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Prevention and Treatment of Alzheimer's Disease Through Electromagnetic Field Exposure

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Arendash et al.

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(45) **Date of Patent:** **Jan. 19, 2016**

(54) **PREVENTION AND TREATMENT OF
ALZHEIMER'S DISEASE THROUGH
ELECTROMAGNETIC FIELD EXPOSURE**

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patent is extended or adjusted under 35
U.S.C. 154(b) by 206 days.

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11, 2009.

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A61N 1/00 (2006.01)
A61N 5/02 (2006.01)

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CPC **A61N 5/02** (2013.01)

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CPC A61N 2/02; A61N 2/002; A61N 2/006;
A61N 5/02
USPC 600/9, 13, 15; 607/2, 115
See application file for complete search history.

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Primary Examiner — Navin Natnithithadha

Assistant Examiner — Kaylee Wilson

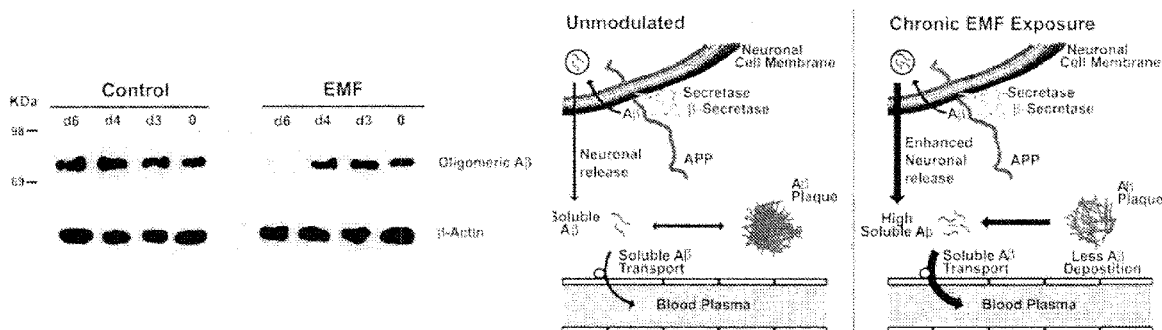
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J. Choksi; Michele L. Lawson

(57) **ABSTRACT**

The invention includes a method of treating and preventing a
neurological disorder, such as Alzheimer's Disease, in a sub-
ject in need thereof by positioning an electromagnetic field
emitting source proximal to the subject and exposing the
subject to an electromagnetic field having a predetermined
frequency for a predetermined absorption period. Preferably,
each individual treatment (comprising exposure to the prede-
termined frequency for the predetermined absorption period)
is continued at a predetermined schedule (preferably daily)
for a predetermined treatment period.

The predetermined frequency, according to a preferred
embodiment, is about 918 MHz with a specific absorption
rate (SAR) of about 0.25 W/kg+/-2 dB. The predetermined
absorption period of this preferred embodiment is about one
hour. The treatment period is long-term, being greater than
about 6 months and preferably between about 7 and 9 months.

