



# OPEN Examining the effects of extremely low-frequency magnetic fields on cognitive functions and functional brain markers in aged mice

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Extremely low-frequency magnetic fields (ELF-MFs) are ubiquitously present in various environments of everyday life. While surveys from the World Health Organization (WHO) have not demonstrated the existence of ELF-MF-induced harmful consequences in healthy subjects, whether older adults are more vulnerable to the effects of residential and occupational ELF-MF exposure, and therefore may be at risk, remains unsettled. Here, we explored this potential health issue by investigating, in aged mice, the effects of chronic exposure to ELF-MFs (50 Hz ELF-MF at 1 mT for 8 h/day, 5 days/week for 12 consecutive weeks) on cognitive functions and expression profile of brain markers typically associated with aggravated aging or the development of Alzheimer's disease (AD). Sham-exposed mice showed a significant age-related decline in spatial memory functions compared to young adult mice. However, this expected pattern was neither exacerbated nor counteracted by chronic exposure to ELF-MFs. No difference in hippocampal expression of APP-695, Aβ<sub>(1-42)</sub>, S100b and GFAP proteins or in the pTau/Tau ratio was observed between sham- and ELF-MF-exposed aged mice, suggesting that chronic exposure to ELF-MFs does not aggravate aging and associated neuroinflammation, or promote pathological pathways involved in the initiation of AD. Because care should be taken in extrapolating these results to older adults with various comorbidities, applying current exposure limits to existing or new sensitive ELF-MF locations is recommended.

**Keywords** Extremely low-frequency magnetic fields, Memory functions, Aging, Alzheimer's disease

In addition to the electric power distribution within residential houses and working places, the twenty-first century faces an exponential increase in environmental exposure to extremely low-frequency magnetic fields (ELF-MFs) generated by a complex mix of domestic appliances and numerous electronic devices. Despite updated guidelines for limiting exposure to electric and magnetic fields<sup>1</sup>, this diversity of electromagnetic sources has become the focus of sanitary concerns and has prompted a growing number of epidemiological and experimental studies designed to investigate the biological effects of exposures to occupational and residential ELF-MFs<sup>2,3</sup>. Unraveling potential consequences on human health and diseases was the primary goal. That exposure to high levels of MFs translates into biological effects, notably electrical stimulation of body tissues for this type of MFs, and can be potentially harmful is not in dispute. In contrast, potential adverse effects of repeated long-term exposures to low or extremely low levels of MFs—that is those below intensities sufficient to trigger acute biological responses—continue to be hotly debated<sup>2-5</sup>.

While surveys under the aegis of the World Health Organization (WHO) failed to demonstrate the existence of ELF-MF-induced harmful consequences in healthy subjects, such an association has been put forward in the event of disease conditions such as childhood leukemia<sup>6,7</sup>, although the underlying biophysical mechanisms remain largely unclear<sup>8,9</sup>. This raises the possibility that other unhealthy population categories might be

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disproportionally sensitive to the effects of ELF-MF exposure, and therefore potentially at risk. One such category is Alzheimer's patients. Alzheimer's disease (AD), characterized by progressive neurodegeneration, is one of the leading causes of dementia and death in economically developed countries that primarily affects the elderly<sup>10,11</sup>. The neuropathological hallmarks of AD brains consist of abundant amyloid plaques and neurofibrillary tangles containing hyperphosphorylated forms of the tau protein<sup>12–16</sup> that are accompanied by astrogliosis<sup>17,18</sup> and microglial cell activation<sup>19,20</sup>. Most people with AD do not exhibit the genetically inherited form of the disease but rather the sporadic form which is thought to be the consequence of a complex interaction between multiple factors such as increasing age, family history, lifestyle and diets<sup>21</sup>. Environmental factors also contribute to the risk of developing the disease. A comprehensive review of these factors identified occupational-related exposures, notably to electric and magnetic fields, as a putative risk factor for dementia<sup>22</sup>. Hence, the increasing number and diversity of ELF-MF sources combined to chronic exposures raise the question of their impacts on the aging process and the etiology of AD.

Several epidemiological studies have analyzed the association between occupational exposure to ELF-MFs and pathological aging. Evidence for such an association is mixed. A meta-analysis of 42 epidemiological studies found only a weak association between occupational exposure to ELF-MFs and AD<sup>23</sup>. In contrast, a recent meta-analysis of 20 epidemiological studies revealed an increased risk of AD amongst workers with ELF-MF-related occupations, albeit with a high heterogeneity between gender and exposure levels<sup>24</sup>. Interestingly, the increased risk of AD has been suggested to be restricted to cases with disease onset occurring by the age of 75 years<sup>25</sup>, which was confirmed in three subsequent studies for occupational<sup>26,27</sup> and residential exposures to ELF-MFs<sup>28</sup>. The risk was found to be higher in older men than in women<sup>26</sup>. Results are equally conflicting in animal models of the disease. While some preclinical investigations in transgenic and non-transgenic models of AD show that long-term exposure to ELF-MFs may be beneficial for memory<sup>29,30</sup>, other studies reveal that long exposure does not seem to change the time course of the disease<sup>31</sup>. Studies in young mice show that ELF-MFs may alter hippocampal neuronal architecture together with impaired working<sup>32</sup> and recognition memory<sup>33</sup>, changes which have also been observed in AD mice<sup>34</sup>. In contrast, in young healthy rats, ELF-MF exposure does not induce AD-related pathology or memory impairments<sup>35</sup>.

AD is the most frequent cause of dementia<sup>11,36</sup>. Consequently, the focus has been placed predominantly on the possible association between environmental factors and the prevalence of the disease, the above-cited studies being just a few examples. However, as the most profound risk factor for AD is age and because AD is thought to be linked to aging<sup>37</sup>, it appears legitimate to wonder whether environmental factors might impact the aging process prior to the onset of the disease. Also, because the impact of a given risk factor on the development of dementia may evolve over time, it has been suggested to apprehend a possible relationship as early as possible<sup>38</sup>. Despite these considerations and the growing aging population, characterization of the potential adverse effects of chronic ELF-MF exposure during the aging process is lacking. The present study was designed to examine the potential effects of chronic ELF-MF exposure in aged mice.

Assuming that aging and AD are part of a continuum<sup>36,39</sup>, some mechanistic links could underlie these two conditions which in turn raises the possibility that aging could adopt an aggravated trajectory toward mild cognitive impairment (MCI) or even dementia by converting into AD when under the influence of specific environmental risk factors<sup>40</sup>. Thus, the objectives of this study were twofold. First, examine whether the biological aging process is accompanied by an increased sensibility to ELF-MFs that can translate into altered cognitive functions. To this end, the memory profile of aged mice was probed following chronic exposure to ELF-MFs. Second, determine whether the aging process is susceptible to aggravation or disease when facing chronic exposure to ELF-MFs. We therefore investigated whether this particular environmental condition had an incidence on the development of AD in aged mice and induced abnormal expression of brain markers typically associated with AD pathology. Since aged rodents do not spontaneously develop extracellular beta-amyloid accumulations and intracellular neurofibrillary tangles<sup>41</sup>, we focused primarily on cerebral markers whose expression precedes these pathological events. These early markers are critical in the pathogenesis of AD and can be detected in aged mice<sup>42</sup>.

## Results

### Body weight and survival of 50 Hz ELF-MF-exposed and non-exposed aged mice

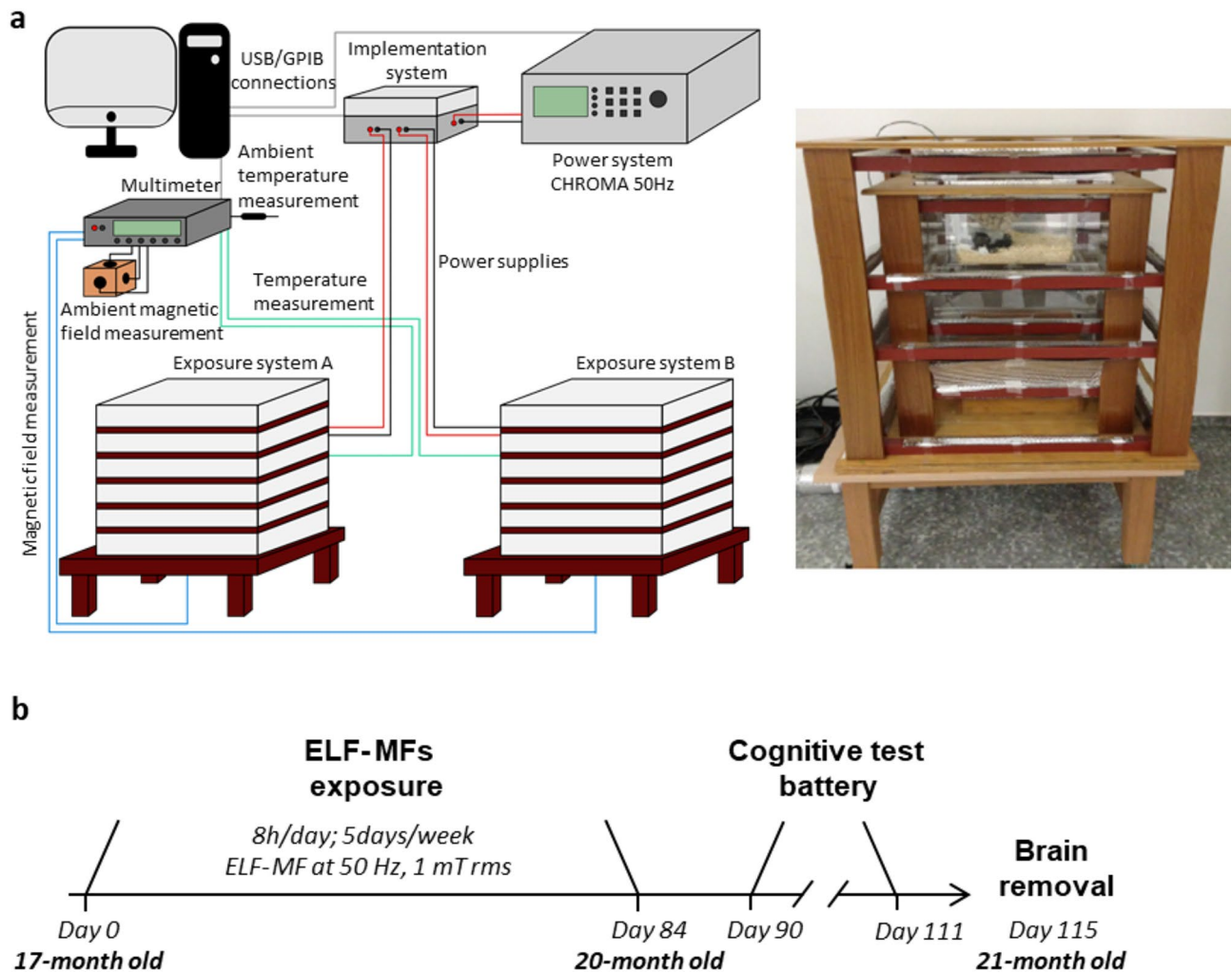
Upon completion of the 3-month exposure (Fig. 1a), the mean body weight of sham- and ELF-MF-exposed aged groups was similar ( $33.46 \pm 0.34$  versus  $33.15 \pm 0.33$  respectively; unpaired t-test:  $t_{109} = 0.64$ ,  $p = 0.53$ , NS), indicating that chronic exposure of aged mice to ELF-MFs did not interfere with body weight regulation. Although the mortality rate was slightly higher in ELF-MF-exposed (11 out of 64) than in sham-exposed (8 out of 64) mice, this difference in frequency was not statistically significant ( $\chi^2_{1,111} = 0.21$ ,  $p = 0.65$ , NS), suggesting that long-term exposure to ELF-MFs did not affect aging-induced mortality and overall survival.

