ABR tests were performed under light anesthesia (1% isoflurane) to minimize electrical activity from the neck muscles, and mice were placed on a heating blanket to avoid hypothermia. Five auditory stimuli were tested in the current study; they consisted of four pure-tone frequencies (4, 8, 16, and 32 kHz, 20 ms in duration, rise-fall time of 2 ms) and a wide-spectrum sound (i.e., a 100- $\mu$ s “click,” which presented monaurally at a rate of 15 Hz using an insert earphone [Knowles Electronics, Itasca, IL, USA] placed in the animal right ear canal). They were played with intensities decreasing from 80 to 0 dB with 5-dB steps. ABRs were recorded using two subcutaneous electrodes (SC25; Neuroservice, Aix-en-Provence, France) located just above the tympanic bulla and skull dorsal midline, and one ground electrode placed in the thigh. The signal was digitized (sampling rate = 32 000 Hz) and filtered (150–1500 Hz). For each frequency and intensity, 500 raw traces were acquired and averaged using the *RTLab* software (Echodia, Clermont-Ferrand, France). ABR thresholds corresponding to the minimal intensities at which an evoked response could be detected on the averaged ABR traces (usually Waves II and III; see details in

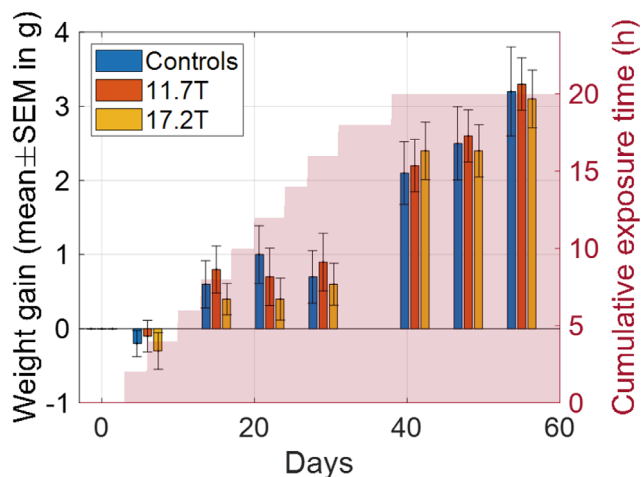
Royer et al.<sup>15</sup>) were assessed for each stimulus. Mice for which “click” stimulus ABR threshold was equal or superior to 70-dB SPL were considered as deaf and removed from analysis. The ABR tests were performed blind of the animals’ exposure status.

## 2.5 | Statistics

Behavioral results are presented as mean over the group  $\pm$  SEM. When multiple runs of one test were performed during a session, the results of the runs were averaged. Statistical analyses were run with *GraphPad Prism* version 9.4.1 (GraphPad Software, San Diego, CA, USA). After a verification of normality using Shapiro–Wilk test, data were analyzed with two-way repeated-measures analyses of variance (ANOVAs) followed by Tukey’s multiple comparisons test, to compare the results of the three groups of mice. For the behavioral tests, the two variables of the two-way ANOVA were the  $B_0$  field (i.e., earth field for the control group, 11.7 T or 17.2 T for the exposed mice) and time. For the ABR test, the two variables were the  $B_0$  field and the sound frequency. Significance threshold was set to  $p < 0.05$ . A summary of the results of the two-way ANOVAs is provided in Table 1.

## 3 | RESULTS

After  $B_0$  exposure, mice were removed from the conical tubes and brought back to their home cages where they could rest. When awaking from anesthesia, the mice exposed at 11.7 T and 17.2 T showed the expected transient rotating walking behavior. Mice exposed parallel to the  $B_0$  field were rotating counter-clockwise, whereas mice exposed antiparallel to the  $B_0$  field were rotating clockwise.



**FIGURE 2** Mice weight gain measured longitudinally over the study and averaged for each group of mice (controls, exposed at 11.7 T and exposed at 17.2 T). The weight gain is measured relative to the first day of the study (i.e., the first day when the behavioral tests were performed). The corresponding cumulative exposure time (i.e., the total time spent by the mice in the scanners) is also displayed in this graph (right vertical axis).

This effect was no longer observable 5 min (or less) after the mice were taken out of the scanners.

