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Impact of Cerebral Radiofrequency Exposures on Oxidative Stress and Corticosterone in a Rat Model of Alzheimer's Disease

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Abstract.
Background: Alzheimer's disease (AD) is the most common type of neurodegenerative disease leading to dementia. Several studies suggested that mobile phone radiofrequency electromagnetic field (RF-EMF) exposures modified AD memory deficits in rodent models.
Objective: Here we aimed to test the hypothesis that RF-EMF exposure may modify memory through corticosterone and oxidative stress in the Samaritan rat model of AD.
Methods: Long-Evans male rats received intracerebroventricular infusion with ferrous sulphate, amyloid-beta 1-42 peptide, and buthionine-sufloximine (AD rats) or with vehicle (control rats). To mimic cell phone use, RF-EMF were exposed to the head for 1 month (5 days/week, in restraint). To look for hazard thresholds, high brain averaged specific absorption rates (BASAR) were tested: 1.5 W/Kg (15 min), 6 W/Kg (15 min), and 6 W/Kg (45 min). The sham group was in restraint for 45 min. Endpoints were spatial memory in the radial maze, plasmatic corticosterone, heme oxygenase-1 (HO1), and amyloid plaques.
Results: Results indicated similar corticosterone levels but impaired memory performances and increased cerebral staining of thioflavine and of HO1 in the sham AD rats compared to the controls. A correlative increase of cortical HO1 staining was the only effect of RF-EMF in control rats. In AD rats, RF-EMF exposures induced a correlative increase of hippocampal HO1 staining and reduced corticosterone.
Discussion: According to our data, neither AD nor control rats showed modified memory after RF-EMF exposures. Unlike control rats, AD rats showed higher hippocampal oxidative stress and reduced corticosterone with the higher BASAR. This data suggests more fragility related to neurodegenerative disease toward RF-EMF exposures.

Keywords: Alzheimer's disease, corticosterone, memory, mobile phone, oxidative stress, radiofrequency

INTRODUCTION

Alzheimer's disease (AD) is the most common type of neurodegenerative disease leading to dementia. Loss of memory is among the first symptoms reported by patients. Working memory and long-term declarative memory are affected early during

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the disease. The individual pattern of impaired memory functions correlates with parameters of structural or functional brain integrity. Typical neurochemical lesions include depositions of amyloid-β $(Aβ)_{1-42}$ peptide which results from an altered proteolytic cleavage of the Aβ protein precursor (AβPP). Increased oxidative stress as heme oxygenase 1 (HO1) was consistently observed in the brain of AD patients as well as AD rodent models [1–6].

Nowadays, the use of mobile phones has gradually increased raising issues about the possible cerebral effects of radiofrequency electromagnetic fields (RF-EMF). Up to date, there is no clear conclusion regarding RF-EMF beneficial or deleterious effects in rodent models, either healthy or pathological.

Several studies showed beneficial RF-EMF effects on transgenic AD mice overexpressing the mutant Swedish form of human APP (APPsw) or containing five mutant human genes associated with AD (5xFAD). These mice are considered to model familial AD which occurs in about 2-3% of all AD cases [6]. Arendash et al. showed that about 8 months of daily whole body RF-EMF exposure at low brain average specific absorption rate (BASAR, 0.25 W/kg, 918 MHz) reversed both cognitive impairment and Aβ deposition in aged APPsw mice [7]. Similar data were reported after shorter exposures (2 months) [8] or at 5 W/Kg using 5xFAD mice [9]. Some mechanisms were proposed including a possible mediation through enhanced neuronal and mitochondrial activity [10].

Alternatively, other authors suggested the absence of cerebral RF-EMF effect. No effect on Aβ aggregation was detected *in vitro* after short-term exposures [11] or after 3 months' exposure at 5 W/kg in 5xFAD mice [12]. No effect on senescence-typical deficits in spatial memory and on brain interleukins was shown in aged rats after a 1-month exposure [13]. Preserved performances in the navigation or working memory tasks were reported after about 20 days exposure at 0.05-1.44 W/kg [14, 15]. Normal cytokine levels or oxidative stress parameters were reported after *in vitro* or *in vivo* RF-EMF exposures [13, 15–20].