### Effects of chronic ELF-MF exposure on the memory profile of aged mice

Memory performance of sham- and ELF-MF-exposed aged groups was assessed across several memory paradigms tailored to various memory forms characterized as sensitive to normal and pathological (such as AD) aging. The timeline and sequence of testing for these two cohorts of animals are presented in Fig. 1b. One group of young mice served to probe the effects of aging throughout the different memory paradigms.

#### *Spatial recognition memory*

Sham- and ELF-MF-exposed mice were first submitted to a modified version of the Y-maze two-trial arm discrimination task. Conducted in an 8-arm radial maze, this paradigm exhibits an increased spatial component and relies heavily on hippocampal function, making it particularly sensitive to the effects of aging and amyloidopathy<sup>43</sup>. Four hours after a single encoding phase of 10 min with only two arms of the maze being



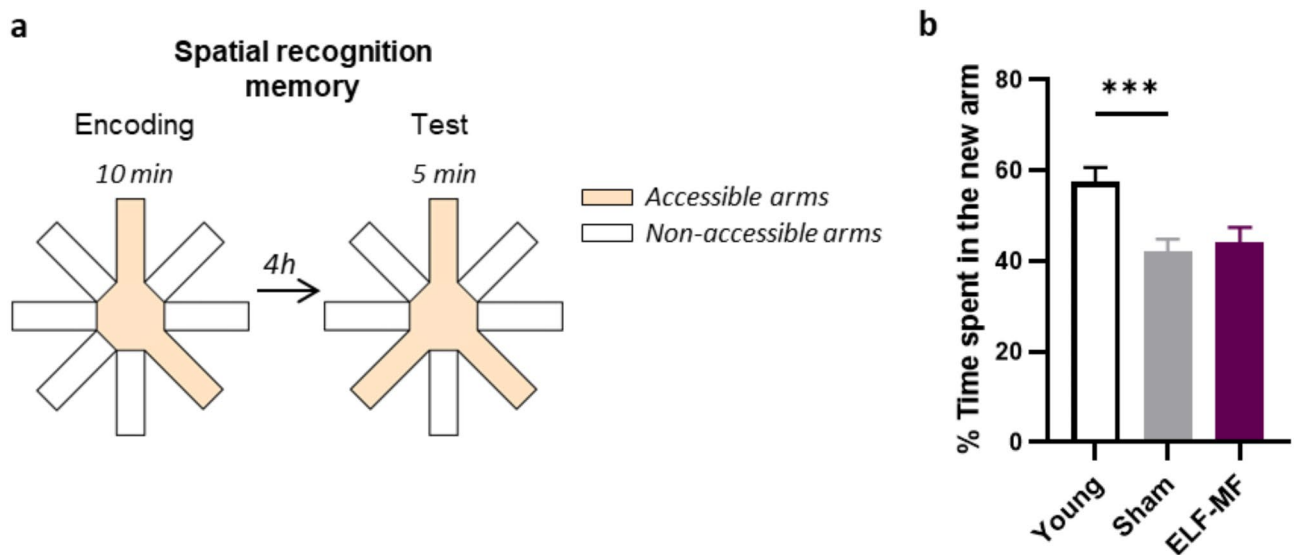
**Fig. 1.** ELF-MF exposure system and experimental timeline. (a) (Left) Schematic diagram of the 50 Hz ELF-MF system. (Right) The exposure system was composed of three superposed modules capable of hosting three separate cages simultaneously. These exposure cages, devoid of any metal content, were placed within Merritt coils delivering a homogeneous ELF-MF (50 Hz, 1 mT) within each cage. During the chronic exposure period of 12 weeks, each cage could house up to 5 mice. Temperature and magnetic flux density in each coil container were monitored in real time and checked daily. (b) Experimental timeline for ELF-MF chronic exposure, cognitive testing and brain removal.

accessible for exploration, mice were submitted to a test phase during which a third arm was made available (Fig. 2a). As expected, young mice outperformed sham-exposed aged mice by exhibiting a robust preference for the unexplored (previously inaccessible) arm (Fig. 2b). Accordingly, the percentage of time spent in the new arm was significantly lower in sham-exposed aged mice than in young mice (unpaired *t*-test:  $t_{58} = 3.48$ ,  $p = 0.0009$ ; Fig. 2b), indicative of an age-related spatial recognition memory deficit as previously reported in aged rodents<sup>44,45</sup>.

However, sham- and ELF-MF-exposed aged mice were equally able to discriminate the newly accessible arm during the test phase, which resulted in a spatial recognition memory performance above chance level (unpaired *t*-test:  $t_{79} = 0.31$ ,  $p = 0.76$ , NS; Fig. 2b). There was no confounding effect of ELF-MF exposure on the total exploration time of arms of the maze during either the encoding phase (sham mice,  $n = 42$ :  $225.32 \pm 11.98$ ; exposed mice,  $n = 39$ :  $225.0 \pm 14.09$ ; unpaired *t*-test:  $t_{79} = 0.02$ ,  $p = 0.99$ , NS) or the test phase (sham mice,  $n = 42$ :  $146.81 \pm 13.76$ ; exposed mice,  $n = 39$ :  $152.09 \pm 10.52$ ; unpaired *t*-test:  $t_{79} = 0.30$ ,  $p = 0.76$ , NS). Similarly, there was no effect of ELF-MF exposure on arm preference (arm preference  $\times$  treatment interaction:  $F_{1,158} = 0.07$ ,  $p = 0.79$ , NS) during the encoding phase (open arm 1: sham mice,  $n = 42$ :  $119.07 \pm 9.31$ ; exposed mice,  $n = 39$ :  $119.92 \pm 12.35$ ; open arm 2: sham mice,  $n = 42$ :  $103.53 \pm 9.81$ ; exposed mice,  $n = 39$ :  $98.93 \pm 9.23$ ). Thus, chronic ELF-MF exposure neither aggravated nor reversed the detrimental effect of aging on spatial recognition memory.

#### Spatial discrimination memory

The same cohorts of mice were subsequently submitted to a more cognitively challenging paradigm in the 8-arm radial maze which required learning and remembering the spatial location of the 3 constantly baited arms of