20 Claims, 13 Drawing Sheets



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Figure 1A

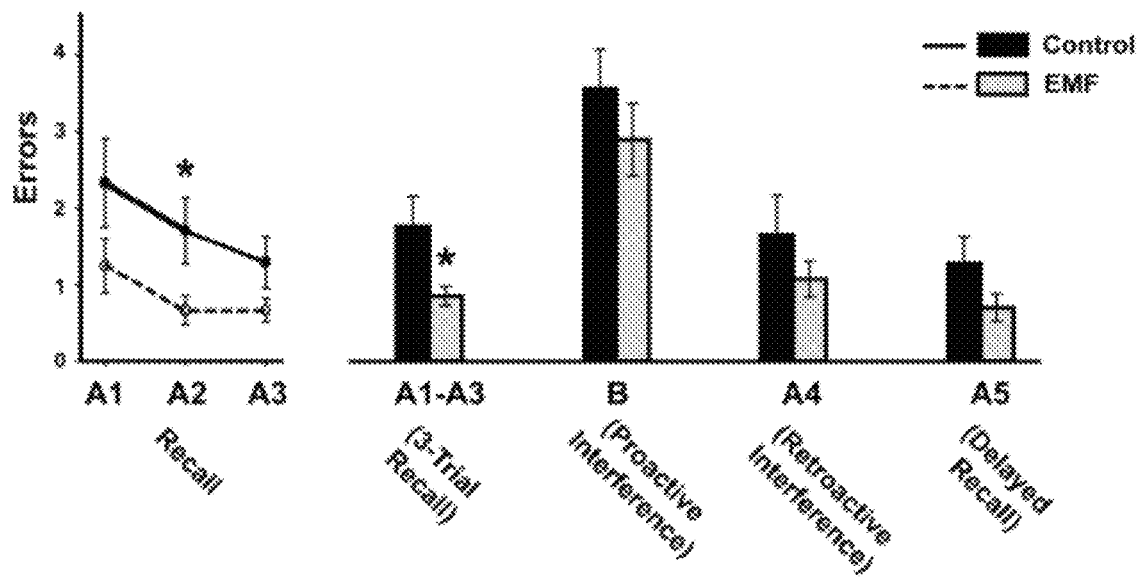