In contrast, other authors suggested possible toxicological effects of RF-EMF exposures. Increased inflammatory cytokines levels and plasmatic corticosterone as well as impaired emotional memory were reported in our laboratory in middle-aged rats after an acute head only exposure at 6 W/Kg [19]. Alterations of spatial and non-spatial memory were shown in young adult rats after 4-5 weeks' exposures [21, 22]. Increased oxidative stress was reported after RF-EMF head exposure for 10 months in adult rats [23].

If daily RF-EMF exposures were shown to modify brain in AD, it would mark an important biological interaction with possible public health consequences. Here we aimed to test the hypothesis that RF-EMF exposure may modify memory through corticosterone and oxidative stress in a rat model of the sporadic development of AD.

The Samaritan rats™ were obtained by a continuous intracerebroventricular infusion of the FAB solution containing ferrous sulphate, $Aβ_{1-42}$, and buthionine-sufloximine. The FAB solution was previously shown to lead to cerebral amyloid plaques and neuritic deposition [2]. Both AD and control rats received repeated head only exposures to mobile phone RF-EMF in restraint (BASAR = 0 W/kg 45 min; 1.5 W/kg 15 min; 6 W/kg 15 min; and 6 W/kg 45 min). Spatial memory in the radial arm maze, plasmatic corticosterone levels, HO1, and amyloid plaques in the brain were assessed.

MATERIALS AND METHODS

Animals

Forty Long-Evans male rats were purchased from Taconic Pharmaceuticals (USA). They were 2-3 months old and weighed approximately 200 g upon arrival. All rats were housed two per cage under controlled conditions of temperature (20-22°C), humidity (40%), and 12 h-light/dark cycle with *ad libitum* access to water. Food was delivered *ad libitum* excepted during deprivation. The cage's environment was enriched with plastic cylinders identical in shape and appearance to RF-EMF exposure rockets. All rats were handled daily and allowed 2 weeks to acclimate to environmental conditions before experimentation began. The protocols were approved by the French State Council guidelines for the care and use of laboratory animals (Decree n° 87-849, October 19, 1987).

Experimental procedure

Control and AD rats were randomly assigned to an exposure group: 0 W/kg 45 min ($n = 6$), 1.5 W/kg 15 min ($n = 5$), 6 W/kg 15 min ($n = 4$), or 6 W/kg 45 min ($n = 5$). Daily exposures were performed from 9 : 00 a.m. till 11 : 00 a.m. starting at day 1 and were repeated 20 times over a 26-day period (Fig. 1). Rats were food deprived to 85% of their *ad libitum* body weight starting one week before day 1 until day 10,

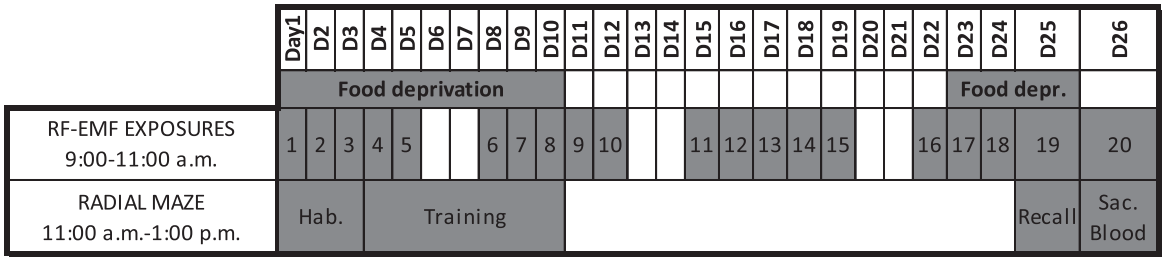


Fig. 1. Experiment design. Rats were exposed in the morning (9:00 a.m. – 11:00 a.m.) for 20 days to RF-EMF. Behavioral tests were scheduled right after the exposures, from 11 : 00 a.m. till 1 : 00 p.m. From D1 to D3 rats were habituated to the radial maze apparatus. From D4 till D10 rats performed the radial maze training. On D25, they were tested for memory recall. Rats were sacrificed (Sac.) on D26 after the last exposure. The brain and blood were collected.

and between day 23 and day 25. The radial maze test was performed between day 1 and day 10 and on day 25. On day 26, rats were sacrificed. Blood and brain samples were collected.