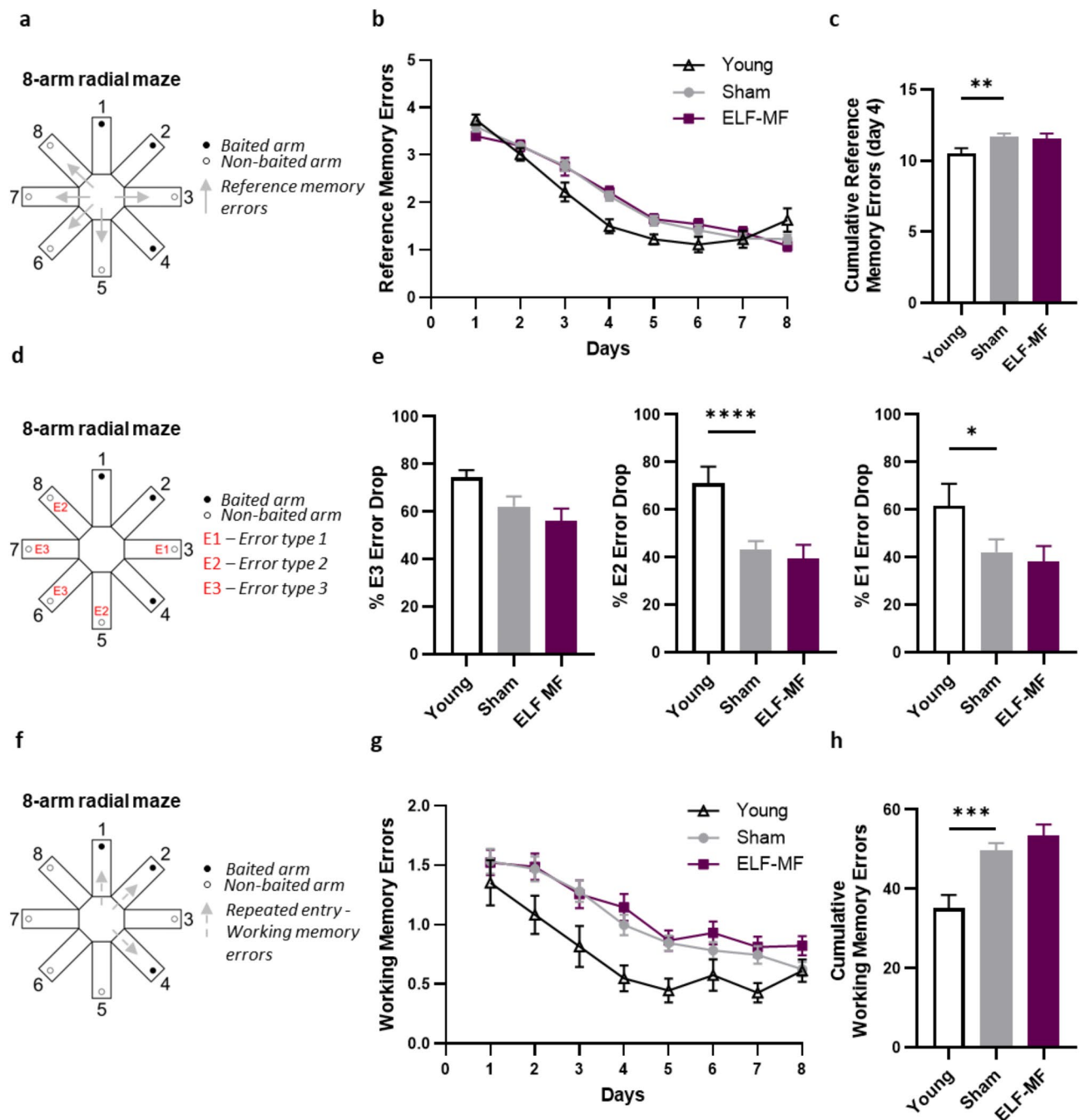


**Fig. 2.** Effects of ELF-MF chronic exposure on spatial recognition memory. (a) Experimental design. Testing occurred in an 8-arm radial maze to increase spatial cognitive demand. Percent time spent in new arm during the testing phase was used as an index of recognition memory performance. (b) Young mice outperformed sham-exposed aged mice. Performance of ELF-MF aged mice was comparable to that of sham-exposed aged mice. \*\*\* $p < 0.001$ ;  $n = 18$ –42 mice/group.

the maze (Fig. 3a). This spatial discrimination paradigm is sensitive to age-related differences in spatial memory performance in old mice<sup>46</sup> or animal models of pathological aging such as the APPswe/PS1dE9 transgenic mouse model of AD (Figure S1a, b). Its configuration enables testing both spatial reference and working memory, depending on the type of errors the animal makes. Acquisition curves show that the performance of young and sham-exposed aged mice improved over the 8 training days, with the daily mean number of reference memory errors (visits to any of the 5 non-baited arms; see Methods) declining significantly as training progressed (two-way ANOVA, main effect of days:  $F_{7,518} = 90.83$ ,  $p < 0.0001$  Fig. 3b). However, aged mice were slower in acquiring the discrimination (group  $\times$  days interaction:  $F_{7,518} = 3.26$ ,  $p = 0.0021$  Fig. 3b). A closer examination of the intersession evolution of performance revealed that the decrease in the number of reference memory errors committed by young mice was not linear. The performance of young mice started to plateau after 4 days of training and stabilized thereafter (Fig. 3b). Post-hoc analyses indeed revealed that the number of reference memory errors on Day 4 was significantly lower than on Day 3 (Tukey's test:  $p = 0.01$ ) and not different from Day 5 (Tukey's test:  $p = 0.87$ , NS Fig. 3b). In contrast, sham-aged mice needed two more training days (i.e., 12 additional trials) to reach a level of task mastery comparable to that of young mice (Fig. 3b). Thus, the cumulated number of reference memory errors after 4 training days was higher in sham-aged mice than in young mice (Mann-Whitney test:  $U = 306.0$ ,  $p = 0.008$  Fig. 3c). A similar aged-related profile emerged when we examined the number of total reference memory errors (including repeated visits to non-baited arms; Figure S2a, b).

We also examined the possibility that aged mice were less precise in locating the spatial position of the 3 baited arms of the maze. To this end, we dissected the reference memory errors committed by the animal during training as a function of their spatial location (Fig. 3d; see also Methods). Young and sham-exposed aged mice were equally quick in abandoning visits to non-baited arms that were the most distant from baited arms (E3 errors: Mann-Whitney test:  $U = 423.5$ ,  $p = 0.27$ , NS; Fig. 3e). A different pattern emerged when we examined E2 and E1 errors which reflect the mouse's ability to distinguish between arms that are closer in space (Fig. 3d). Young animals were also prompt in reducing these two types of errors (Fig. 3e). In contrast, sham-exposed aged animals exhibited difficulties and persisted in visiting non-baited arms adjacent to baited arm (E2 errors: Mann-Whitney test:  $U = 162.0$ ,  $p < 0.0001$ ) or the only non-baited arm surrounded by two baited arms (E1 errors: Mann-Whitney test:  $U = 295.5$ ,  $p < 0.005$ ; Fig. 3e), thus indicating a deficit in pattern separation.

Exposing aged mice to ELF-MFs exerted no detectable effect on spatial discrimination performance. All spatial discrimination parameters analyzed for sham- and ELF-MF-exposed aged mice were similar as revealed by two-way ANOVAs for the rate of acquisition (reference memory errors, main effect of days:  $F_{7,763} = 148.70$ ,  $p < 0.0001$  with no treatment effect:  $F_{1,109} = 7 \times 10^{-6}$ ,  $p = 0.99$ , NS and no group  $\times$  treatment interaction:  $F_{7,763} = 0.64$ ,  $p = 0.72$ , NS; Fig. 3b), the number of cumulative reference memory errors at day 4 (Mann-Whitney test:  $U = 1395.0$ ,  $p = 0.40$ , NS; Fig. 3c), memory precision (E3 errors: Mann-Whitney test:  $U = 1351.0$ ,  $p = 0.43$ , NS; E2 errors: Mann-Whitney test:  $U = 1472.0$ ,  $p = 0.70$ , NS; E1 errors: Mann-Whitney test:  $U = 1489.0$ ,  $p = 0.78$ , NS; Fig. 3e) or the number of total reference memory errors (Figure S2b). Notably, at the beginning of training (Day 1), the level of performance of sham- and ELF-MF-exposed aged mice was comparable to that of young mice (number of reference memory errors,  $3.60 \pm 0.11$ ,  $3.40 \pm 0.09$  and  $3.74 \pm 0.11$ , respectively; one-way ANOVA,  $F_{2,126} = 2.0$ ,  $p = 0.14$ , NS; Fig. 3b), indicating that aged mice were capable of processing the visuospatial components of the task (absence of a confounding performance effect of aging condition or ELF-MF exposure).



**Fig. 3.** Effects of ELF-MF chronic exposure on spatial discrimination memory. **(a)** Experimental design. Mice needed to locate and remember the spatial location of the three constantly baited arms of the maze. **(b)** Learning curves of young, sham- and ELF-MF-exposed aged groups. Sham aged mice were slower in acquiring the spatial discrimination task compared to young mice. ELF-MF exposure did not affect reference memory performance.  $n = 18-53$  mice/group. **(c)** Cumulative number of reference memory errors after 4 days of training.  $**p < 0.01$ ;  $n = 18-53$  mice/group. **(d)** Experimental design. The type of reference memory errors (E1, E2 and E3) was used to assess memory precision in the form of an error drop index (see Methods). **(e)** Sham-exposed aged mice committed more E1 and E2 errors compared to young mice, indicating impairment in pattern separation-dependent memory. ELF-MF chronic exposure did not exacerbate or attenuate this aged-related deficit.  $****p < 0.0001$ ;  $*p < 0.05$ ,  $n = 18-53$  mice/group. **(f)** Experimental design used to assess working memory. Repeated entries into baited arms were scored as working memory errors. **(g)** An aged-dependent memory deficit in working memory was observed in sham-exposed aged mice compared to young mice. ELF-MF chronic exposure did not exacerbate or rescue this deficit.  $n = 18-53$  mice/group. **(h)** The cumulative number of working memory errors across the 8 days of testing was higher in sham-exposed aged mice compared to young mice but was not altered by ELF-MF chronic exposure.  $***p < 0.001$ ;  $n = 18-53$  mice/group.