Figure 1B

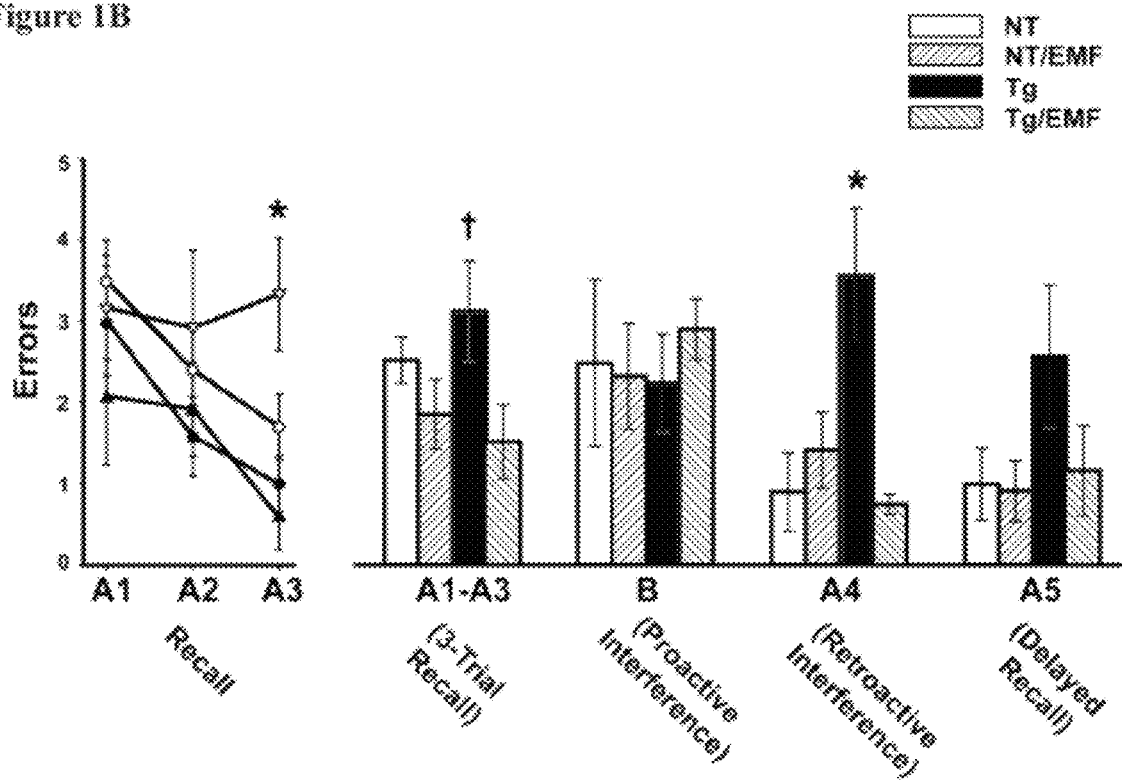


Figure 1C

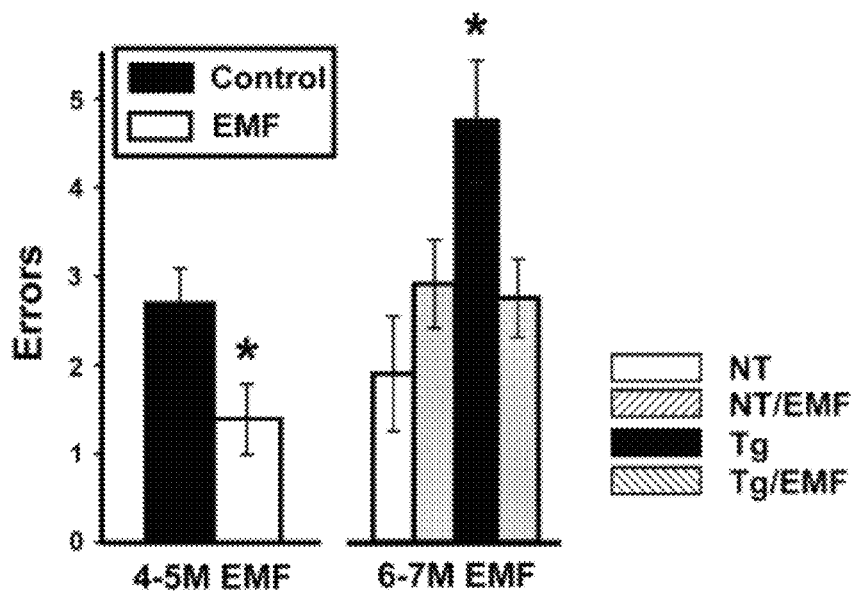
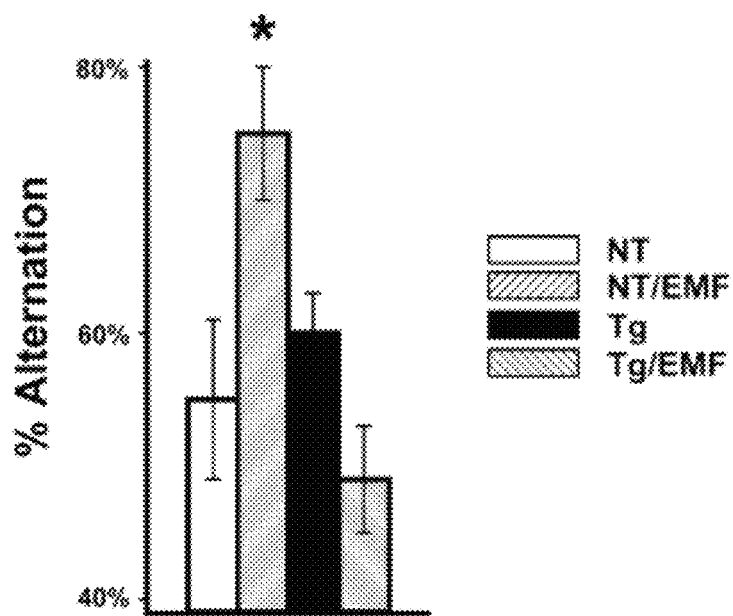