Model of Alzheimer's disease

At Taconic Pharmaceuticals (USA), 20 Samaritan rats™ were implanted with osmotic pump connected to a permanent cannula inserted into the left cerebral ventricle. They were infused chronically for 4 weeks with the FAB solution: ferrous sulfate heptahydrate (1 mM), $A\beta_{1\text{-}42}$ (15 μM), and L-buthionine-(S, R)-sulfoximine (12 mM) solution [2]. Twenty control rats were infused with artificial cerebrospinal fluid. Rats were received at INERIS two weeks after the osmotic pump implantation.

Radiofrequency exposure system

Exposure set-up was previously described [24]. A RF generator (900–64 type, Radio Frequency Power Amplifier, France) emitting a 900 MHz RF (1/8 duty factor, pulse repetition rate of 217 Hz) was connected to a four-output divider. Each output was connected to a loop antenna allowing local exposure of four animal's heads simultaneously. During exposure, animals were placed in individual Plexiglas rockets (5 mm thick, 6 cm diameter, and 15 cm length) capped with a truncated cone on which a loop antenna was fixed. Rockets were lined with holes to minimize the rise in body temperature. Exposed and sham-exposed animals were placed at the same time in two identical anechoic chambers during the exposure session. BASAR calculations were made with segmented rats and experimental confirmation measurements were performed with homogeneous phantoms [25].

Radial maze test

Spatial memory was measured in the eight-arm radial maze. The apparatus was an octagonal central platform with eight identical arms numbered from 1 to 8. Extra maze visual cues were placed on the walls for orientation.

Habituation

Habituation sessions were performed once daily on day 1, 2, and 3. Each rat was placed in the central platform with food pellets. The habituation session lasted until the rat ate all food pellets with a maximum duration of 6 min.

Training

Training sessions were performed once daily from day 4 to day 10. The arms 1, 2, 4, and 7 were baited for all rats through all training sessions. In each session, the rat was placed in the central platform and allowed to freely explore the radial arms until he ate the 4 pellets, or 10 min passed. Between each animal, the maze was cleaned with 70% alcohol solution.

Recall

On day 25, the rats were tested as during a training session.

A *success* was counted when the rat ate a baited arm. A *reference memory error* (RME) was counted when an animal visited a never baited arm (3, 5, 6, and 8). A visit was counted when rat's forepaws crossed the half of arm's length. A *working memory error* (WME) was counted when an animal visited more than once a baited arm. The performance was equal to:

$$\text{Performance} = \frac{\text{Number of success}}{\text{Maximum number of success (4)} + \text{RME} + \text{WME}}$$

Blood and brain tissue processing

Rats anesthetized with isoflurane were transcardially perfused with 0.1% phosphate buffered saline (PBS). Blood was collected from *venae cavae* in heparin vials and centrifuged at 10 000 rpm for 10 min at 4°C to separate plasma. Plasma was stored at –80°C until assayed. The brains were quickly removed. The left hemisphere was assigned for immunohistochemistry. The brains were fixed in 4% paraformaldehyde (PFA) for 4 days, incubated in 30% sucrose solution, frozen in isopentane at –50°C, and stored at –80°C. Twenty μm sagittal brain slices were performed using a cryostat microtome, and stored at –21°C in cryoprotectant solution. The section allowed to check cannula implantation. Given the fact that infusion was into the ventricle, the FAB solution also impacted the right hemisphere which was assigned for Enzyme Linked Immuno Sorbant Assay (ELISA). The cortex and the hippocampus were snap-frozen on a bed of dry ice, grounded into powder and stored at –80°C.

Thioflavin-S immunohistochemistry

Thioflavin-S staining was performed to bind to amyloid fibrils. The protocol was adapted from Vallet et al. [26]. Briefly, sections were mounted on superfrost positive slides and dried for 5 h at 37°C. Sections were washed 3 times with PBS and incubated in 0.03% potassium permanganate (KMnO4), 1% oxalic acid, and 1% metabisulfite potassium for 5 min. The sections were run in 0.02% thioflavin-S for 8 min in the dark and rinsed in 80% ethanol. After washing with PBS, sections were incubated in the Hoechst solution for 10 min in order to stain the nuclei in blue. Thioflavin-S gives a distinct spectral shift revealed in green fluorescence. Slides were observed under a fluorescence microscope coupled to Axiovison software.