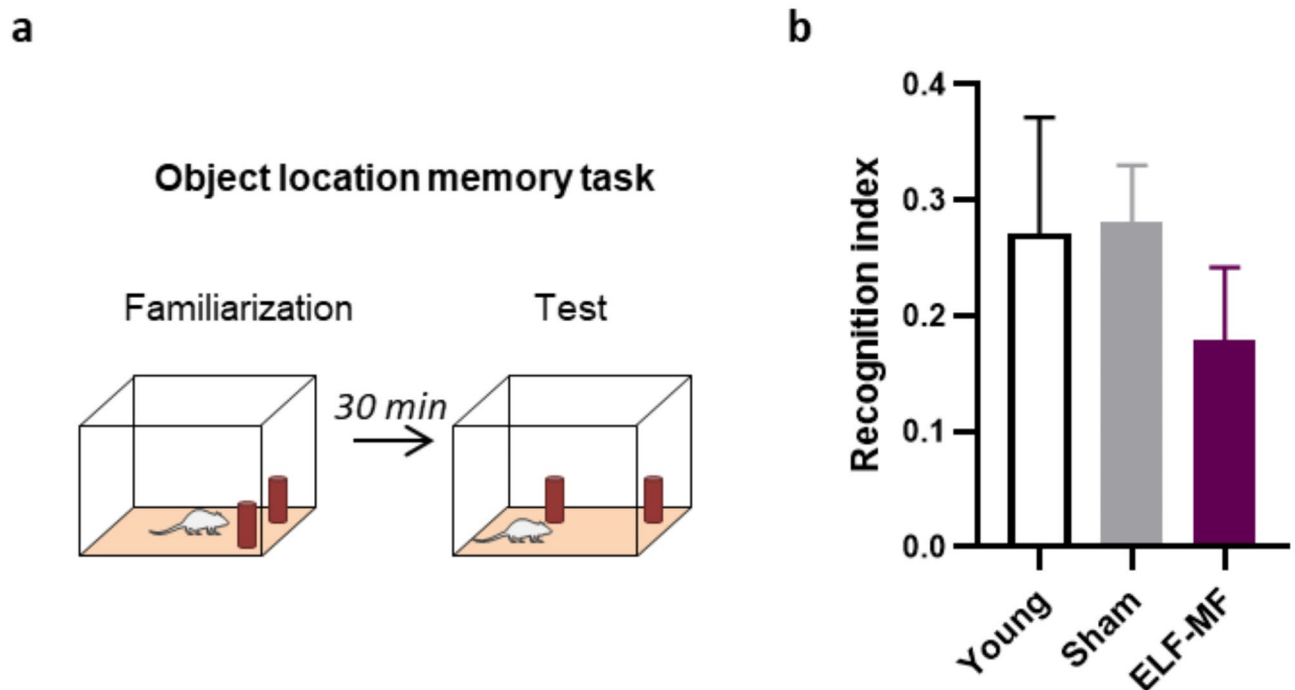
Also, the duration of training trials on Day 1 was similar across groups (young mice:  $424.20 \pm 29.60$ ; aged sham:  $425.60 \pm 16.28$ ; aged ELF-MF-exposed:  $458.80 \pm 17.92$ ;  $F_{2,126} = 1.09$ ,  $p = 0.34$ , NS), further suggesting that motor ability was not a confounding factor in these experiments. Exposure of aged mice to ELF-MFs also failed in altering memory precision, regardless of the error type (sham- versus ELF-MF-exposed for E3: Mann-Whitney test:  $U = 1351.0$ ,  $p = 0.43$ , NS; for E2: Mann-Whitney test:  $U = 1472.0$ ,  $p = 0.70$ , NS; for E1: Mann-Whitney test:  $U = 1489.0$ ,  $p = 0.78$ , NS; Fig. 3e).

We next examined working memory errors (Fig. 3f). While young and sham-exposed aged mice progressed over training days (two-way ANOVA, main effect of days:  $F_{7,518} = 17.46$ ,  $p < 0.0001$ ; Fig. 3g), there was a significant main effect of age ( $F_{1,74} = 14.33$ ,  $p < 0.0003$ ) with no significant age  $\times$  days interaction ( $F_{7,518} = 0.99$ ;  $p = 0.44$ , NS; Fig. 3g), indicating that aged mice were slower in avoiding repeated visits into baited arms. Accordingly, upon training completion on Day 8, a higher number of cumulative working memory errors relative to young mice was observed ( $t_4 = 3.79$ ,  $p = 0.0003$ ; Fig. 3h). As for reference memory, exposure of aged mice to the ELF-MFs did not significantly affect working memory performance. Numbers of working memory errors for sham- and ELF-MF-exposed aged mice were similar as revealed by two-way ANOVAs for the rate of acquisition (main effect of days:  $F_{7,763} = 29.32$ ,  $p < 0.0001$  with no treatment effect:  $F_{1,109} = 1.02$ ,  $p = 0.31$ , NS and no group  $\times$  treatment interaction:  $F_{7,763} = 0.51$ ;  $p = 0.83$ , NS; Fig. 3g) and for the number of cumulative working memory errors upon training completion on Day 8 ( $t_{109} = 1.13$ ,  $p = 0.26$ , NS; Fig. 3h). Performance of all groups was similar on Day 1 (one-way ANOVA,  $F_{2,126} = 0.38$ ,  $p = 0.68$ , NS; Fig. 3g), thus ruling out a performance effect due to aging or ELF-MF exposure.

#### Object location memory

When previously exposed to two identical objects, mice recognize the situation when an object has been relocated. Heavily dependent on hippocampal function, this spatial memory task is appropriate for assessing differences in aging and neurodegenerative studies<sup>47–49</sup>. As a validation of our experimental design, we established that wildtype mice explored significantly more the object in the new position while APPswe/PS1dE9 mutant mice spent a similar amount of time exploring both displaced and non-displaced objects. This memory impairment resulted in a significantly different discrimination index between wildtype and APPswe/PS1dE9 AD mice (Figure S3a, b). However, sham-exposed aged mice submitted to the same procedure were not impaired (t-test:  $t_{57} = 0.09$ ,  $p = 0.92$ , NS Fig. 4a, b), indicating that their performance in object location was not sufficiently affected to differentiate them from young adult mice, possibly because of a spatially rich environment (i.e., insufficiently challenging) favoring recall of object-context associations or a delay too short between the object familiarization phase and the test phase.

To rule out potential confounding effects due to between-group differences in the object exploration time during the familiarization phase (i.e., differential encoding and initial memory strength), the exploration trial was stopped when the total object exploration reached 1 min. During the test trial with a moved object administered 30 min after initial exploration, sham- and ELF-MF-exposed aged mice showed similar discrimination indexes



**Fig. 4.** Effects of ELF-MF chronic exposure on object location memory. (a) Experimental design. (b) Detection of the change in the object location was similar in young, sham- and ELF-MF-exposed aged mice.  $n = 12\text{--}47$  mice/group.

( $t$ -test:  $t_{83} = 1.30$ ,  $p = 0.20$ , NS; Fig. 4b). Thus, chronic exposure to ELF-MFs did not affect object location memory in aged mice.

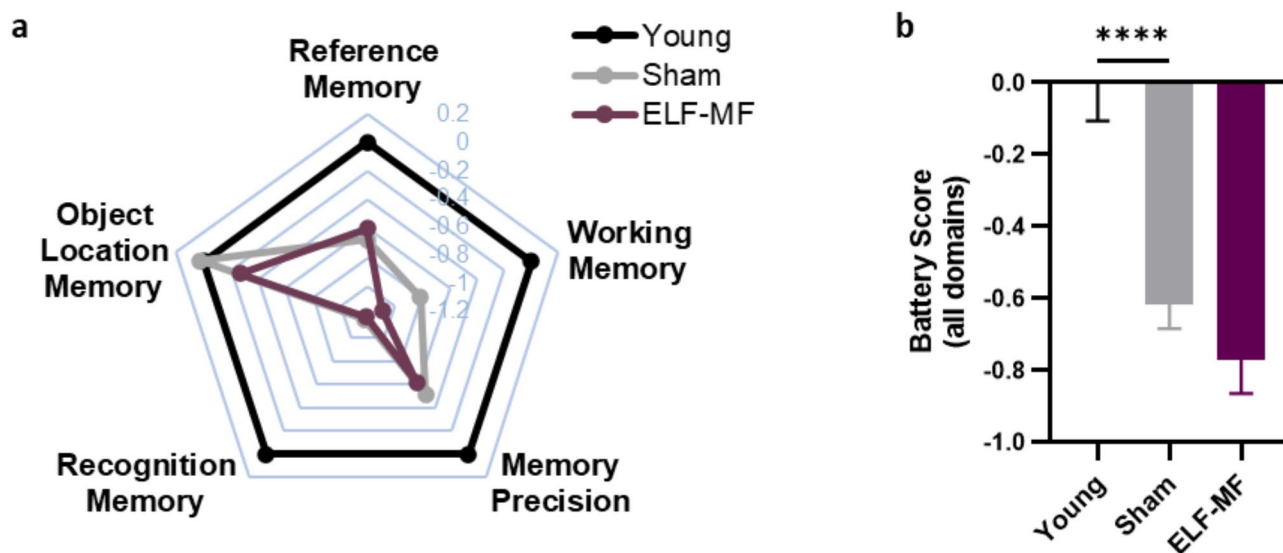
#### Composite test scores

Normative z-scores for each group (Table S1) were first plotted on a spider graph for all tested cognitive domains (spatial recognition memory, cumulative reference and working memory errors, memory precision and object place recognition). As shown in Fig. 5a, young adult mice outperformed sham-exposed aged mice across all the selected domains, with a normalized battery score of 0 compared to -0.67 for sham-exposed aged mice (Mann-Whitney  $U = 7164.0$ ;  $p < 0.0001$  Fig. 5b). ELF-MF-exposed aged mice performed similarly to sham-exposed aged mice across all domains (Fig. 5). Accordingly, these two groups exhibited a comparable battery score (Mann-Whitney  $U = 30525.0$ ;  $p = 0.59$ , NS Fig. 5b). Overall, this extended analysis further confirmed the absence of positive or negative effects of ELF-MF chronic exposure on cognitive processes in aged mice.