Figure 1D



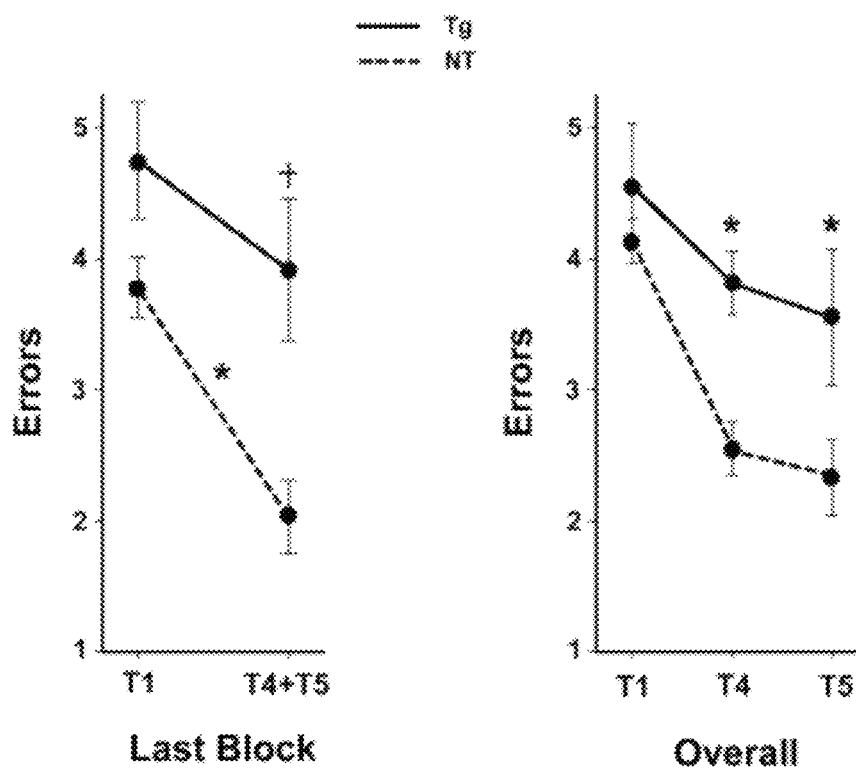


Figure 2

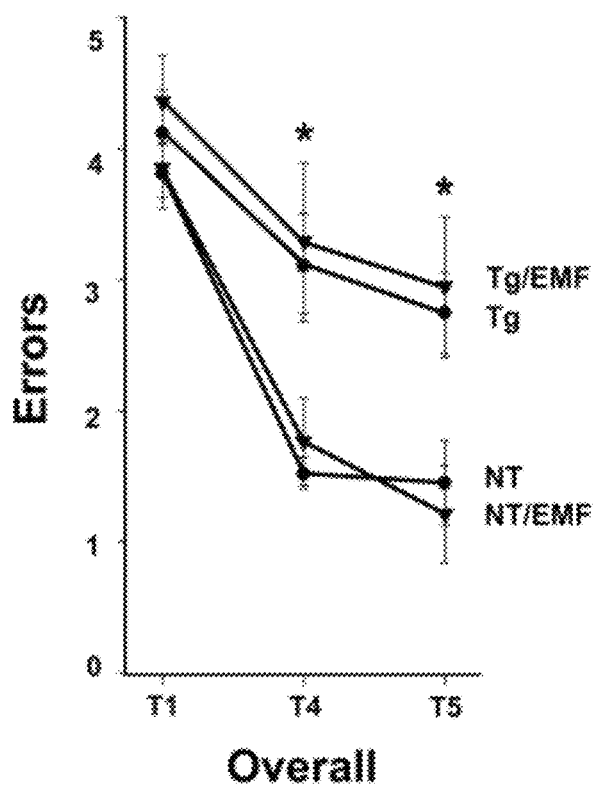


Figure 3