HO1 enzyme-linked immunosorbent assay (ELISA)

Brain samples were homogenized by sonication in ice-cold Trisma/Base buffer containing protease inhibitor cocktail 4% (Roche) and phosphatase inhibitor cocktail 1% (Sigma). Samples were centrifugated at 9800 rpm for 15 min and the supernatant was collected. A Bradford test was used to measure total protein content in each sample. Brain HO1 assessment was performed using a sandwich ELISA development kit (Immunoset™ HO1 rat, Enzo Life Sciences) according to the manufacturer's instructions. Briefly, plates were coated overnight with HO1 monoclonal capture antibody and then blocked for at least 1 h with 1% bovine serum albumin blocker. After adding standards and samples, plates were incubated with the detection antibody for 2 h. Plates were washed three times with PBS-0.05% and Tween-20. TMB development solution was added to the wells and plates were incubated for 10-15 min in the dark. A stop solution was added, and the optical density was read at 450 nm. HO1 concentrations were determined using standard curves and were expressed as ng/mg of total protein content.

Plasmatic corticosterone

A corticosterone competitive assay was performed with a commercially available kit (assay designs, correlate-EIA corticosterone, R&D) according to the manufacturer's instructions. Briefly, polyclonal anti-corticosterone antibody and alkaline phosphatase-labeled corticosterone were added to the pre-coated wells. After 2 h incubation at room temperature, the excess reagents were washed away, and substrate was added. After 1 h incubation, the enzymatic reaction was stopped with trisodium phosphate solution. Corticosterone concentrations (ng/ml) were inversely proportional to the intensity of the yellow color read at 405 nm.

Statistical analysis

Statistical analyses were performed using the SPSS 16 software (Inc., Chicago, IL, USA). Values were given as mean ± standard deviation of mean (SEM) per group. Levene's test was used for variance homogeneity. Analyses were performed using Mann-Whitney tests, correlations (Spearman coefficient), 3 and 2-ways analysis of variance (ANOVA). Time was treated as within subject factor while rat model and BASARs were treated as between subject factors. Effects were considered significant when $p < 0.05$.

RESULTS

Increased thioflavine tangles in the AD rats

Data reported in Fig. 2 showed thioflavine tangles in the control and FAB-injected rats. RF-EMF exposures had no effect on the number of thioflavine tangles ($p > 0.05$). Data from each rat model were pooled. The number of thioflavine tangles was

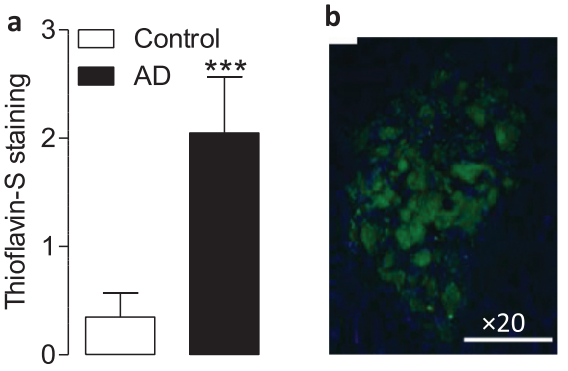


Fig. 2. Thioflavine tangles. Amyloid plaques were detected using the thioflavine staining in control and AD rats. a) There was no RF-EMF effect. Data from each rat model were pooled. ***$p < 0.0008$: The AD rats showed more thioflavine plaques than the controls ($n = 20$/group). b) Picture of the striatum of a sham rat (0 W/kg) perfused with the FAB solution (×20), showing an amyloid plaque labeled in green fluorescence with cell nuclei in blue.

