SUPPORTING INFORMATION

S1. SUPPORTING INFORMATION - METHODS

S1.1. Animals

All experiments were performed according to procedures approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Minnesota. Male and female C57BL/6 mice (Charles River Laboratories, Wilmington, MA, USA) were used for all experiments. Animals were maintained under standard laboratory conditions with free access to food and water and 12 h light-dark cycle.

S1.2. Magnets and animal holders

The MR magnets used for these studies were a whole-body human 10.5T magnet (Agilent/Magnex) with 88-cm clear bore and a 60-cm patient/subject accessible bore and the animal, horizontal bore 16.4T magnet horizontal bore magnet (26 cm ID clear bore and 12 cm ID accessible bore) for small and medium size animals. (Varian/Magnex). The mouse holders were designed to hold up to 12 mice. The overall dimensions of the holder were limited by the 16.4T magnet bore size (inner diameter = 12 cm), and also by the axial dimension of the bore volume with variations in the B_0 field homogeneity < 1% (± 42 cm from the magnet center). The complete holder consisted of six small plastic bins (height = 8 cm, width = 7 cm, length = 14 cm) attached to a holder support piece (Figure S1). The bottom of each plastic container was filled with rodent cage bedding. The temperature was maintained at 24°C using warm water circulating through attached hoses. The air exchange in mouse containers was maintained by continuous air flow through silicone tubing connected to mouse containers. Each bin was used only for two mice to allow them enough space for such a long exposure time (3 hours). This was required by the IACUC guidelines on physical restrain of mice to minimize their discomfort and distress. Mice were awake during the exposure and could freely move inside the container. Neither general anesthesia nor sedation of mice was used. An identical copy of the mouse holder was used for the control group of mice, but for the exposure the holder was placed in a plastic tube mimicking the visual sense of confinement experienced in the magnet bore. The same holder was also used for the 10.5 T field exposure.

S1.3. *In vivo* [1]H MR spectroscopy

During the MRI/MRS experiment, spontaneously breathing animals were anesthetized with 1.0 – 1.5% isoflurane. The depth of anesthesia was monitored using continuous respiratory rate monitoring (SA Instruments, Stony Brook, NY). Uniform temperature was maintained inside the magnet using circulating warm water in tubes surrounding the cradle containing the animal. All MR experiments were performed using a 9.4T/31cm horizontal bore magnet (Varian/Magnex Scientific, Yarnton, UK) equipped with a 15-cm gradient/shim coil (Resonance Research, Billerica, MA, USA) and interfaced to a DirectDrive console (Agilent/Varian, Palo Alto, CA, USA). The homogeneity of the magnetic field was adjusted by FASTMAP automatic shimming (1). The ^{1}H MR spectra were acquired using an ultra-short echo-time STEAM (TE = 2 ms, TR = 5 s, number of averages = 320) localization sequence combined with VAPOR water suppression (2). The ^{1}H MRS data were acquired from the volume-of-interest (VOI = 2.0 x 1.2 x 1.6 mm^3) centered in left hippocampus using the multi-slice fast spin-echo imaging for a precise VOI positioning. Metabolites were quantified using LCModel with the spectrum of fast relaxing macromolecules included in the basis set. The unsuppressed water signal was used

as an internal reference, assuming 80% brain water content. Only metabolites that were consistently quantified with the Cramèr-Rao lower bounds below 50% were included for further analysis.

S1.4. Morris water maze test

The Morris water maze (MWM) tests were used to evaluate the spatial learning of B_0 exposed mice. A hard plastic 120-cm diameter round pool was filled with water to the depth of 40 cm. The water was maintained at room temperature (~24°C) and was made opaque by adding 100 mL of non-toxic latex white paint. The pool contained a square-shaped acrylic platform (13 cm x 13 cm) submerged 1 cm below the water line. The water pool was virtually divided into four quadrants (zones): north (N), east (E), south (S), and west (W), and large visual cues (white square, circle, triangle and cross with 4-cm wide, black outline) were posted on each of four walls of the room surrounding the water pool.