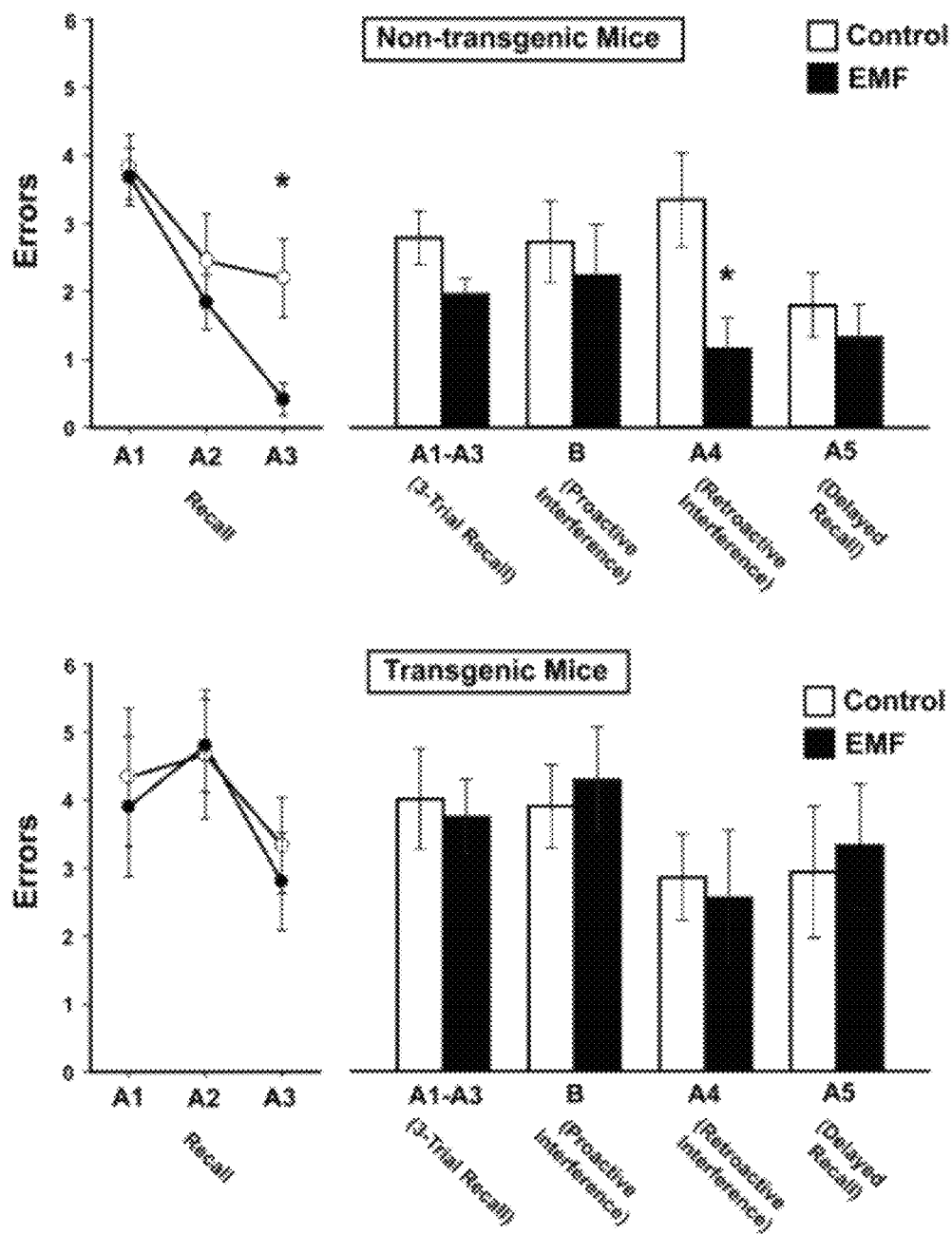


Figure 4

Figure 5A

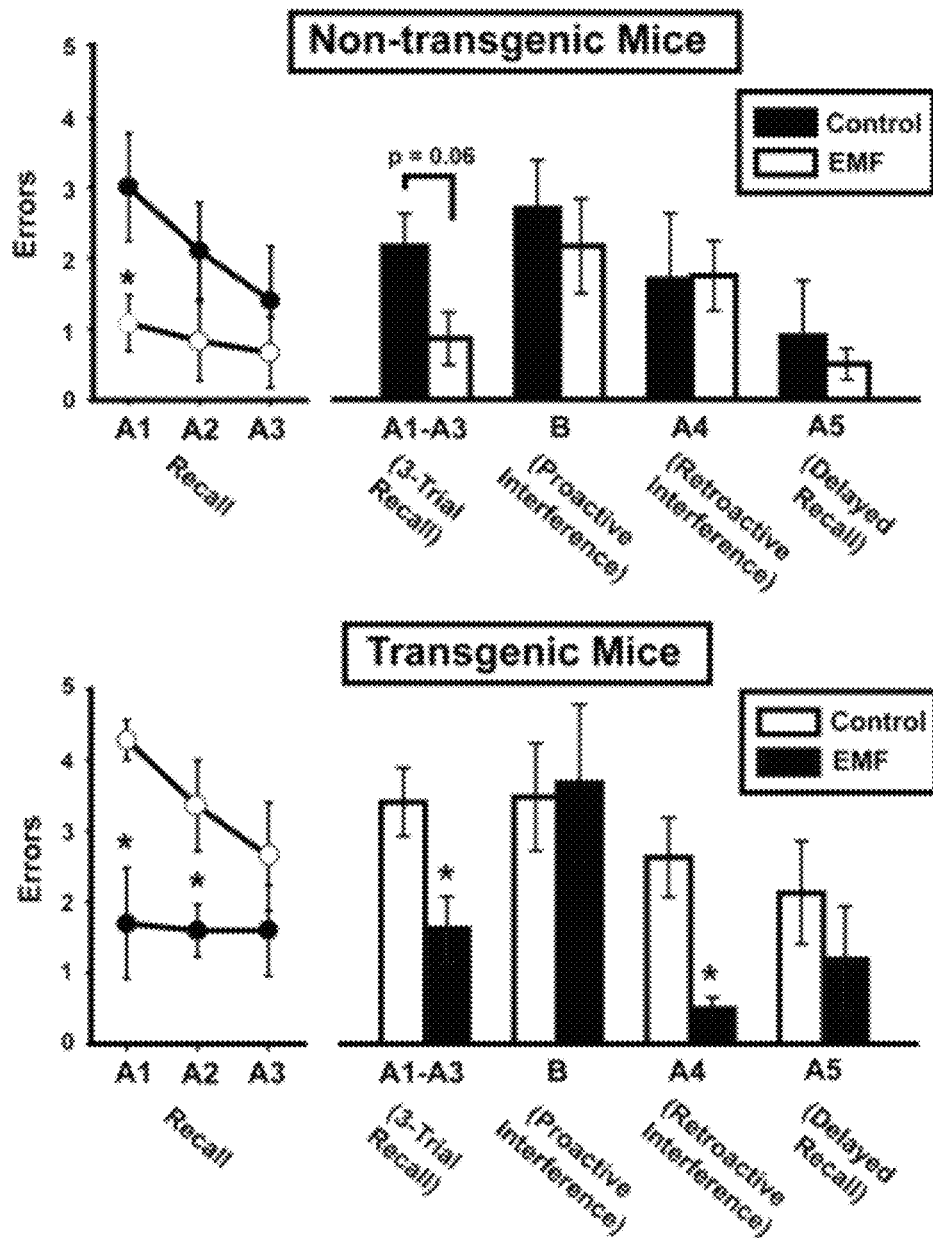


Figure 5B