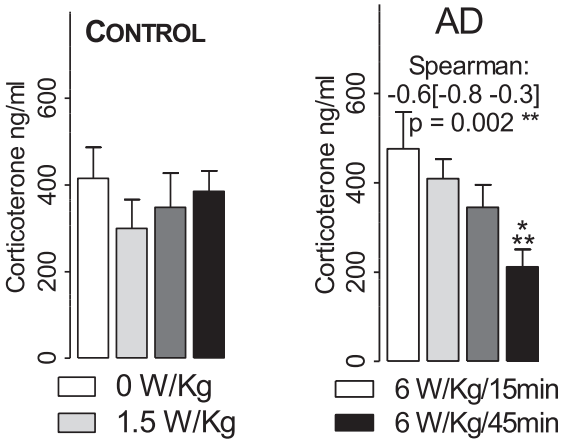


Fig. 3. Effects of RF-EMF on plasmatic corticosterone. Basal (sham) corticosterone levels were not different between control and AD rats. In the controls, the BASARs had no effect on corticosterone. **$p = 0.002$: corticosterone of AD rats was significantly inversely correlated to the BASARs (Spearman -0.6 [-0.8, -0.3]). **$p = 0.002$ and *$p = 0.015$: the 6 W/kg 45 min exposed AD rats showed significantly less corticosterone than the sham AD rats, and than the 6 W/kg 45 min exposed controls, respectively. ($n = 4$-6/group).

higher in the AD rats compared to the control rats ($p = 0.0008$) (Fig. 2).

BASAR-dependent correlative decrease of corticosterone in the AD rats

Data reported in Fig. 3 showed plasmatic corticosterone level in control and AD rats. Basal (sham) levels of corticosterone were not different between AD and control rats ($p > 0.05$). In the control groups, corticosterone levels were not dependent on BASARs ($p > 0.05$). However, in the AD groups, there was a correlative reduction of corticosterone at the higher BASAR (significant inverse correlation: Spearman coefficient -0.6 [-0.8, -0.3] $p = 0.002$).

BASAR-dependent increase of HO1

Data reported in Fig. 4a showed HO1 levels in the hippocampus in control and AD rats. In the control group, hippocampal HO1 was not dependent on the BASARs ($p = 0.4$). Basal (sham) HO1 level was higher in the AD rats compared to the control rats ($p < 0.01$). In AD rats, there was a correlative increase of HO1 at the higher BASARs (significant correlation: Spearman coefficient 0.5 [0.1, 0.8] $p = 0.01$).

Data reported in Fig. 4b showed HO1 levels in the cortex in control and AD rats. In the control group, cortical HO1 was dependent on the BASARs (significant correlation: Spearman coefficient 0.4 [0.005, 0.7], $p = 0.04$). Basal (sham) HO1 level was higher in the AD rats compared to the control rats ($p = 0.03$). In AD rats, cortical HO1 was not dependent on the BASARs ($p = 0.6$).

Absence of effect of RF-EMF exposures on spatial memory

Data reported in Fig. 5 indicated (a) the performance, (b) the RME, and (c) the WME. The 3-ways ANOVA did not show any BASAR effect on performance, RME and WME ($p > 0.05$). Thus, data were pooled for each rat model. The 2-ways ANOVA indicated that both memory performance and RME were significantly dependent on the rat model (respectively, $F(1,45)=19.6$, $p < 0.0001$ and $F(1,45)=17.2$, $p = 0.0001$), a time effect ($F(7,315)=5.9$, $p < 0.0001$ and $F(7,315)=8.1$, $p < 0.0001$) and the rat model×time interaction ($F(7,315)=2.3$, $p = 0.03$ and $F(7,315)=2.2$, $p = 0.04$). Compared to the controls, AD rat showed reduced performances at the 7th training session ($p < 0.01$) and on the recall test ($p < 0.001$) and commit more RME on the recall test ($p < 0.001$) (Fig. 5a, b). The 2-ways ANOVA performed on WME indicated a significant rat model effect ($F(1,45)=10.2$, $p = 0.002$) but no time effect ($F(7,315)=1.8$, $p = 0.09$) or rat model×time interaction ($F(7,315)=0.4$, $p = 0.09$).