Except for the first MWM test of Protocol I, all MWM tests started the week following the last B_0 exposure (Figure 1). During the first four testing days, animals were trained to find a hidden platform. The platform was re-located between three MWM sessions in Protocol I (NW – before B_0 exposure, SW – 4 week B_0 exposure, SE – 8 week B_0 exposure). For all MWM tests of Protocol II, the platform was placed in the center of W quadrant. Each day consisted of four trials in each of which animals started in a different zone (Protocol I – NW, NE, SE or SW; Protocol II – N, E, S or W). Animals were given a maximum time of 60 s (Protocol I) or 90 s (Protocol II) to locate and escape onto the submerged platform. If an animal failed to reach the platform within the allotted time, it was gently guided to the platform and allowed to sit there for about 5 s before being removed from the pool. In such case, the escape latency was set to the maximum test duration, 60 s and 90 s for protocols I and II, respectively. Mice were tested subsequently in groups of four using an interleaved study design (i.e. the following mouse number order: (1 2 3 4) repeated four times, (5 6 7 8) repeated four times …). On the fifth day of MWM testing, the hidden platform was removed from the pool for a probe trial in which the mice were allowed to swim freely for 60 s. The fractions of the total test time spent in different zones of the MWM pool were evaluated.

The trajectories of swimming mice were automatically recorded by the mouse motion video tracking system (ANY-maze, Stoelting Co, USA). Parameters, such as the time (latency) to reach the platform, total distance travelled, and time spent in different zones were evaluated directly by the ANY-maze data processing tools. The angular velocity was calculated from the raw ANY-maze tracking data using in-house software. Only rotations completed within 4 seconds (angular velocity > 90°/s) were counted for tight circling evaluation.

S1.5. Balance beam walking

The balance beam (BB) walk task assesses an animal's ability to maintain balance while traversing a narrow beam to reach a safe platform. Mice were placed on the beam at one end and allowed to walk to the goal box (20 x 20 cm, with a 4 x 5 cm entrance hole). Recorded measurements included the latency to cross the beam and the number of hindpaw fault or slips. Mice underwent three days of beam walk training prior to the B_0 exposure. During this training period, the mice were trained (four trials per day) to cross a 17-mm wide rod of a square cross-section (100 cm in length, elevated 50 cm above a table surface). After 4-week exposure to the B_0 field, the animals were tested in a single day (the next day after the last B_0 exposure, Figure 1) on three types of rods of varying widths (8 – 17 mm)

and varying cross-sectional shapes (square or round): 15 mm square, 17 mm round, 8 mm square. Two trials were carried out for each rod type.

S1.6. Rotarod test

The Rotarod apparatus (Ugo Basile S.R.L., Italy) with a rotating cylinder of a 3-cm diameter was used to evaluate the motor coordination and balance. The rotational speed accelerated from 5 to 50 rpms over a maximal trial length of 300 sec. Latency at which mice fell off the rotating cylinder was measured. Prior to the B_0 field exposure, mice were trained on Rotarod for 3 days, four trials per day. After the 4-week exposure to the B_0 field, mice were tested on Rotarod using four trials in one day. Rotarod testing was performed two days after the last B_0 exposure (Figure 1).