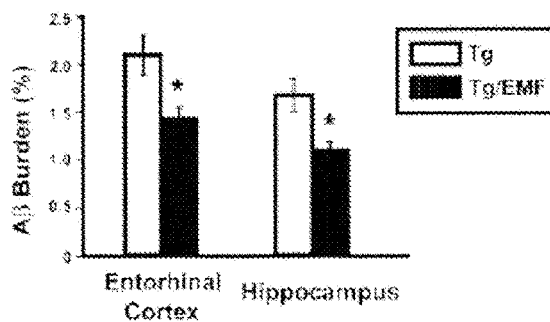
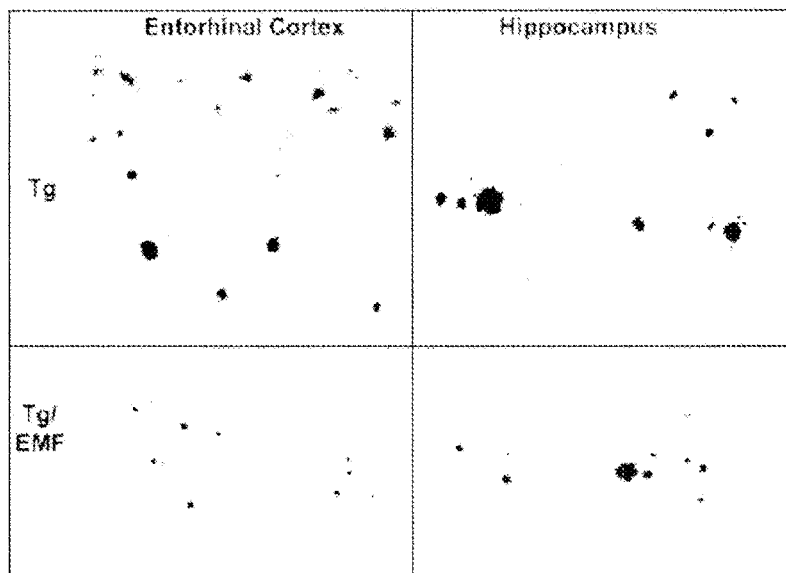
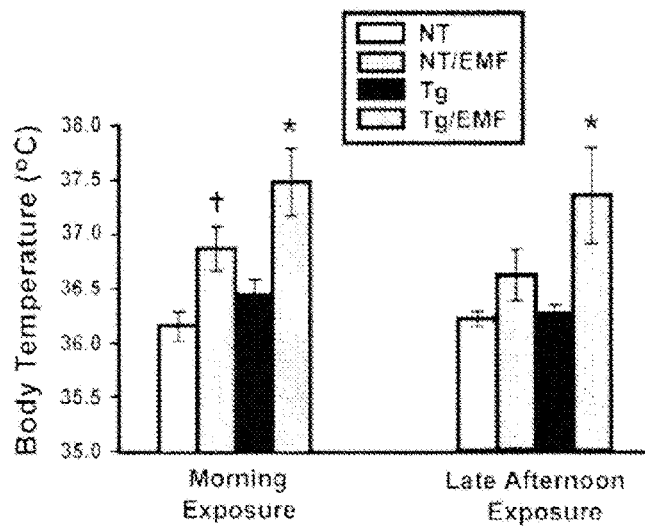