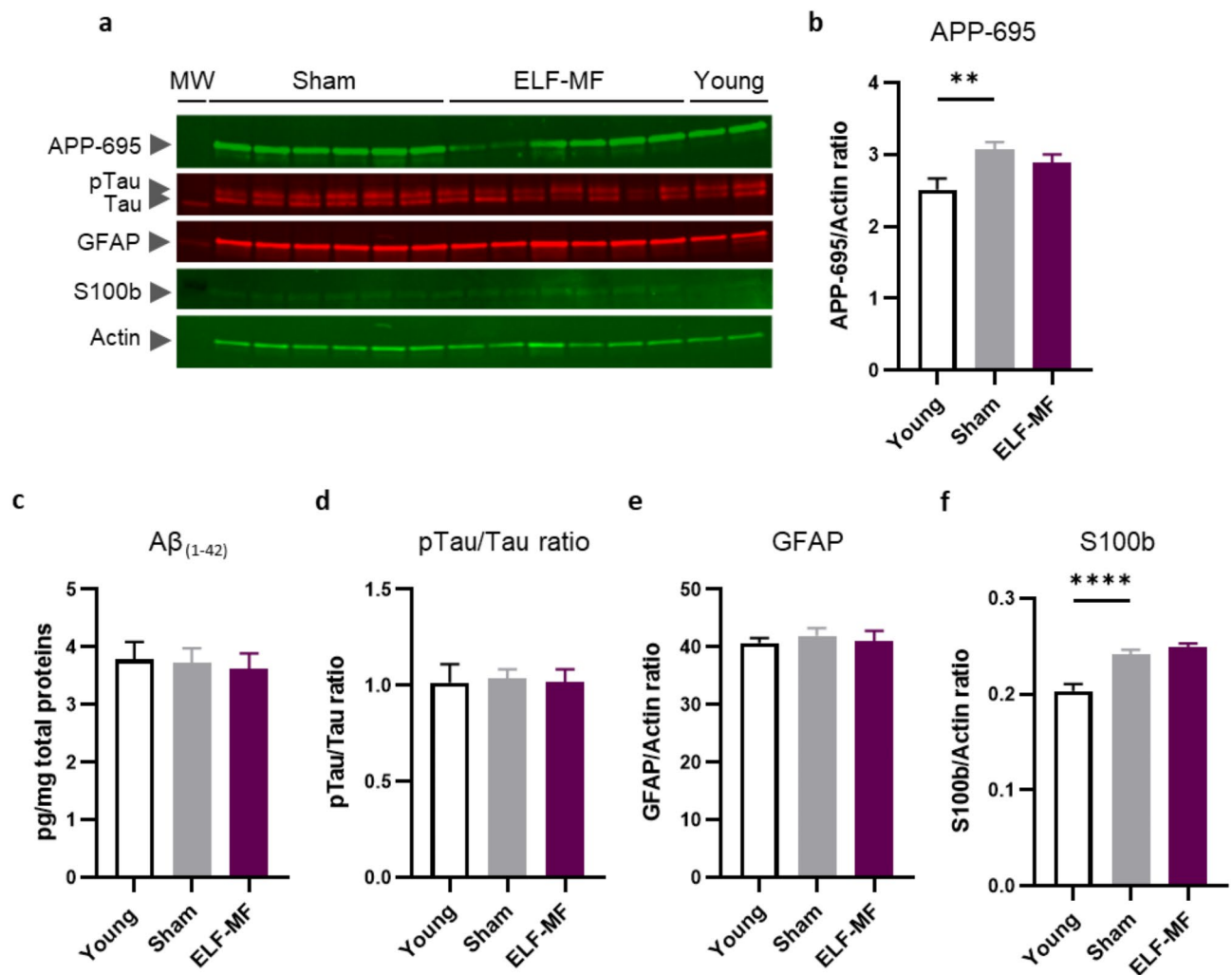
#### Effects of 50 Hz ELF-MF exposure on the expression of cerebral markers of pathological aging

To determine whether chronic ELF-MF exposure may trigger an AD-like pathology in aged mice, we analyzed the expression of pre-symptomatic AD markers in the hippocampus, which stands among the first brain areas affected in AD<sup>50</sup>. Three days after the last behavioral paradigm (Fig. 1B), mice were euthanized and the total hippocampal proteins were extracted to analyze the expression of preclinical AD markers. Because the pathological amyloid cascade is related to an imbalance between A $\beta$  production from amyloid precursor protein (APP-695) and clearance/degradation processes<sup>51</sup>, we first analyzed the impact of chronic ELF-MF exposure on APP-695 protein expression. Although sham-exposed aged mice expressed significantly more APP-695 than young mice (unpaired  $t$ -test:  $t_{61} = 2.93$ ,  $p = 0.0047$ ; Fig. 6a, b), chronic exposure to ELF-MFs did not affect hippocampal APP-695 expression with similar levels of APP-695 proteins in sham- and ELF-MF-exposed aged mice (unpaired  $t$ -test:  $t_{85} = 1.20$ ,  $p = 0.23$ , NS; Fig. 6a, b). We next measured the impact of chronic ELF-MF exposure on extracellular A $\beta$  accumulation. We used an ELISA kit that specifically detects rodents' A $\beta_{(1-42)}$  with negligible cross-reactivity to A $\beta_{(1-40)}$  and APP-695. We found that the concentration of A $\beta_{(1-42)}$  in hippocampi from young and sham-exposed aged mice was not significantly different (Mann-Whitney  $U = 263.0$ ;  $p = 0.45$ , NS; Fig. 6c) and as observed for APP-695 expression, ELF-MF chronic exposure did not affect the A $\beta_{(1-42)}$  concentration in aged mice (ELF-MF- versus sham-exposed aged mice: Mann-Whitney  $U = 671.0$ ;  $p = 0.60$ , NS; Fig. 6c).

We also analyzed the impact of chronic ELF-MF exposure on the phosphorylation status of Tau proteins in aged mice. As validated in APPswe/PS1dE9 mice (Figure S4a), the Tau5 antibody detects two specific bands in Western blots corresponding to the phosphorylated (pTau, upper band) and non-phosphorylated forms (Tau, lower band) of the Tau protein<sup>52</sup>. Confirming previous observations, densitometric quantification of hippocampal extracts revealed a significant molecular shift towards a predominance of the tau phosphorylated form in APPswe/PS1dE9 mice compared to wild-type controls (Figure S4b). In contrast, we did not find any



**Fig. 5.** Composite battery scores of young, sham- and ELF-MF-exposed aged groups. **(a)** Spider graph showing normalized z-score performance across the different cognitive domains of the test battery. Means and SEM of z-scores data for each group are provided in Table S1. Young-mice outperformed aged mice from the sham- and ELF-MF-exposed groups. ELF-MF chronic exposure did not alter the pattern of performance expressed by sham-exposed aged mice. **(b)** The overall battery score of sham-exposed aged mice was lower compared to young mice, indicating an age-dependent effect. Scores of sham- and ELF-MF-exposed groups were similar, indicating not effect of ELF-MF chronic exposure. \*\*\*\* $p < 0.0001$ ;  $n = 18$ –53 mice/group.



**Fig. 6.** Effects of ELF-MF chronic exposure on the hippocampal expression of cerebral markers of aging and AD. **(a)** Representative Western blots of the different brain markers analyzed in young, sham- and ELF-MF-exposed aged groups. Shown is a gel which contains 6 aged sham mice out of a total of 45, 6 aged ELF-MF-exposed mice out of a total of 42 and 2 young mice out of a total of 18 mice. Each band represents an individual mouse for each of the groups. **(b)** APP-695 expression.  $**p < 0.01$ . **(c)** A $\beta$ <sub>(1-42)</sub> expression. **(d)** pTau/Tau ratio. **(e)** GFAP expression. **(f)** S100b expression.  $****p < 0.0001$ . ELF-MF chronic exposure did not alter hippocampal expression of any of these markers.  $n = 18$ –45 mice/group.

change in the pTau/Tau ratio of sham-exposed aged mice compared to young mice (unpaired *t*-test:  $t_{61} = 0.25$ ,  $p = 0.81$ , NS; Fig. 6d) or in ELF-MF-exposed compared to sham-exposed aged mice (unpaired *t*-test:  $t_{86} = 0.27$ ,  $p = 0.79$ , NS; Fig. 6d), suggesting that the chronic ELF-MF exposure does not affect the tauopathy process.

Astroglisis is thought to play a role not only as a pathological consequence of AD but also as a potential trigger and accelerator of amyloid plaque formation and tau-related neuronal injury<sup>19,53,54</sup>. Indeed, astrocyte activation, reflected by an increased expression of intermediate filament proteins such as glial fibrillary acidic protein (GFAP), can occur early, even before the onset of memory decline<sup>55</sup>. As shown in Figure S4a, c, we report a significant increase in GFAP expression in APPswe/PS1dE9 compared to wildtype mice. In contrast, we did not observe any changes in GFAP levels in young mice compared to sham-exposed aged mice (unpaired *t*-test:  $t_{61} = 0.62$ ,  $p = 0.54$ , NS; Fig. 6e) or between sham- and ELF-MF-exposed aged mice (unpaired *t*-test:  $t_{85} = 0.45$ ,  $p = 0.66$ , NS; Fig. 6e). Next, we analyzed the expression of the S100b protein which is also mainly expressed in astrocytes. In contrast to GFAP, we demonstrated that the expression of S100b was altered by aging, with a significant increase of S100b protein expression in sham-exposed aged mice compared to young mice (unpaired *t*-test:  $t_{61} = 4.44$ ,  $p < 0.0001$ ; Fig. 6f). However, ELF-MF chronic exposure did not modify expression of this astroglial marker (unpaired *t*-test, sham- versus ELF-MF-exposed aged mice:  $t_{85} = 1.07$ ,  $p = 0.29$ , NS; Fig. 6f). Taken collectively, these results indicate that chronic exposure to ELF-MFs does not aggravate cellular processes involved in the progression of aging or induction of AD. In considering the possibility that some aged mice may be differentially sensitive to the effects of ELF-MF exposure, we examined data variability across the level of expression of the different cerebral markers. Box plot graphs depicting the distribution of individual values for young, aged sham- and ELF-MF-exposed mice revealed that data variability was similar between these groups,

suggesting that individual differences were not exacerbated in aged mice in response to ELF-MF exposure (Figure S5).