Figure 2 shows the body-weight variation across time for each group of mice as compared with the first day of the study (i.e., the first day when the behavioral tests were performed). The weight loss measured in the first week can be related to a stress caused by the mice undergoing anesthesia for the first time. All mice then put on weight regularly, with an average of  $3.2 \pm 0.4$  g in 55 days over all groups. Mice weight gain did not show a significant variation with  $B_0$  field (two-way ANOVA,  $p$ -value for  $B_0$  field = 0.90). The corresponding interaction term for time  $\times B_0$  field was 0.52. Furthermore, multiple comparison tests did not show a significant difference among the three groups of mice at any time point.

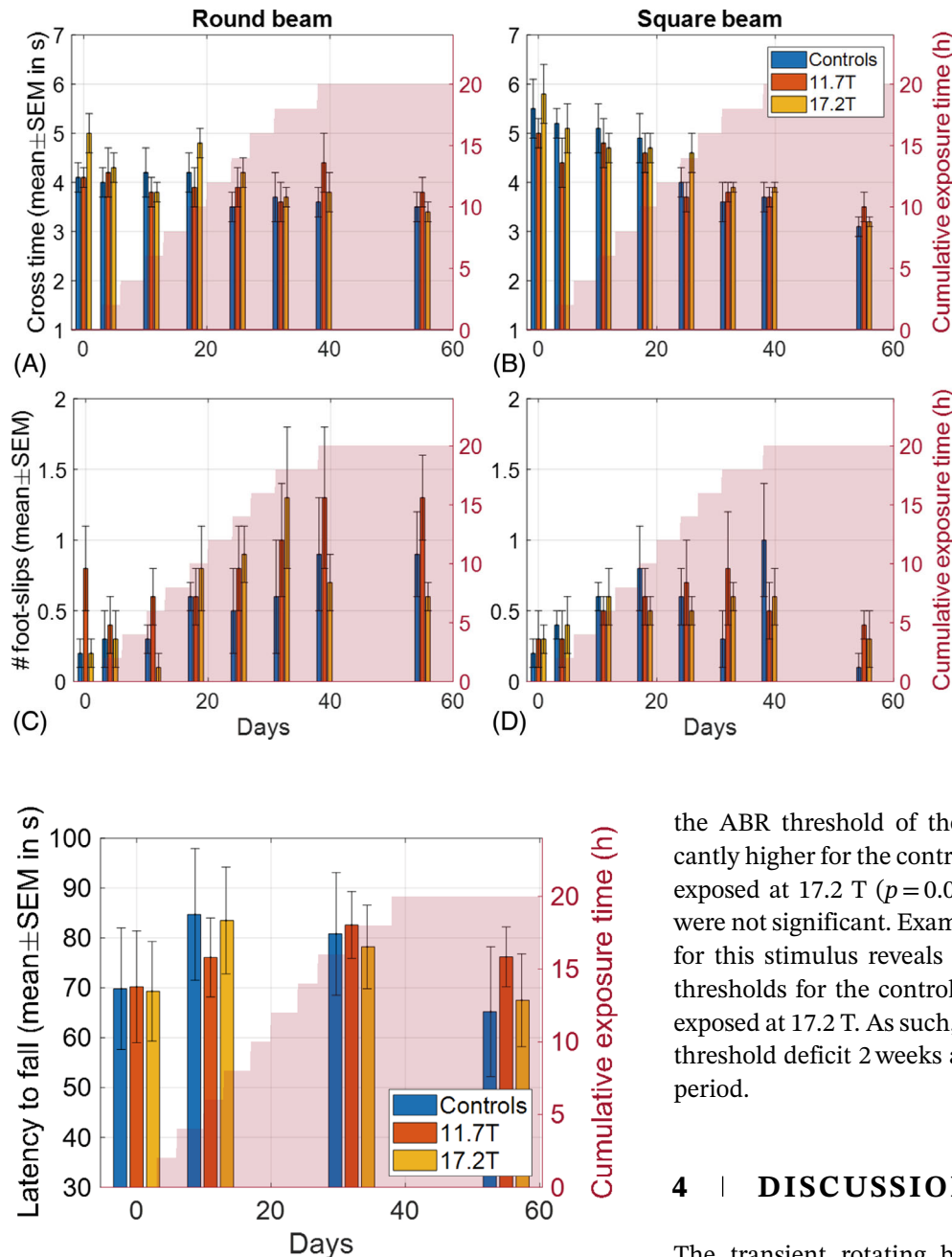
Behavioral tests (balance beam, rotarod, and swimming test) were performed longitudinally over the study. The results of the balance-beam tests, which the mice performed once per week on two different beams (a round one and a square one), are presented in Figure 3. During the  $B_0$  exposure period, these tests were performed on the next day following a  $B_0$  exposure session. All groups of mice showed a decreasing latency to cross the beams over time (Table 1), and no significant difference with  $B_0$  field was observed ( $p$ -value for  $B_0$  field = 0.69 for the round beam and 0.70 for the square beam). The corresponding interaction terms for time  $\times B_0$  field were 0.35 and 0.77, respectively. The mean number of foot-slips on the beams showed a significant increase over time (Table 1), which can be explained by the increased speed of the mice when