S1.7. Fear conditioning test

The contextual and cued fear-conditioning (FC) test was used to assess the ability of mice to learn and remember an association between environmental context and discrete cues paired with an aversive experience (3). Freezing behavior during the test was measured as an index of fear memory. Data collection and analysis were automated via a video-monitoring fear-conditioning apparatus (Med Associates, Inc.). Fear conditioning and testing was performed in two days at the end of behavioral testing battery, approximately 10 days after the last B_0 field exposure (Figure 1). On day 1 (conditioning), mice were exposed to a series (5 pairings, 60 s inter-trial interval) of cue presentations (80 dB white noise tone and light, 20 s duration) that co-terminated with a mild foot-shock (electrical current of 0.7 mA, 1 s duration). Mice were re-exposed to the test context (3 min) 24 hours later to assess context-induced freezing. After this test, mice were returned to their home cages for two hours, and then re-tested for freezing behavior in a novel context (altered visual, tactile and olfactory design of the chamber). This second test consisted of a three minute baseline (non-specific freezing behavior) and three minute cue exposure (cued fear) period.
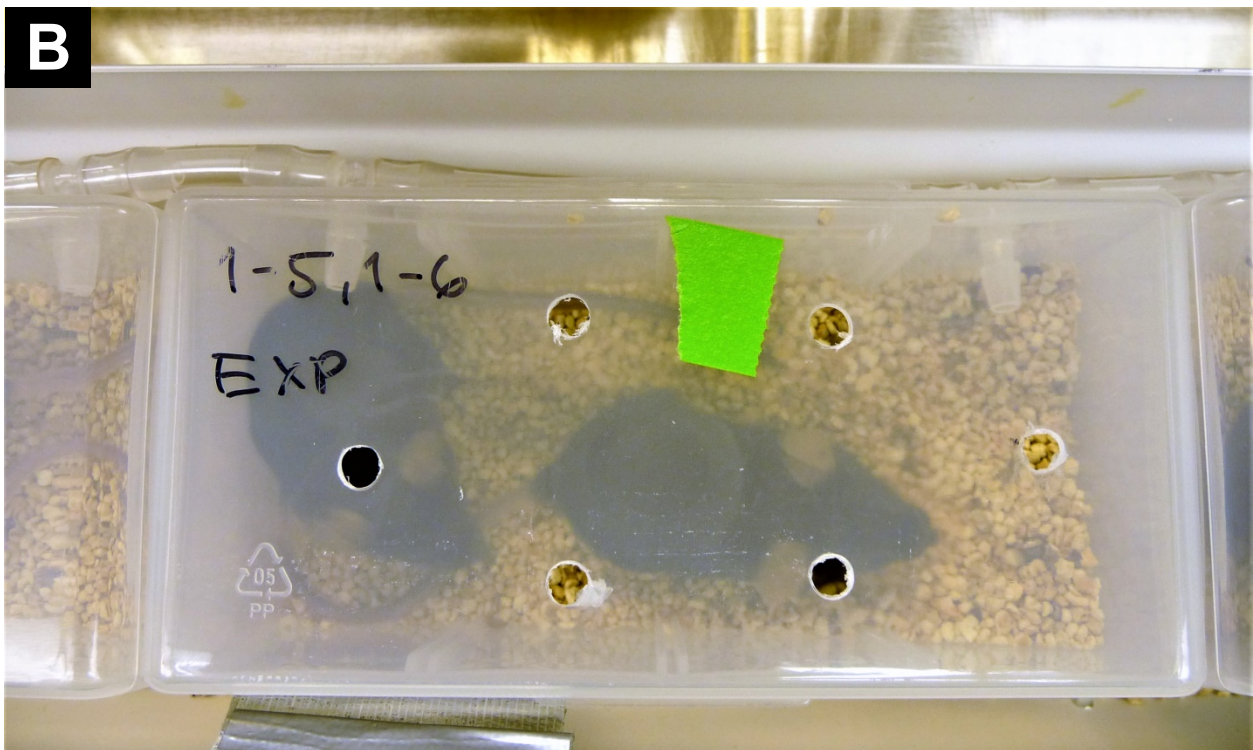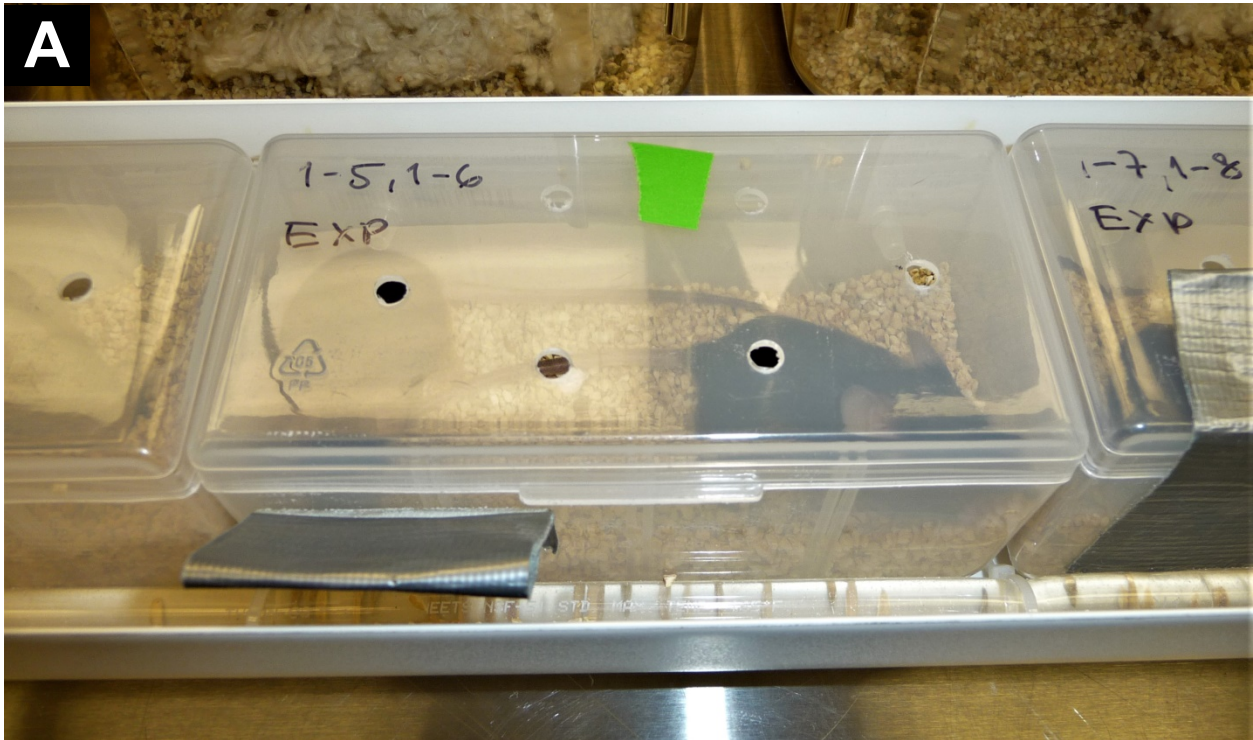
S1.8. Statistical analysis