Figure 5C

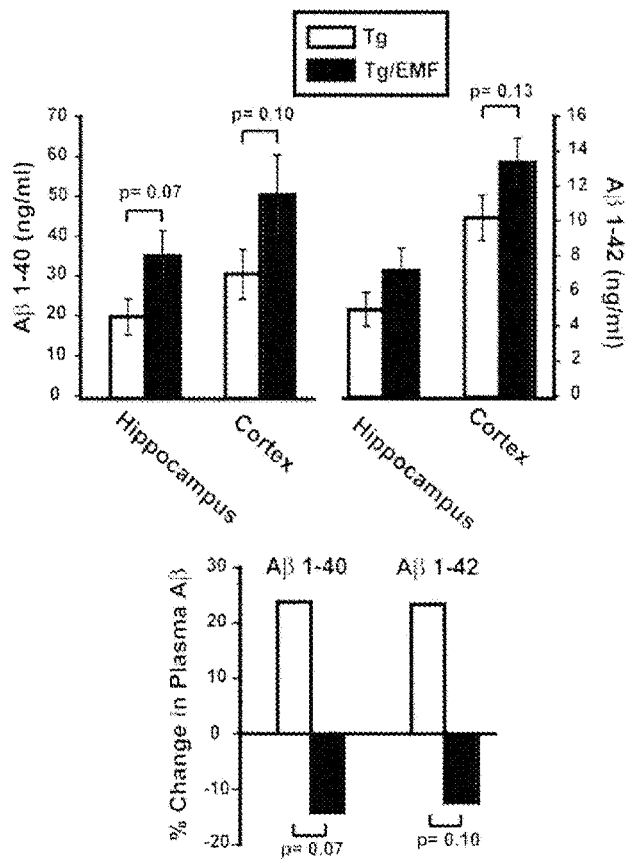
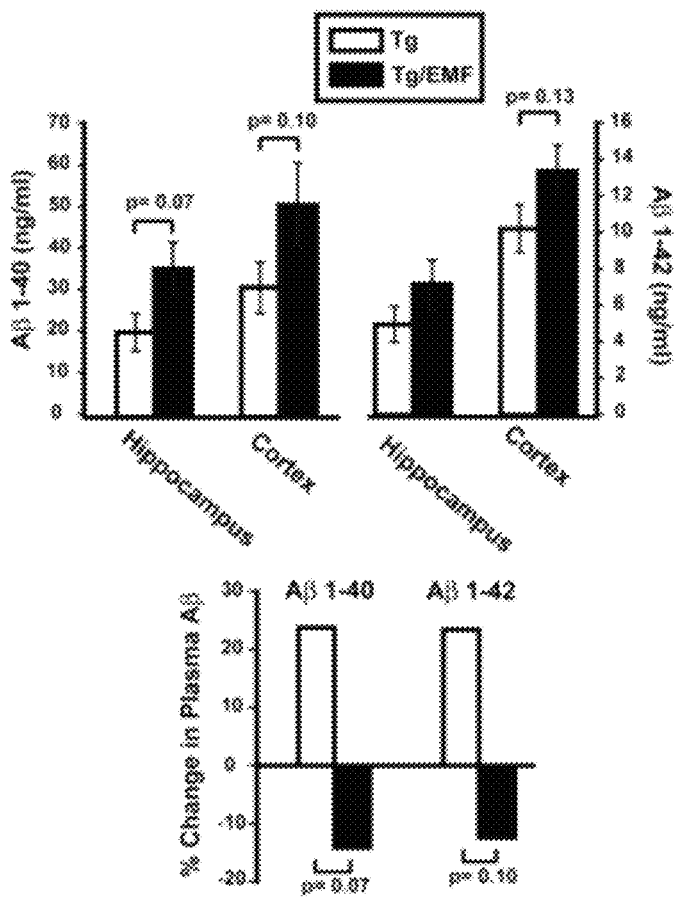