## Discussion

This study focused on the impact of exposure to chronic ELF-MFs during the course of the aging process and explored whether such environmental treatment may constitute a risk determinant for aggravating aging or triggering the AD symptomatology in aged mice. Aging is considered the leading risk factor for both genetic and sporadic AD<sup>56</sup>. Yet, the mechanisms underlying the age-related vulnerability to AD remain largely unclear, mainly because aging is sensitive to a plethora of interactive genetic and environmental risk factors, as well as comorbidities, that may impact the aging trajectory towards either healthy or pathological aging<sup>21,37</sup>. This delicate balance is thought to be dependent on several biological processes such as inflammation, impaired autophagy and mitochondrial dysfunction, epigenetic alterations or altered neuronal plasticity including synaptic loss which are thought to act, either independently or synergistically, as crucial determinants of the susceptibility to AD<sup>56,57</sup>.

Environmental factors may exert deleterious effects via an acceleration of the biological age<sup>58</sup>. Meta-analyses of epidemiological studies incorporating occupational exposure to ELF-MFs as a risk factor for pathological aging report highly heterogeneous results which prevents the establishment of a clear association between occupational exposure to ELF-MFs and the development of chronic disorders, particularly neurodegenerative diseases<sup>23,24,59,60</sup>. In the case of case-control studies, the results generated are equally inconsistent<sup>61</sup>, possibly because of their retrospective nature and potential confounds when matching cases of AD or dementia and selected controls<sup>62</sup>. The aging trajectory can lead to multiple endpoints ranging from healthy cognitive decline, subjective cognitive complaints despite satisfactory performance at cognitive tests, MCI and dementia<sup>63–65</sup>. While not as prominent as in AD, neuronal loss occurs during physiological aging conjointly with a reduction of cortical synapses in the same brain circuits that degenerate during AD and can thus contribute, at least in part, to this detrimental spectrum<sup>66</sup>. Such an age-related cellular decline can thus make the aging brain particularly vulnerable to environmental conditions, including ELF-MFs. In sharp contrast, and certainly intriguing, are the preclinical and clinical reports pointing to the beneficial effects of ELF-MF exposure in some neurological disorders including AD<sup>29,67,68</sup>. This pattern of effects has prompted the exploration of transcranial electromagnetic treatments as a potential therapy<sup>69</sup> that could act by normalizing aging-induced inflammatory responses<sup>70</sup> or by favoring, via proteolytic pathways, the clearance of misfolded proteins involved in the progression of neurodegenerative diseases<sup>71</sup>.

By using a rigorously controlled ELF-MF environmental setting coupled to a prospective design in aged mice with a known history, we here provide novel insights into the characterization of the health effects associated with chronic exposure to ELF-MFs in aged mice submitted to various memory challenges. Experimenters in this study were blind to the experimental conditions and relatively large sample size for the different groups was used to increase the statistical power of analyzes as well as the accuracy and reliability of our data. We also generated z-scores which enabled to (i) integrate measures along the same cognitive dimension in different behavioral paradigms; (ii) reduce the intrinsic variability of single tests and (iii) provide a robust characterization of the underlying memory profile of individual mouse in each experimental group. Altogether, our investigations failed to identify any deleterious or beneficial effects of chronic ELF-MF exposure on cognitive processing and did not pinpoint any detectable change in the expression of hallmark cerebral markers of aging or the occurrence of an imbalance in the amyloid cascade that could potentially lead to AD. Thus, this type of electromagnetic field delivered chronically at the intensity of 1 mT did not alter the aging process or the vulnerability of the aged brain to AD.

Frequency and magnetic flux density are two key experimental parameters in ELF-MF studies. Since most European countries, including France, utilize alternating current with a nominal utility frequency of 50 Hz, people are most frequently exposed to this frequency during daily living activities. We therefore selected it in the present study. The ICNIRP Guidelines recommends general public and occupational exposure thresholds of 0.2 mT and 1 mT, respectively<sup>1</sup>. We therefore chose to expose aged mice to the highest magnetic flux density of 1 mT and mimicked human discontinued exposure by delivering the ELF-MF only 8 h/day and 5 days per week. This regimen was applied for 12 successive weeks to achieve chronicity.

Cognitive functions are mediated by multiple systems organized into interconnected brain regions that can operate independently, synergistically or even in competition<sup>72,73</sup>. Because the whole mouse brain was exposed to ELF-MFs in the present study, we incorporated in our cognitive test battery several memory paradigms tailored to specific memory forms known to be dependent on memory systems distributed throughout interconnected superficial (e.g., prefrontal cortex) and deeper (e.g., hippocampus) regions of the brain. We selected memory forms reported to be sensitive to aging or impaired during AD, such as spatial working and reference memory<sup>74,75</sup> or recognition memory, a subdivision of episodic memory, which is typically affected during the early stages of this neurodegenerative disease<sup>76</sup>. We were particularly attentive in implementing memory paradigms that were sensitive enough to detect potential changes associated with aging or AD. For instance, our modified recognition memory paradigm adapted to the 8-arm radial maze enhanced the spatial cognitive demand, making it more sensitive in detecting potential aged-related memory impairments otherwise more difficult to observe in the classical Y-maze<sup>77</sup>. In the spatial discrimination task, we further used the APPswe/PS1dE9 mouse model of AD to validate the ability of this paradigm to detect memory impairments. To control for the potential confound of ELF-MF exposure on motivation, we used appetitive (radial maze in which arms display emotional valence) and innate (Y-maze and object location which engage a minimal motivational component besides the innate preference to investigate novel stimuli) paradigms. In addition to innate memory tasks based on preference for novelty<sup>43,78</sup>, we address the effects of ELF-MF exposure on the learning rate in the radial maze wherein spatial training requires multiple imposed training sessions delivered over several days. Overall, integration of

these multi-level control parameters makes unlikely the possibility that the observed lack of effects of ELF-MF exposure on cognitive performance is due to a lack of sensitivity of our behavioral profiling.

Besides reference and working memory forms, we also dedicated our attention to specific hippocampal processes that could potentially be altered by exposure to ELF-MFs in aged mice. Hippocampal function enables the formation of distinct spatial representations of similar or interfering inputs<sup>78</sup>. This so-called pattern separation process under the guidance of the dentate gyrus is particularly crucial in spatial discrimination paradigms requiring to process information presented closely in space, in our case, accurately distinguishing in the 8-arm radial maze between baited and non-baited arms located close to each other. We found memory precision to be altered in sham-exposed aged mice, mainly when these mice had to locate the baited arm surrounded by two adjacent non-baited arms, indicative of a possible inability to pattern-separate during spatial training. However, chronic exposure to ELF-MFs did not alter, either negatively or positively, memory precision in these aged mice. At the cellular level, newborn neurons have been shown to be necessary for normal pattern separation function in the DG of adult mice<sup>79</sup> and adult hippocampal neurogenesis is impaired by aging<sup>80</sup>, raising the possibility that altered neurogenesis could contribute, at least in part, to the reduced memory precision of sham-exposed aged mice. While ELF-MF exposure (1 mT; 50 Hz) can promote *in vivo* adult hippocampal neurogenesis<sup>81</sup>, including long-term survival of newborn neurons in young adult mice<sup>82</sup>, our findings indicate however that such an ELF-MF neurogenic boosting mechanism appears insufficient to rescue impaired memory precision in the aged brain in which a plethora of structural and cellular changes are ongoing<sup>36,83</sup>.

As they age, wild-type rodents exhibit behavioral alterations and cognitive decline that correlate with those observed in AD. However, they do not spontaneously develop the two main histopathological hallmarks of AD, namely extracellular beta-amyloid aggregates and intracellular accumulation of hyper-phosphorylated tau proteins that compose neurofibrillary tangles<sup>41</sup>. Accordingly, we did not observe these pathological features in aged C57BL/6J mice and found that an external inducer such as ELF-MFs could not trigger these brain markers. However, the observations that intraneuronal A $\beta$  accumulation can occur early in the disease progression and precede the formation of extracellular amyloid plaques<sup>84</sup> and that cognitive deficits of AD patients are correlated to soluble A $\beta$  levels rather than plaque-development *per se*<sup>85</sup> prompted us to examine hippocampal levels of soluble A $\beta$ . These levels were not modified by ELF-MF chronic exposure. Similarly, the increase in Tau phosphorylation, which precedes the accumulation of neurofibrillary tangles and is detectable in both adult and aged mice experiencing stressful conditions<sup>42,86,87</sup>, was not influenced by chronic exposure to ELF-MFs.

Interestingly, a causal relationship between the accumulation of aggregated intracellular proteins and inflammation during aging has been emphasized, suggesting that co-occurrence of these two cellular processes can initiate AD<sup>88</sup>. Inflammation is a hallmark of normal aging<sup>89</sup>. Accordingly, we found that S100b proteins were upregulated in sham-exposed aged mice, confirming previous studies<sup>90</sup>. However, while sham-exposed aged mice showed signs of inflammation as revealed by upregulation of inflammatory brain markers, ELF-MF chronic exposure did not exacerbate or reduce neuronal inflammation.