The neurochemical data were analyzed using the t-test with a modest correction for multiple comparisons (False discovery rate, Benjamini-Hochberg procedure, q = 0.2). All behavioral test data (except rotarod and FC) were analyzed using two-way ANOVA using the B_0 exposure as a first factor and the test day (MWM – escape latency, swim distance, tight circling), the MWM zone (MWM – Probe) or session type (FC – baseline, context, cue). The ANOVA tests were accompanied by the multiple comparison analysis (relative to the control group) corrected by Dunnett's method (Prism, GraphPad Software, Inc.). Rotarod and BB walking tests were analyzed using one-way ANOVA. All bar plots show the mean and error bars representing the standard deviation (SD) for spectral quantification and standard error of the sample mean (SEM) for the behavioral data.

S1.9 References

1. Gruetter R, Tkac I. Field mapping without reference scan using asymmetric echo-planar techniques. Magn Reson Med 2000;43(2):319-323.

2. Tkac I, Starcuk Z, Choi IY, Gruetter R. In vivo 1H NMR spectroscopy of rat brain at 1 ms echo time. Magn Reson Med 1999;41(4):649-656.

3. Shoji H, Takao K, Hattori S, Miyakawa T. Contextual and cued fear conditioning test using a video analyzing system in mice. J Vis Exp 2014(85).