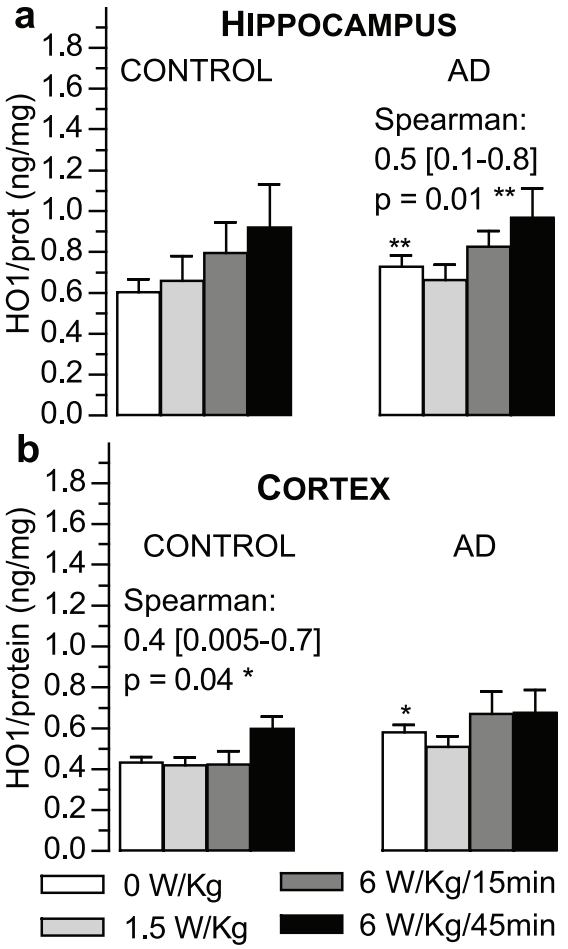


Fig. 4. BASAR-dependent increased cerebral HO1. HO1 ELISAs were performed on (a) hippocampus and (b) cortical samples in control and AD rats. a) In the hippocampus of controls, the BASARs had no effect on HO1. $^{**}p < 0.01$, basal (sham) levels of hippocampal HO1 were higher in AD rats than in controls. $^{**}p = 0.01$: hippocampal HO1 in the AD rats was significantly correlated to the BASARs (Spearman: 0.5 [0.1-0.8]). b) $^{**}p = 0.01$: cortical HO1 in the control rats was significantly correlated to the BASARs (Spearman: 0.5 [0.1-0.8]). $^{*}p < 0.05$: basal (sham) levels of cortical HO1 were higher in AD rats than in controls. In the cortex of AD rats, the BASARs had no effect on HO1 ($n = 4$-6/group).

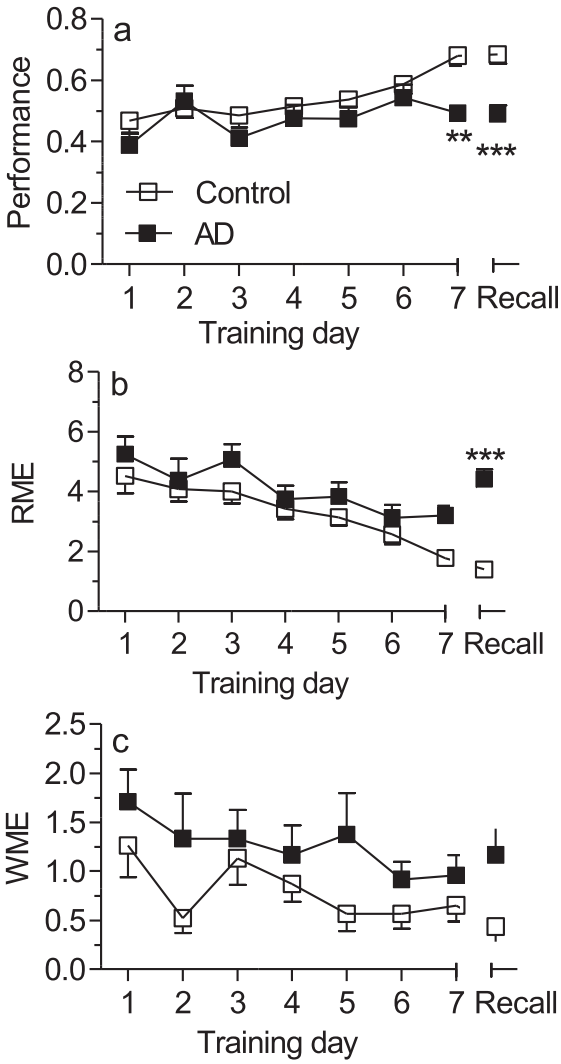


Fig. 5. Spatial memory in the radial maze. Control and AD rats were tested in the radial maze. There was no RF-EMF effect. Data from each rat model were pooled. a) $^{**}p < 0.001$, $^{***}p < 0.0001$: AD rat performances were reduced compared to controls on the 7th training session and on the recall session. b) $^{***}p < 0.0001$: AD rats committed more reference memory errors (RME) than the controls on the recall session. c) AD rats showed more working memory errors (WME) than controls ($n = 4$-6 rats/group).