While still debated due to the lack of solid experimental and mechanistic evidence, magnetic hypersensitivity has been reported by some individuals following acute or chronic exposures to ELF-MFs<sup>91,92</sup>. We considered the possibility that some aged mice may be disproportionately sensitive to the effects of ELF-MF exposure. However, when examining the variability in memory performance or expression of brain markers in these mice, we did not find evidence for abnormally large individual differences in sensitivity to ELF-MF exposure.

## Conclusion and limitations

In summary, our study does not provide conclusive evidence for an association between chronic exposure to ELF-MFs and aggravated aging or initiation of the AD symptomatology in aged mice. However, we acknowledge potential limitations and perspectives. We cannot formally exclude that a more prolonged exposure to ELF-MFs or additional exposure of aged mice during the three weeks of behavioral testing could have translated into possible effects on cognitive processes or alterations in some of the cerebral markers examined. The use of older mice with a potentially more vulnerable brain at the time of ELF-MF exposure could have been impacted more heavily by our ELF-MF regimen with the downside, however, of introducing important mortality biases. Of note, this study was restricted to male mice due to the impossibility of housing separately male and female mice during ELF-MF exposure and behavioral testing. Whether female aged mice could be more sensitive to ELF-MFs would deserve further investigation, although data from animal and human case-control or cohort studies have not pinpointed an apparent gender effect. For occupational exposure, gender differences in risk associations have been reported but these differences may be related to distinct occupations for men and women resulting in gender imbalances for certain specific activities and potential sample biases<sup>24</sup>.

Also, we cannot exclude the existence of compensatory phenomena that could influence the response of aged mice to ELF-MF exposure, and thus account, at least in part, for the absence of effect of ELF-MFs. Compensation may have been triggered in the brain of ELF-MF-exposed aged mice, particularly when extended periods of exposure, over weeks, are experienced. Underlying mechanisms are thought to involve neurofunctional plasticity<sup>93</sup>. The cognitive reserve, which reflects the ability of the brain to cope with external harmful stimuli, may also act as a form of preventive compensation and therefore contribute to the protection of the cerebral tissue<sup>93,94</sup>. While at play in the human brain hosting an extensive library of previous and continuous experiences, such a protective mechanism was, however, less likely to operate in the brain of our aged mice which were experimentally naive before undergoing ELF-MF exposure. Replicating this study using a different mouse strain, such as the senescence-accelerated mouse-prone 8, could be of interest since these mice exhibit age-related learning and memory decline associated with the hallmark features of AD pathogenesis including cerebral inflammation, amyloid- $\beta$  (A $\beta$ ) deposits and tau hyperphosphorylation<sup>95</sup>. Though the focus of our test battery was on dissecting the effects of ELF-MF chronic exposure on memory processes, this approach could be extended to explore complementary behavioral domains including, for instance, anxiety, sociability,

depression or food consumption for which reliable rodent tasks and specific brain markers are available. Because care should be taken in extrapolating the present findings to older adults with various comorbidities, applying current precautionary policies on magnetic fields from power lines across various countries to existing or new sensitive ELF-MF locations is recommended.

## Materials and methods

### Animals

One hundred and twenty-eight 17-month-old male C57BL/6J mice were obtained from Janvier Laboratories (Le Genest-Saint-Isle, France). These aged mice were housed four per plastic cage in our *in vivo* facility at IMS on a 12 h/12 h light/dark cycle (lights on at 7:00 AM), with water and food available *ad libitum*. Mice were 17 months of age at the start of ELF-MF exposure and 20 months when they underwent behavioral procedures. At this age, these mice express senescent changes in almost all biomarkers in all animals<sup>96</sup>. Strain-specific diseases that can affect brain marker expression in a non-specific manner are minimized compared to older mice that are also more likely to die, creating a sample bias within the surviving animals. Behavioral testing was conducted during the light phase of the cycle. Eighteen young adult male mice (C57BL/6J from Janvier Laboratories, 10 weeks of age) served as an additional control group to probe the effect of aging. APPswe/PS1dE9 transgenic mice (9-month-old,  $n=9$ ) and their wild-type littermates ( $n=8$ ) were bred in-house and used to establish the sensitivity of our behavioral and molecular approaches to detect AD-related changes. Experiments were conducted blind to the treatment condition and according to protocols approved by the ethical committee of the University of Bordeaux (protocol A50120159). All experimental procedures complied with official European guidelines for the care and use of laboratory animals (Directive 2010/63/UE) and followed the ARRIVE guidelines for animal research<sup>97</sup>.

### ELF-MF exposure

The exposure system consisted of two identical sets of Merritt coils powered by the mains via a voltage controller and installed in a dedicated temperature- and humidity-controlled room (Fig. 1a). Freely moving aged mice were placed in 25 cm x 20 cm exposure cages made of PVC with walls and cover lids perforated with holes. Cages were located in the center of each independently-activated set of coils as previously described<sup>98</sup>. Each cage accommodated 5–6 mice randomly assigned to either a sham-exposed ( $n=64$ ) or a 50 Hz ELF-MF-exposed ( $n=64$ ) group. Exposure cages and water bottles were without metal content. When activated, the exposure system generated a vertical ELF-MF at 50 Hz calibrated at an intensity of 1 mT root mean square (rms). This ELF-MF was homogenous inside the cage with a variability of  $\pm 5\%$ <sup>98</sup>. Temperature and magnetic flux density in each coil container were monitored in real time. Experimental groups were exposed 8 h daily, 5 consecutive days a week for 12 successive weeks. Sham control groups were treated in parallel exactly as the ELF-MF-exposed group. Sham mice were placed in an identical exposure system with inactive coils that did not deliver any ELF-MF. C57BL/6J mice ranging from 18 to 24 months of age are typically considered old<sup>96</sup>. However, the early (18 to 21 months) and late (22 to 24 months) phases of this aging period exhibit different survivorship and aging kinetics. To minimize these potential confounding factors and because emerging age-related alterations start to manifest by late adulthood<sup>99,100</sup>, we therefore chose to target preferentially the early aging phase by exposing mice for 12 weeks from 18 to 20 months of age.

After 12 weeks of exposure (Fig. 1b), sham controls and ELF-MF-exposed aged mice were removed from the exposure system and housed four per cage with *ad libitum* access to food and water. They were handled to minimize stress-related responses and their free-feeding weights were taken daily. We were limited in the number of animals that could be tested daily in the cognitive test battery described below. To adapt to this time-constraint, we exposed consecutive subgroups of 12 mice (6 sham and 6 ELF-MF-exposed), each subgroup starting to be exposed every month, which resulted in a total of 109 aged animals tested behaviorally over a period of 10 months. Each of these subgroups was submitted to the different paradigms of the cognitive test battery that were administered sequentially over a period of 3 weeks (Fig. 1b).

Mouse subgroups, including young mice ( $n=18$ ) and APPswe/PS1dE9 transgenics ( $n=17$ ), were transferred each day to the experimental room 30 min prior to behavioral testing. All apparatuses listed below were cleaned with 70% ethanol and dried between subjects. Given converging evidence from human and animal studies indicating that healthy young subjects are not affected by ELF-MF exposure<sup>1</sup>, only aged mice underwent ELF-MF exposure. Thus, main effects of age were examined between young versus sham-exposed aged groups and potential effects of ELF-MF exposure on aging were assessed by comparing separately sham- and ELF-MF-exposed aged groups for behavioral and cerebral readouts.

### Y-maze

The Y-maze two-trial procedure has been routinely used to examine spatial recognition memory and takes advantage of the innate tendency of rodents to explore novel environments<sup>101</sup>. To increase its spatial cognitive demand, we adapted this procedure to an automated 8-arm radial maze made of grey PVC (Imetronic, Marcheprime, France) in which only three arms were used to form a Y-shape (90°–135°–135° between the arms; e.g. arms 8, 2 and 5; Fig. 2a). Each arm was 62 cm long and 12 cm wide and radiated from a central platform (32 cm in diameter). This previously validated behavioral procedure<sup>43</sup> was composed of an exploration (encoding) phase and a recognition phase which were separated by an inter-trial interval of 4 h. During the exploration trial, one of the three available arms was closed. A mouse was positioned on the central platform of the maze and allowed to explore the two available arms during 10 min. During the recognition trial, the mouse was again placed on the central platform and could explore all three arms during 5 min. The time the animal spent in each of the three arms of the maze was automatically recorded during encoding and recognition trials by video-tracking (Poly software; Imetronic, Marcheprime, France). Discriminating the novel arm from the two familiar arms was considered as an index of spatial recognition memory. Memory performance was thus

expressed as the percentage of time spent in novel arm calculated as follows: (time spent in novel arm/time spent in all three arms)  $\times$  100. The time spent on the central platform of the maze was excluded from the calculation of performance. Chance level was set at 33% of the exploration time. The time spent by the animal in each of the three arms of the maze was recorded during encoding and test phases, with the entrance into an arm being scored when the first half of the animal's body was inside that arm.

### 8-arm radial maze

Mice were submitted to spatial discrimination testing in the same 8-arm radial maze used for the spatial recognition memory procedure. Training lasted for 10 days (2 days of habituation and 8 days of testing). Prior to the beginning of the discrimination procedure, a progressive food restriction regimen was applied over 8 days until mice reached 85–90% of their free-feeding weights. This restriction was maintained during the whole testing period by adjusting the daily amount of food provided. Once in the maze, each mouse was videotracked (Poly software; Imetronic, Marcheprime, France) to capture arm choices and latencies. Sucrose pellets were used as rewards placed at the end of the chosen arms (one single 20 mg pellet per arm; Dustless Precision Pellets, BioServ, Flemington, New Jersey, USA). During the first two days of habituation, mice were allowed to freely explore the maze with all arms accessible and baited with pellets. The two daily trials were terminated when the animal collected all 8 rewards. Food-restricted mice were then tested the next day for spatial discrimination. During the discrimination procedure, each mouse was assigned a constant set of 3 baited arms out of 8. Arms were chosen such that the 3 angles separating them were always 45°–90°–135° (for instance arms 1–2–4, 2–3–5, 3–4–6, etc.; Fig. 3a). Mice underwent 6 consecutive daily trials separated by an inter-trial interval of 1 min for 8 consecutive days. The first trial of a session began with placing a mouse on the central platform, initially with all the doors closed. After one minute had elapsed, all doors were simultaneously opened. The mouse was allowed to choose freely among the 8 arms of the maze. Upon reaching the central platform of the maze after each arm visit, all arms were briefly closed for 4 s and then opened again to allow the animal to make its next choice. This prevented the use of clockwise or counterclockwise motor strategies during exploration of the maze. The trial ended when the mouse collected the pellets from all three baited arms. The animal was then confined to the central platform with all doors closed until the next trial.