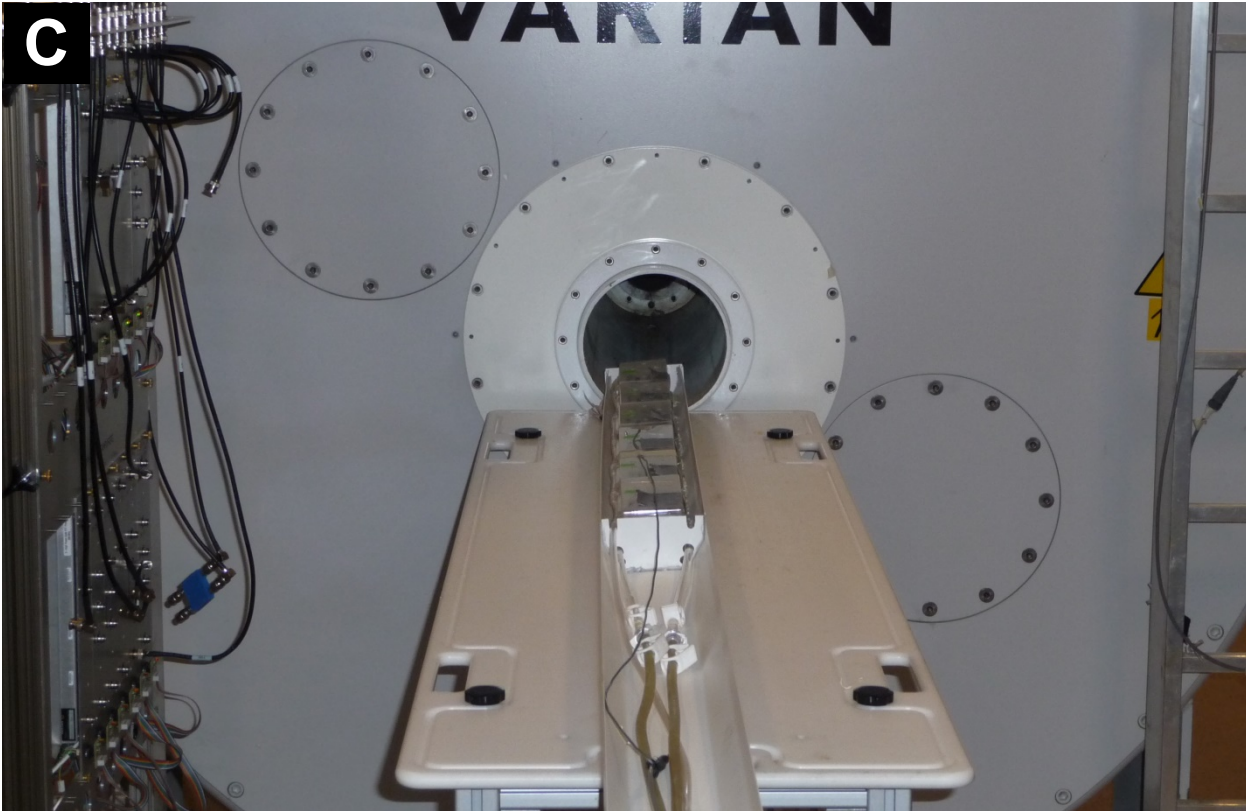## S2. SUPPORTING INFORMATION – FIGURES

Figure S1. Images of the animal holder. (**A**,**B**) Plastic bins of the animal holder, two mice were kept inside each bin during exposures to magnetic field. (**C**) The animal holder in front of the 16.4 T magnet. The temperature was maintained at 24°C using warm water circulating and the air exchange in mouse containers was maintained by continuous air flow through silicone tubing connected to mouse containers.
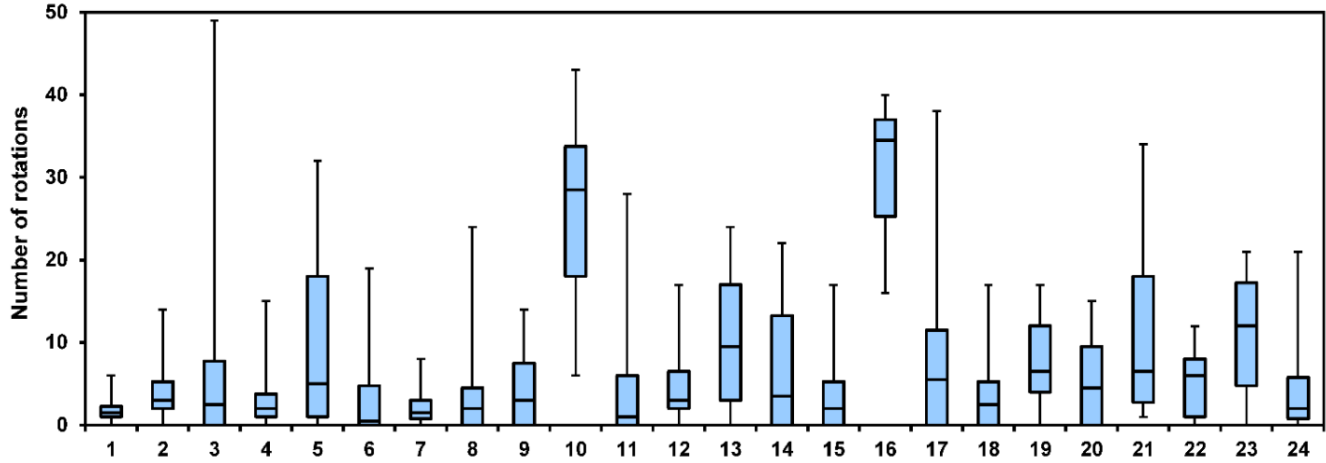
Figure S2. Morris water maze test – Protocol II. Angular velocity analysis – number of tight-circling 360° rotations for all mice chronically exposed to 16.4 T. The box plot represents data of 16 MWM trials (4 trial per test day, 4 days of testing). Boxes indicate median with upper and lower quartiles, whiskers indicate maximum and minimum values.

S3. SUPPORTING INFORMATION – TABLES

Table S1. Protocol I: Morris water maze tests performed on mice before exposure to magnetic field. Neither control (N = 12) nor 16.4T* group (N = 12) were exposed to any magnetic field. The label 16.4T* means that this group of mice was later exposed to 16.4 T magnetic field. Submerge platform was located in the north-west (NW) quadrant (zone). Table values represent mean ± SD.