running on the beams. No significant difference with  $B_0$  field was observed ( $p$ -value for  $B_0$  field = 0.27 for the round beam and 0.94 for the square beam), and the corresponding interaction terms for time  $\times B_0$  field were 0.62 and 0.56, respectively. Furthermore, multiple comparison tests did not show a significant difference among the three groups of mice at any time point, neither for the latency to cross the beams nor for the number of foot-slips.

In addition to balance beam tests, mice performed four sessions of the rotarod test (Figure 4). These tests were performed consecutively after a balance beam session, after a few hours of rest. The latency to fall off the rod showed a significant variation over time (Table 1), and no significant difference with  $B_0$  field was observed ( $p$ -value for  $B_0$  field = 0.99). The corresponding interaction term for time  $\times B_0$  field was 0.77. Furthermore, multiple comparison tests did not show a significant difference among the three groups of mice at any time point, confirming the apparent absence of short-term or long-term abnormalities in motor functions.

The mice performed three sessions of the swimming test (Figure 5) to detect potential circling behaviors. The two first sessions were performed 2 days following a  $B_0$  exposure, and the last one 2 weeks following the last  $B_0$  exposure. The data from 19 of these 144 runs (6 runs for the control mice and 13 runs for the mice exposed at 11.7 T) were not considered in the analysis: they were considered as failed, because the mice escaped the water tank from its side. The swimming distance to the platform decreased over time (Table 1), but no significant difference with  $B_0$  field was observed ( $p$ -value for  $B_0$  field = 0.86). The corresponding interaction term for time  $\times B_0$  field was 0.11. While swimming to the platform in the water tank, the mice did less than one rotation, on average, and the number of rotations showed a decreasing tendency over time (Table 1), likely caused by the decreased swimming distance. No significant difference with  $B_0$  field was observed ( $p$ -value for  $B_0$  field = 0.46), and the corresponding interaction term for time  $\times B_0$  field was 0.65. Furthermore, multiple comparison tests did not show a difference among the three groups of mice at any time point, neither for the swimming distance nor for the number of rotations. Overall, no behavioral impairment resulting from the chronic exposure at ultrahigh field was observed, neither during, nor 2 weeks after the end of the  $B_0$  exposure period.

ABR tests were performed after the end of behavioral tests. The results of these tests are presented in Figure 6. One mouse of the control group was considered deaf and as such was excluded from the analysis, and a technical problem occurred for one mouse of the group exposed at 11.7 T for 8-kHz and 16-kHz stimuli, so these two data points were removed from the analysis. Two-way ANOVA results showed a significant variation



**FIGURE 4** Results of the four sessions of the rotarod test performed longitudinally over the study. The ability of mice to stand on the rotating rod reflects their motor coordination. Results are averaged for each group of mice (controls, exposed at 11.7 T and exposed at 17.2 T). The cumulative exposure time (i.e., the total time spent by the mice in the scanners) is also displayed in this graph (right vertical axis).