Solving this spatial discrimination task requires a combination of spatial reference and working memory. The first visit to a non-baited arm during any trial was considered a reference memory error, with a maximum of 5 per trial. Total reference memory errors included repeated visits to non-baited arms. Repeated arm visits during a trial are typically considered as working memory errors. Since the knowledge that some arms are never rewarded (reference memory) can be sufficient to avoid visits to these never-baited arms, only repeated visits to baited arms reflect genuine working memory performance. Therefore, re-entries into baited arms within a trial were scored as working memory errors. The spatial configuration of baited arms also enabled to distinguish three subtypes of reference memory errors: type 3 errors (E3) were scored when visits were made to non-baited arms that were away (not adjacent) from baited arms (e.g., in the 1, 2, 4 baited arms configuration, visits to arms 6 and 7); type 2 errors (E2) were scored when visits were made to non-baited arms adjacent to baited arms (e.g., in the 1, 2, 4 baited configuration, visits to arms 5 and 8); and type 1 errors (E1) were scored when the animal entered the only non-baited arm surrounded by two baited arms (e.g., in the 1, 2, 4 baited configuration, visits to arm 3). Because non-baited arms are located either close in space or more distant (i.e., separated) to baited arms, scoring these different types of errors enables to probe pattern separation-dependent memory<sup>79</sup>, with a decreased number of E1 and E2 errors across training days reflecting a good ability to pattern separate. Thus, for each type of E3, E2 or E1 errors, the following index was generated: Error drop = % errors on Day 1 - % errors on Day 4. The number of errors committed by each group on the first day (Day 1) and fourth day (Day 4) of training was first normalized to 100% and the difference in the percentage of errors between Day 1 and Day 4 was then calculated, with Day 4 corresponding to the training day where performance of young mice reached a plateau.

### Object location memory task

The ability of mice to recognize that an object encountered before had changed spatial location was assessed<sup>102</sup>. For three consecutive days, mice were individually habituated to a 40 cm  $\times$  40 cm empty square-shaped open field with transparent Plexiglas walls for 10 min. On the fourth day, mice were placed in the open field with two identical copies of a novel object and allowed to explore the two similar objects until the sum of time spent exploring both reached 60 s. After a delay of 30 min in a home cage, mice underwent a test trial during which one object was moved to the corner opposite to its original position. The location of the moved object was counterbalanced between mice. Time spent exploring the two objects was recorded until the sum spent exploring both reached 30 s. Memory performance was expressed as a discrimination index using the following formula: (seconds on novel object position minus seconds on familiar object position)/30 seconds; the higher the discrimination index, the better the memory.

### Composite test scores

To generate composite test scores capturing performance across the different memory procedures of our battery<sup>103</sup>, we converted individual performances to normative z-scores using the following formula:  $z = (X - M)/SD$ , where X is the animal's raw score for a given memory paradigm, and M and SD are respectively the mean and standard deviation of the control group (young mice). Z-scores served to establish a spider graph illustrating the performance of each group for spatial recognition memory, reference memory, working memory, memory precision and object location memory. The directionality of the scores was adjusted so that decreased z-score values reflected poor performance<sup>104</sup>. An overall score for the entire battery was also calculated for each group by averaging z-scores across memory paradigms, with each individual memory component receiving equal weightage.

### Euthanasia and brain extraction

For biochemical analysis, a total of 105 mice (young:  $n = 18$ ; aged sham:  $n = 45$ ; aged ELF-MF-exposed:  $n = 42$ ) were euthanized by cervical dislocation. Brains were extracted, and hippocampi were separated in an ice-cold solution of phosphate-buffered saline solution (pH = 7.4) and dissociated by using a 18G needle in ice-cold buffer containing 0.32 M sucrose supplemented with 10 mM HEPES (pH 7.4) and protease and phosphatase inhibitors (Sigma, L'Isle d'Abeau, France). Samples were kept at  $-80^{\circ}\text{C}$  until further analyses. Frozen hippocampal samples were diluted 1:1 with 2X lysis buffer (20 mM HEPES, 0.15 M NaCl, 1% triton X100, 1% deoxycholic acid, 1% SDS, pH 7.5) for Western blotting. Samples were then centrifuged 15 min at 10 000 g. The protein concentration of the supernatants was measured with the DC protein assay (Bio-Rad, Marnes-La-Coquette, France). Protein content was normalized to 50  $\mu\text{g}$  of protein per sample. Proteins were separated by SDS/PAGE on Tris-Glycine precast 4–15% gel (BioRad, Marnes-La-Coquette, France) and transferred to a polyvinylidene difluoride (PVDF) membrane using Transblot Turbo<sup>®</sup> and Transblot Turbo RTA<sup>®</sup> transfer kit (Bio-Rad, Marnes-La-Coquette, France). After blocking with a solution of Tris-Tween-buffered solution (TTBS containing 0.05% Tween 20, 200 mM NaCl and 10 mM Tris, pH 7.4) supplemented with 5% non-fat dry milk for 1 h at room temperature, the membranes were incubated with the appropriate antibodies diluted in 5% non-fat dry milk-TTBS, at  $4^{\circ}\text{C}$  overnight under gentle agitation. The following primary antibodies were used: anti-APP-695 (Clone 22C11; Millipore; 1:1000); anti-GFAP (Chemicon; 1:2000); anti-Tau5 (Invitrogen; 1:1000), anti-S100b (Abcam; 1:500) and anti-Actin (Sigma Aldrich, 1:5000). Membranes were then incubated with anti-IgG secondary antibodies conjugated to IRDye<sup>™</sup> for 1 h at room temperature. Goat anti-Rabbit (IRDye<sup>™</sup> 800CW, LI-COR; 1:2000) and goat anti-Mouse (IRDye<sup>™</sup> 680 RD, LI-COR, 1:2000) were used. Finally, membranes were scanned with the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA) and protein readouts were expressed as density light units. Respective averages were then determined across the Western blots and normalization was performed against the actin housekeeping protein to ensure that variations in sample homogeneity and loading were accounted for. This normative ratio enhanced consistency across groups. Animals from each group (young ( $n = 18$ ), aged sham- ( $n = 45$ ) and aged ELF-MF-exposed ( $n = 42$ )) were represented on each gel that contained 6 animals as shown in Fig. 6a. Several gels were generated similarly so as to process the total number of mice in each group (Figure S6).

### Quantification of A $\beta$ (1–42) peptide

The hippocampal concentration of A $\beta$ <sub>(1–42)</sub> peptide was evaluated by ELISA (Amyloid Beta 42 mouse ELISA Kit, Thermofisher, France, catalog number KMB3441) according to the manufacturer's instructions<sup>105</sup>. A total of 92 mice (young:  $n = 16$ ; aged sham:  $n = 38$ ; aged ELF-MF-exposed:  $n = 38$ ) were processed. This kit detects specifically soluble forms of mice A $\beta$ <sub>(1–42)</sub> peptides with negligible cross-reactivity with mouse A $\beta$ <sub>(1–40)</sub> forms. A $\beta$ <sub>(1–42)</sub> concentration in samples was determined by comparing the samples to a standard curve (0–250 pg/ml). The absorbance at 450 nm was read using a microplate reader.

### Statistical analyzes

Data sets were tested for normal distribution with the Kolmogorov–Smirnov test. When variables were normally distributed, the following parametric statistics were used: Student t-tests for two-group comparisons where appropriate and one-way or two-way ANOVAs with repeated measures followed by Bonferroni-corrected two-group post-hoc Student t-test or Tukey's test. When variables failed to reach normality, the nonparametric Mann-Whitney's test was used. The chi-square ( $X^2$ ) test was used to assess differences in frequency. Statistical tests were conducted using Prism 10.0 (GraphPad Software, Boston, USA). Data were expressed as mean  $\pm$  standard error mean (SEM), with  $n$  indicating the number of animals analyzed. Values of  $p < 0.05$  were considered as significant.

### Data availability

The datasets generated during and analysed during the current study are available from the corresponding authors on reasonable request.

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Conceptualization: B.B., O.N., S.H. and I.L.; Methodology: O.N., S.H., B.B., F.P.d.G., A.H. and I.L.; Investigation: S.H., O.N., V.A., F.P.d.G., A.H. and B.B.; Visualization: S.H., B.B. and O.N.; Funding acquisition: B.B., O.N. and I.L.; Project administration: B.B., O.N. and I.L. Supervision: O.N. and B.B.; Writing – original draft: B.B. and S.H.; Writing – review and editing: O.N., I.L., F.P.d.G., S.H. and B.B.

## Declarations

## Competing interests

The authors declare no competing interests.

## Ethical approval

This study has no human data. Experiments were conducted according to protocols approved by the ethical committee of the University of Bordeaux (protocol A50120159). All experimental procedures complied with official European guidelines for the care and use of laboratory animals (Directive 2010/63/UE) and followed the ARRIVE guidelines for animal research.

## Additional information

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