MWM Test parameter	Group	Day 1	Day 2	Day 3	Day 4
Escape latency (s)	Control	35.3± 8.0	23.3±15.5	26.2±15.0	21.0±13.4
	16.4T*	42.7± 9.4	25.2± 8.8	17.4±8.7	15.4± 9.5
Swim distance (m)	Control	6.08±1.62	3.45±2.04	4.07±2.29	2.99±2.27
	16.4T*	7.95±1.62	4.20±1.47	2.83±1.35	2.46±1.35
Tight circling (360° rotations)	Control	2.26±0.94	1.35±1.33	1.00±1.15	1.35±1.11
	16.4T*	2.08±1.74	1.60±1.30	0.88+0.66	0.98±0.78

MWM - Probe Test parameter	Group	SE	SW	**NW**	NE
Time spent in quadrant (s)	Control	10.0±5.3	19.2±5.0	20.9±6.4	10.0±3.4
	16.4T*	11.2±4.7	18.8±8.0	17.0±5.5	13.0±4.9

Table S2. Protocol I: Morris water maze tests performed on mice after 4-week long period of a chronic exposure to 16.4 T magnetic field. Two mice groups were exposed to B_0 fields for a period of 4 weeks (3-hour long exposures, 2 exposures per week): Control group (sham exposures, N = 12), 16.4T group (N = 12). Submerge platform was located in the south-west (SW) quadrant (zone). Table values represent mean ± SD.