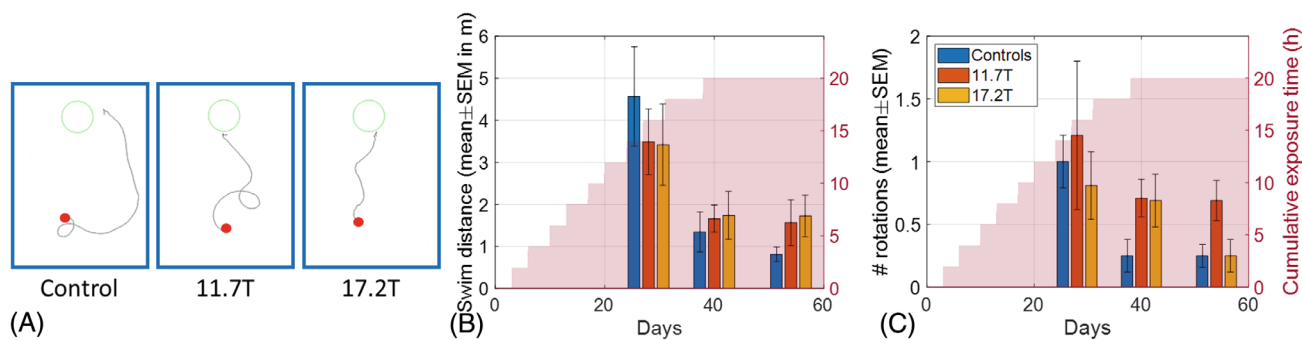
between the brainstem response thresholds and  $B_0$  field ( $p < 0.001$ ), but not a significant interaction term between  $B_0$  field and sound frequency ( $p = 0.99$ ), corresponding to the mice exposed at high  $B_0$  fields having evoked potentials generated for stimuli played at slightly lower intensities. Multiple comparison tests showed that only

**FIGURE 3** Results of the eight sessions of the balance beam test performed longitudinally over the study as an assessment of mice balance. (A,B) Latency to cross the round-section and square-section beams, respectively. (C,D) The corresponding number of foot-slips. Results are averaged for each group of mice (controls, exposed at 11.7 T and exposed at 17.2 T). The cumulative exposure time (i.e., the total time spent by the mice in the scanners) is also displayed in these graphs (right vertical axes).

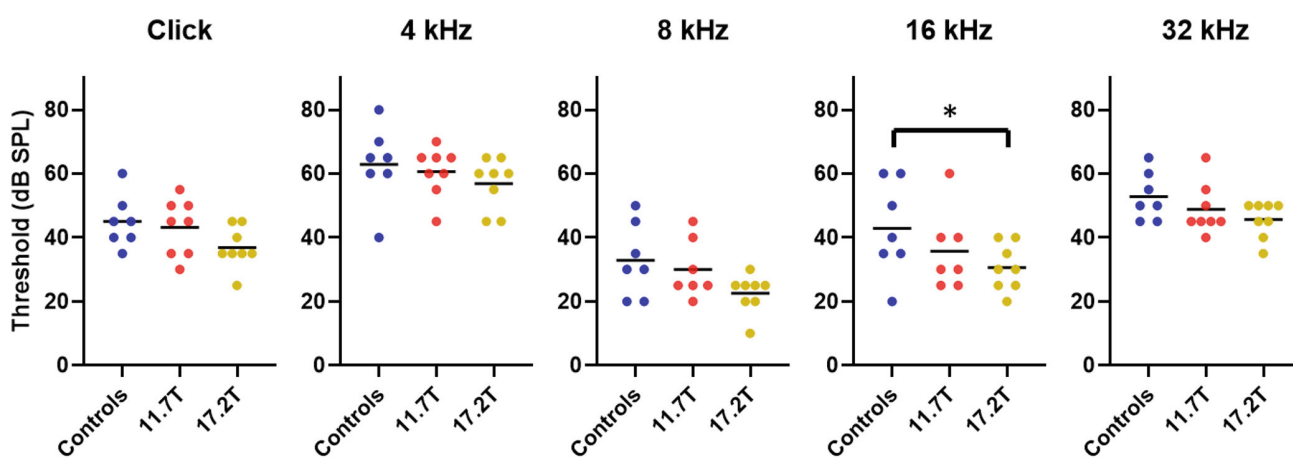
the ABR threshold of the 16-kHz stimulus was significantly higher for the control mice compared with the mice exposed at 17.2 T ( $p = 0.03$ ), whereas other comparisons were not significant. Examination of the individual results for this stimulus reveals a wider dispersion of the ABR thresholds for the control mice compared with the mice exposed at 17.2 T. As such, there was no apparent auditory threshold deficit 2 weeks after the end of the  $B_0$  exposure period.