DISCUSSION

To our knowledge, this is the first report to assess the neurobiological effects of RF-EMF exposures in the FAB Samaritan rats™ model of sporadic form of AD. Compared to control rats, the Samaritan AD rats presented more memory errors, more amyloid plaques, and more oxidative stress in the brain. Our data suggested different BASARs-dependent effects in control or AD rats. AD rats showed correlative increased HO1 in the hippocampus and correlative decreased corticosterone, while the only effect in control rats was a correlative increased oxidative stress in the cortex.

The system of loop antennas in restrained rats allowed similar brain exposure as for a human brain during a phone call [27]. We precisely evaluated numerical and experimental dosimetry [25]. Higher BASARs compared to ICNIRP limits were used to look for hazard thresholds. Limits are 2 W/kg super-

ficial (equivalent to 0.5 W/kg BASAR in the rat). Here, 6 W/kg BASAR in the rat was about 24 W/kg superficial in Human [28].

The physiological role of Aβ peptide on learning and memory has been hypothesized to regulate activity-dependent synaptic vesicle release [29, 30]. In previous *in vivo* studies in healthy rats, acute or repeated RF-EMF exposures for 10 months had no effect on cerebral Aβ [23, 31]. Our data confirmed these results as 20 daily RF-EMF exposures did not induce thioflavine tangles in the healthy controls.

Aβ was largely studied because its aggregates into neuritic plaques are the hallmark of AD and play a central role in neurobiochemical impairments [32]. Here more Aβ aggregates were detected in the FAB Samaritan rats™ compared to control brains.

Several *in vivo* studies on AD models using 1 to 8 months 918 MHz exposures at 0.25 to 5 W/kg suggested a beneficial impact of RF-EMF, i.e., the reduction of Aβ deposition in AD transgenic mice [7–10]. The proposed mechanism was Aβ oligomers disaggregation into soluble $A\beta_{1\text{-}40}$. To the contrary, at 1950 MHz and about 5 W/kg, Aβ depositions remained unchanged *in vivo* after 3 months' exposure in 5xFAD mice or *in vitro* after 3 days exposure in mouse neuronal cells and human neuroblastoma cells [11, 12]. Our data in the Samaritan rats confirmed these negative results as thioflavine tangle levels were stable after 20 daily exposures even at high BASARs. Results discrepancies with Arendash's studies may come from different AD models leading to different type of Aβ depositions, whole body or head only RF-EMF exposures, exposure duration and side effects as temperature increase.

HO1 is a highly sensitive protein to oxidative stress. Many *in vivo* and *in vitro* studies have suggested that RF-EMF may trigger the formation of reactive oxygen species ([33–36], for review: [37, 38]). A previous study using the GSM 900 MHz signal showed that the increase of cerebral HO1 was dependent on the duration of 0.016 W/kg exposures [39]. Our data added further support to this hypothesis: there was a correlative cortical HO1 increase at the longer RF-EMF exposure and the higher BASAR. This correlative increase was only detected in the cortex and there was a trend in the hippocampus probably not powerful enough to be significant. This discrepancy could be explained by a higher absorbed energy in the cortex than in the hippocampus, due to the shorter distance from the antenna to the cortex than to the hippocampus. Heme oxygenase expression is induced by diverse stimuli (including inflammatory cytokines and factors that promote oxidative stress). Its expression may be protective through the antioxidant machinery or by producing molecules involved in the signal transduction pathways which is very important for brain neuroprotective. Here, following RF-EMF exposure, its response may have been beneficial for protecting neurons against oxidative-stress.

The Samaritan AD rats showed higher cerebral HO1 compared to the control rats suggesting cerebral oxidative stress resulting from neurodegenerative process as consistently observed in human tissues and animal model brains of AD [2–5]. In this FAB rat model, Lecanu and collaborators reported that the infusion of buthionine-sufloximine or ferrous sulphate alone, or in combination, failed to show the histological modifications seen with FAB suggesting that, these two chemicals were not neurotoxic [2]. However, as in the present study protocols and endpoints were slightly different from Lecanu's, we cannot exclude that buthionine-sufloximine or ferrous sulphate may play a toxicological role by themselves. Our data suggested different BASARs-dependent effects in control or AD rats. RF-EMF exposed Samaritan AD rats showed that HO1 was dependent on the BASARs in the hippocampus. The hippocampus may have been rendered particularly fragile in response to RF-EMF exposures due to the neurodegenerative process. In the cortex, the HO1 levels in the exposed AD group were not further increased by RF-EMF exposures but were still higher than the exposed control group, suggesting a possible sealing effect. Similarly, previous data indicated that oxidative damage in the somatosensory cortex region of middle aged mice was not further modified by RF-EMF exposures (1950 MHz for 8 months, 5 W/kg) but was remarkably higher than in young mice [40].