MWM Test parameter	Group	Day 1	Day 2	Day 3	Day 4
Escape latency (s)	Control	11.9±4.5	11.6±3.8	14.6±7.4	11.1±5.0
	16.4T	15.1±7.6	16.5±7.6	19.0±6.8	16.0±8.7
Swim distance (m)	Control	1.46±0.58	1.47±0.40	2.35±1.19	1.21±0.48
	16.4T	2.33±1.38	2.67±1.22	2.99±0.85	2.02±1.15
Tight circling (360° rotations)	Control	1.67±1.13	1.51±1.41	0.74±0.94	N/A
	16.4T	2.26±1.72	2.66±3.07	2.74±2.31	N/A

MWM - Probe Test parameter	Group	SE	**SW**	NW	NE
Time spent in quadrant (s)	Control	11.9±3.4	29.7±10.6	10.4±4.8	8.1±5.7
	16.4T	13.8±2.2	21.0±4.9	14.0±5.8	11.2±3.7

Table S3. Protocol I: Morris water maze tests performed on mice after 8-week long period of a chronic exposure to 16.4 T magnetic field. Two mice groups were exposed to B_0 fields for a period of 8 weeks (3-hour long exposures, 2 exposures per week): Control group (sham exposures, N = 12), 16.4T group (N = 12). Submerge platform was located in the south-east (SE) quadrant (zone). Table values represent mean ± SD.

MWM Test parameter	Group	Day 1	Day 2	Day 3	Day 4
Escape latency (s)	Control	25.3±10.3	25.2± 7.6	19.3± 8.3	18.3± 7.4
	16.4T	31.0±14.6	28.1±13.7	28.7±11.4	24.9±14.0
Swim distance (m)	Control	3.92±1.66	3.76±1.53	2.75±1.37	2.74±1.48
	16.4T	4.25±1.67	4.46±2.08	4.46±2.12	3.78±2.42
Tight circling (360° rotations)	Control	3.58±2.20	2.67±1.25	1.84±1.17	1.69±1.15
	16.4T	8.21±7.27	5.46±5.16	5.13±3.33	2.19±2.49

MWM - Probe Test parameter	Group	**SE**	SW	NW	NE
Time spent in quadrant (s)	Control	24.7±5.0	15.0±5.6	11.0±5.3	9.4±3.0
	16.4T	22.2±5.3	15.1±6.0	11.9±7.4	10.0±4.3

Table S4. Protocol II: Morris water maze tests performed on mice chronically exposed to magnetic fields. Four mice groups were exposed to B_0 fields for a period of 4 weeks (3-hour long exposures, 2 exposures per week): Control group (sham exposures, N = 23), 16.4T group (N = 24), 10.5T group (N = 22), 16.4T Motion group (N = 21, instead of 3 hours only 2 min exposures with intensive motion in and out of magnet). Submerge platform was located in the west (W) quadrant (zone). Table values represent mean ± SD.

MWM Test parameter	Group	Day 1	Day 2	Day 3	Day 4
Escape latency (s)	Control	47.0±20.8	22.3±16.5	18.8±14.4	15.2± 7.1
	16.4T	45.8±20.9	43.5±23.4	35.1±20.2	30.3±20.6
	10.5T	38.9±20.2	26.1±17.2	26.3±11.5	20.5±11.5
	16.4T Motion	34.7±15.7	21.0±12.6	24.9±15.9	23.6±13.7
Swim distance (m)	Control	8.36±3.54	4.02±2.62	3.11±1.93	2.59±1.18
	16.4T	6.15±2.05	6.02±2.52	5.55±2.72	5.00±2.52
	10.5T	7.05±3.65	4.58±3.03	4.69±1.98	3.48±1.87
	16.4T Motion	5.84±2.22	3.68±1.93	4.22±2.55	3.71±1.97
Tight circling (360° rotations)	Control	2.39±2.32	1.57±1.62	1.46±1.84	1.45±1.11
	16.4T	9.88±7.17	8.26±6.73	6.25±8.02	4.82±7.04
	10.5T	2.82±1.95	2.47±2.12	2.34±1.68	1.67±1.33
	16.4T Motion	2.57±2.14	1.79±1.20	1.42±1.24	2.07±2.23