## 4 | DISCUSSION

The transient rotating behavior observed immediately after  $B_0$  exposure at 11.7 T and 17.2 T indicates a compensatory response to a vestibular stimulation that persists a few minutes after the end of  $B_0$  exposure. The direction of rotation observed here (counter-clockwise for mice exposed parallel to  $B_0$  and clockwise for mice exposed antiparallel to  $B_0$ ) is in agreement with observations previously reported in the literature.<sup>10,11</sup> According to Houpt et al.,<sup>11</sup> where mice were exposed to the  $B_0$  field restrained in conical tubes similar to the ones used in the current study, maximal vestibular stimulation was obtained when mice were positioned parallel or antiparallel to  $B_0$  and minimal when mice were exposed orthogonally to  $B_0$ , which means that the mice in our study experienced the maximal achievable vestibular stimulation. Despite this strong and chronic exposure to  $B_0$  field, only this transient



**FIGURE 5** Results of the swimming test. (A) Example of swimming trajectories of three mice (one per group) from the starting point (red dot) to the platform (green circle) in the water tank (blue rectangle). (B,C) Swimming distance (B) and number of rotations (C) measured longitudinally over the study and averaged for each group of mice (controls, exposed at 11.7 T and exposed at 17.2 T). The cumulative exposure time (i.e., the total time spent by the mice in the scanners) is also displayed in these graphs (right vertical axes).



**FIGURE 6** Individual results of the ABR test performed 3 weeks after the last  $B_0$  exposure session, to assess mice cochlea functional properties. Five audio stimuli were presented to the mice (four pure-tone frequencies and a wide-spectrum “click”). The thresholds represent the intensity of the audio stimuli that generated evoked potentials in the mice auditory brainstem, measured for each mouse (controls, exposed at 11.7 T and exposed at 17.2 T). The mean ABR thresholds are also indicated for each group of mice. \* $p < 0.05$ .

disturbance of the inner ear could be observed with our experimental protocol. Indeed, the results of the behavioral tests performed 1 or 2 days following a  $B_0$  exposure session were not statistically different between control and exposed mice, even after a cumulative exposure time of 20 h at 11.7 T or 17.2 T. In addition, 2 to 3 weeks following the last  $B_0$  exposure session, the behavioral and ABR tests did not reveal any residual long-term disturbance of the inner ear. As such, even if it is unclear how these results translate to humans, our observations do not point to major contraindications to the human exposure at 11.7 T, or even higher.

Abnormal weight gain could be a marker of stress for mice. Although the experimental protocol of the current study may have induced a stress because of the repeated anesthesia and behavioral tests, the chronic exposure to ultrahigh  $B_0$  fields did not result in body-weight gain deficit over time, as compared with the control mice. These

results are in line with Lv et al.<sup>16</sup> and Khan et al.,<sup>17</sup> where mice exposure to ultrahigh  $B_0$  fields was shown to attenuate anxiety in the long term.

Only a few studies have explored the long-term effects of  $B_0$  exposure on the vestibular function. In Houpt et al.,<sup>18</sup> awake mice were exposed for 30 min in conical tubes to a  $B_0$  field of 14.1 T. The swimming test revealed a tight circling behavior for all the mice tested immediately after  $B_0$  exposure, whereas the results of the mice tested 60 min to 4 days after  $B_0$  exposure did not differ from the control group. In Houpt et al.,<sup>19</sup> rats pre-exposed for 30 min to a  $B_0$  field of 14.1 T and re-exposed 36 days later for 30 min presented a reduced circling behavior compared with the rats that were not pre-exposed, suggesting a long-term habituation to the behavioral effects. In Tkáč et al.,<sup>14</sup> the intensive and chronic exposure of mice to a  $B_0$  field of 16.4 T with a cumulative exposure time of 24 h resulted in long-term behavioral effects in some mice that were still