Plasmatic corticosterone is a well-used marker for stress. Its secretion is increasing between a.m. and p.m. and follows a circadian oscillation [41]. It was previously shown that corticosterone reaches a peak within the first 30 min of an acute inescapable stress as restraint in our protocol [41]. A wide discrepancy among results indicated that RF-EMFs could either have no effect, reduce, or increase corticosterone after acute exposures at high SARs (5-10 W/kg) [18, 19, 42]. Our previous data also suggested no RF-EMF effect on corticosterone levels after repeated head only exposures in adult rats [13]. Here, we comforted this data as control rat corticosterone was not modified after 20 RF-EMF exposures even at high BASARs. Data discrepancies may come from transitory effects, different acute restraint stress reactions,

corticosterone measurement time in the day or time after the end and/or the start of RF-EMF exposures [18].

In AD, hypercortisolemia and loss of plasmatic corticosterone circadian oscillation were shown to be related to AD clinical progression [43–48]. Here, sham Samaritan rats did not show more corticosterone than the controls suggesting similar stress levels. Hypercortisolemia seems to be an integrative aspect of AD which is not commonly reproduced in rodent models.

Our previous experiment in aging rats suggested that repeated head only RF-EMF exposures did not modify the levels of corticosterone [13]. Our present data in Samaritan rats suggested that 6 W/kg exposures induced a correlative reduction of plasmatic corticosterone. In our previous data [19], we reported increased corticosterone after a single acute RF-EMF in aged rats. Such an hormetic response may suggest that RF-EMF exposure was perceived as a stress. This stress could elicit increased corticosterone levels after an acute exposure. To the opposite, repeated exposures could lead to adaptation shown by decreased corticosterone. On the other hand, one may hypothesize that RF-EMF indirectly reduced restraint stress. It could happen through processes related to the neurodegenerative events, possibly involving high BASARs-induced hippocampal HO1.

Up to date, there is no consensus regarding the possible memory deficits after RF-EMF exposures. Several studies reported deleterious effect [7, 49–52]. Using 2450-MHz microwaves in healthy young adult rats, several authors reported spatial memory deficits when a RF-EMF exposure preceded each training session of the radial or the Morris water maze [53, 54]. On the other hand, several studies suggested no RF-EMF effect on memory performance [14, 15, 24, 55–60]. According to other authors, RF-EMF could also cause transitory spatial memory alterations, thus giving a possible explanation for data discrepancies between studies [22]. Here RF-EMF exposures at high BASAR levels prior to each training had no effect on spatial memory in control rats.

The FAB Samaritan rats™ showed a significant decrease in spatial memory, transposable to the memory decline typical of AD. Previously, toxicological RF-EMF effects were suggested in neurodegenerative mice by reduced swimming speed in the Morris water maze or in middle-aged rats, by disrupted emotional memory at 6 W/kg [14, 19]. Alternatively, several studies from Arendash et al. suggested RF-EMF-induced beneficial effects by the reversion of cognitive impairments in AD transgenic mice [7]. Meanwhile, senescence-typical deficits on spatial memory were not modified in aging rats after daily exposures at high BASARs levels [13]. Here, the Samaritan AD rats showed no memory amelioration or degradation related to RF-EMF exposures. Discrepancies between studies may come from model-type specificities and protocols (memory tests, RF-EMF exposures) or temperature increase. In our system, no modification of skin temperature was reported at 6 W/kg compared to the sham-exposed rats [61].

To conclude, here, neither AD nor control rats showed modified memory after RF-EMF exposures. However, unlike control rats, AD rats showed higher hippocampal oxidative stress and reduced corticosterone with the higher BASARs suggesting a possible increased fragility related to neurodegenerative disease toward RF-EMF exposures. Further studies should be performed for mechanism assessment.

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Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/19-0593r1).

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