MWM - Probe Test parameter	Group	N	E	**W**	S
Time spent in quadrant (%)	Control	23.6± 8.5	18.1± 8.3	29.6±10.1	28.7±12.6
	16.4T	22.6±10.3	20.6±12.6	27.5±10.7	29.4±14.7
	10.5T	23.8± 7.5	20.3± 9.6	29.3±10.7	26.5± 7.0
	16.4T Motion	22.8± 9.9	23.7±11.2	25.0± 9.5	28.5± 9.2

Table S5. Protocol II: Battery of behavioral tests performed on mice chronically exposed to magnetic fields. Four mice groups were exposed to B_0 fields for a period of 4 weeks (3-hour long exposures, 2 exposures per week): Control group (sham exposures, N = 23), 16.4T group (N = 24), 10.5T group (N = 22), 16.4T Motion group (N = 21, instead of 3 hours only 2 min exposures with intensive motion in and out of magnet). Balance beam walking (BB), fear conditioning (FC). Table values represent mean ± SD.

Behavioral test	Test parameter	Control	16.4T	10.5T	16.4T Motion
Rotarod	Latency to fall (s)	191.5±47.0	182.4±57.7	204.3±45.1	206.1±45.5
BB 15 mm square	Latency to cross (s)	6.61±2.94	6.02±4.08	8.60±4.96	10.83±9.37
BB 17 mm round	Latency to cross (s)	7.55±2.78	7.47±4.88	10.01±6.41	12.35±7.82
BB 8 mm square	Latency to cross (s)	10.69±5.21	8.74±4.13	16.59±14.53	17.44±11.03
BB 15 mm square	Number of foot-slips	0.25±0.48	0.71±0.66	0.37±0.55	0.24±0.41
BB 17 mm round	Number of foot-slips	1.20±1.26	1.71±1.24	1.20±1.07	0.98±1.02
BB 8 mm square	Number of foot-slips	1.14±0.88	2.33±1.71	0.76±1.20	0.60±0.90
FC Baseline	Freezing (s)	17.4±13.6	18.1±16.2	17.1±14.6	15.2±11.3
FC Context	Freezing (s)	44.1±21.4	36.4±21.2	56.2±23.2	40.6±18.8
FC Cue	Freezing (s)	73.5±13.8	72.5±17.7	5.9±13.7	78.4±11.4

Table S6. Protocol II: Morris water maze test performed six weeks later after the last B_0 exposure. Subgroup of mice exposed to 16.4 T (N = 12) and control group (sham exposures, N = 12). Submerge platform was located in the west (W) quadrant (zone). Table values represent mean ± SD.

MWM Test parameter	Group	Day 1	Day 2	Day 3	Day 4
Escape latency (s)	Control	27.35±18.85	26.49±21.26	31.91±24.11	22.93±19.96
	16.4T	34.45±15.50	31.60±12.52	30.56±16.20	25.03±20.77
Swim distance (m)	Control	4.00±2.34	3.21±1.82	3.51±1.65	2.71±1.36
	16.4T	5.37±1.92	4.91±1.65	4.71±2.21	3.89±2.85
Tight circling (360° rotations)	Control	3.41±2.17	1.96±1.29	2.48±2.52	1.00±0.92
	16.4T	7.06±4.87	6.44±5.99	6.10±5.97	4.46±5.67

MWM - Probe Test parameter	Group	N	E	**W**	S
Time spent in quadrant (%)	Control	22.4±10.0	18.6±14.5	27.8±11.8	31.2±17.1
	16.4T	21.0±7.6	22.2±7.5	29.8±8.9	27.0±14.4