observable 6 weeks after the last  $B_0$  exposure session. In the current study, we chose to also apply such an extreme exposure protocol, to exacerbate any physiological effect occurring due to  $B_0$  field exposure. The idea of repeating the behavioral tests over time was to detect a potential cumulative dose effect, above which a vestibular deficit could occur. However, no abnormal behavior was observed during the chronic exposure to  $B_0$  field, nor 2 weeks after the last exposure session with the balance beam, rotarod, and swimming tests. In Tkáč et al.,<sup>14</sup> the behavior of the mice chronically exposed at 16.4 T was significantly different compared with the control mice for the balance beam test (increased number of foot-slips) and the swimming test (tight circling trajectories). The differences in the experimental protocols may explain the different results obtained in the two studies. In Tkáč et al.,<sup>14</sup> the mice were moving freely in the  $7 \times 14 \text{ cm}^2$  holders in which they were exposed by groups of  $N = 2$  animals, whereas in the current study, the mice were static during the exposure, as during human scanning. In Tkáč et al.,<sup>14</sup> the mice were awake, whereas in the current study they were anesthetized. Chronic exposure to anesthetics has been shown to affect balance in the long term.<sup>20</sup> Nevertheless, because the mice were restrained for 2 h in conical tubes, sedation was mandatory in order to comply with the local ethical protocol. In Tkáč et al.,<sup>14</sup> the mice were exposed in 3-h-long sessions, whereas the sessions lasted 2 h in the current study, although at higher field strengths. The last difference of interest lies in the number of animals exposed in the two studies: The reduced number of mice used in the current study may have hindered the detection of a vestibular deficit. This sample size, however, was favored to perform the cumbersome behavioral tests every week. As such, the repetition of the measurements increased the statistical power of these tests, thus partially compensating for the reduced number of animals. Some settings of the experimental protocol may also have interfered with mice performance in the behavioral tests, thus hampering the detection of a vestibular deficit: As mice were anesthetized and constrained during the exposure, they may not have fully recovered their motor functions on the day following the exposure, when the balance beam and rotarod tests were performed. However, the results of the balance beam and rotarod tests performed before and during the  $B_0$  exposure period do not show a decreased performance. Regarding the swimming test, the comparison between the results obtained in the current study and in Tkáč et al.<sup>14</sup> is not straightforward because of significant differences in the experimental setups. The swimming duration was substantially shorter in the current study due to the smaller size of the water tank and the presence of a visible platform. This shorter swimming duration, combined with the presence of visual cues and the learning

of the exercise, may have facilitated mice orientation and diminished the pure vestibular behavioral guidance, thus minimizing the probability to observe tight circling trajectories.

ABR thresholds depend particularly on the mechanotransduction capabilities of cochlea hair cells and represent an assessment of cochlea properties. The endolymph in the cochlea is subject to ionic currents as well as the endolymph in the vestibular system, and as such to Lorentz forces when placed in a magnetic field.<sup>3</sup> The results of the current study show that the chronic exposure to ultrahigh  $B_0$  fields did not result in an increase of the ABR thresholds. More surprisingly, the ABR thresholds were even slightly (but significantly) higher for the control mice than for the exposed mice. We do not have an explanation for this result, which is beyond the scope of the current article. Given the SD of 8 dB SPL measured over the control group of eight mice, we were able to detect an effect size affecting the cochlea of 12 dB SPL with a significance level of 0.05 and a power of 0.8. To what extent these results can be extrapolated to the hair cells of the vestibular system remains to be determined, as the anatomical differences in these two structures (e.g., hair cells number, orientation) may result in different effects from Lorentz forces. Still, given the lack of methods available to study *in vivo* the vestibular system, the ABR test was used in the current study as an attempt to evaluate the consequences of Lorentz forces on hair cells. The optical coherence tomography method appears to be a promising tool for imaging *in vivo* the inner ear,<sup>21</sup> but it would require thorough developments to be used in such conditions. The ABR results are nonetheless in line with the behavioral results in the sense that no long-term impairment of the inner ear was detected after chronic exposure to an ultrahigh field in our experimental setting (with anesthesia and no head movement).

## 5 | CONCLUSION

Immediately after exposure to an ultrahigh static magnetic field, the mice experienced a transient disturbance of the vestibular system demonstrated by their temporary rotating behavior when they returned in their home cage. However, on the day following  $B_0$  exposures, no alteration of the mice inner ear could be detected with behavioral tests sensitive to balance and motor coordination, even after chronic exposure of the mice (i.e., 20 h of  $B_0$  exposure distributed over a period of 5 weeks). Finally, no long-term impairment of the mice inner ear properties could be detected in the weeks following the last  $B_0$  exposure session, as assessed with behavioral and ABR tests.

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