Figure 5D

Figure 5E



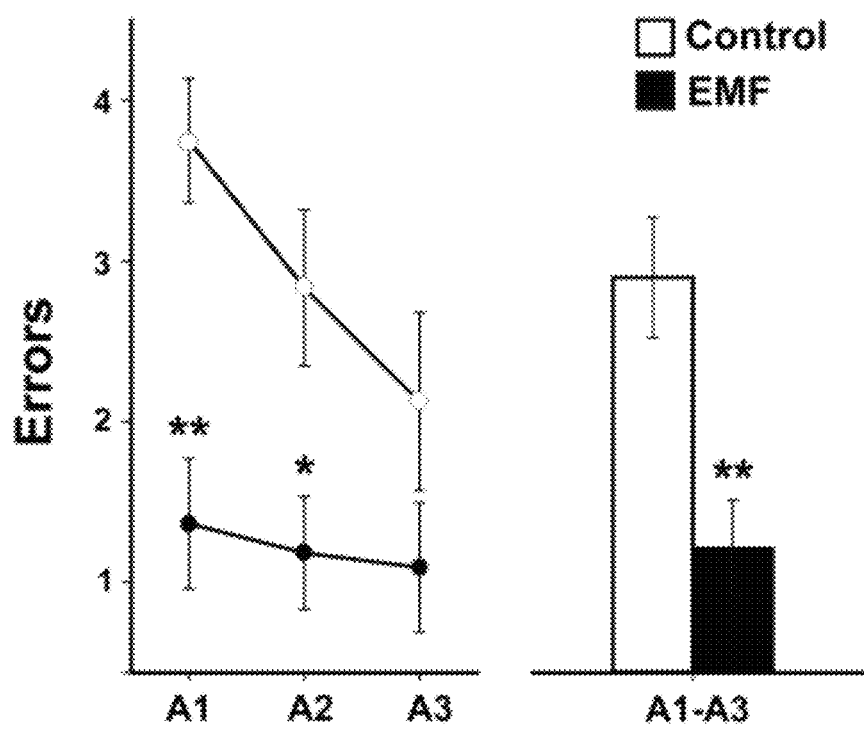


Figure 6

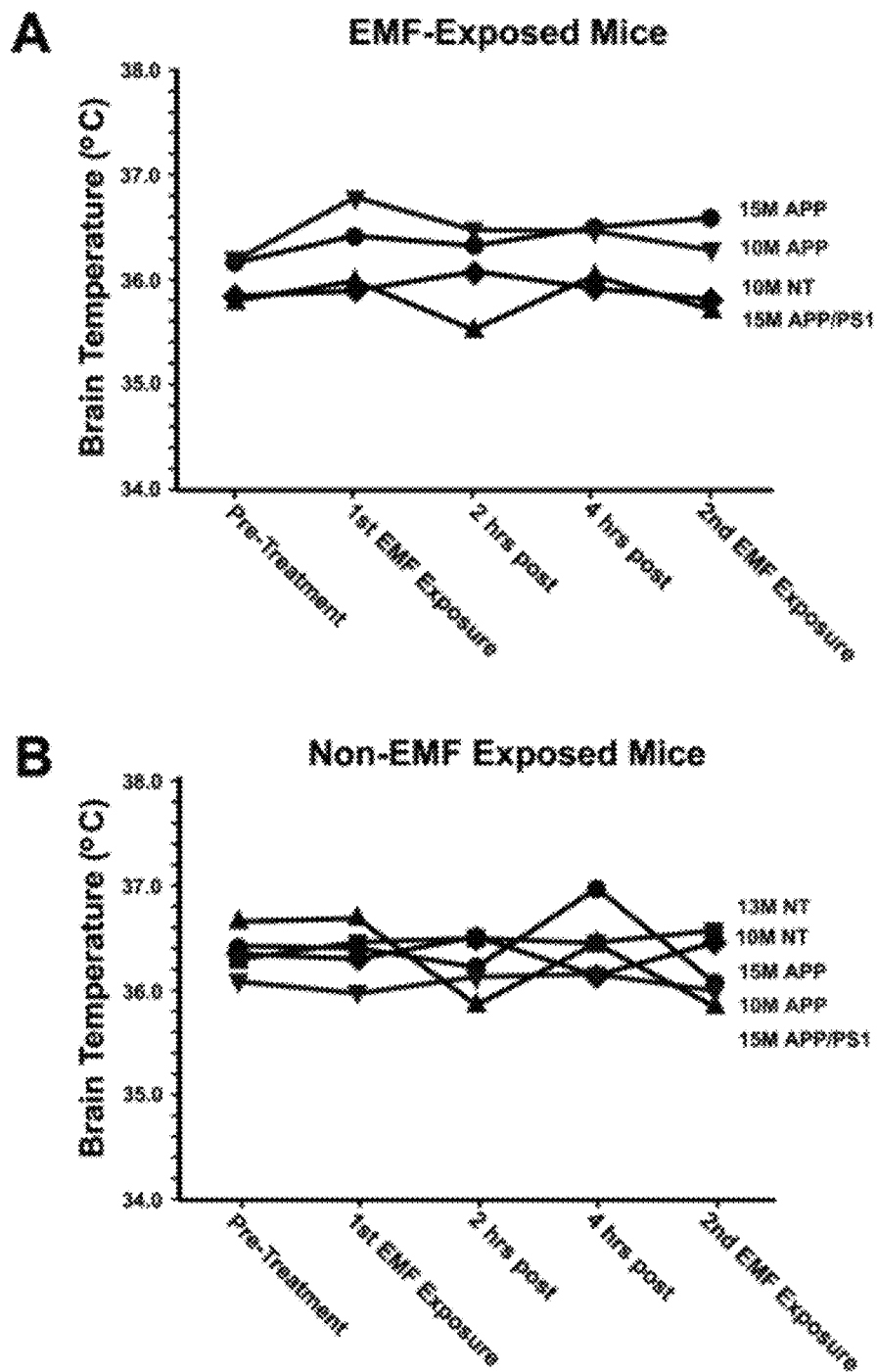


Figure 7

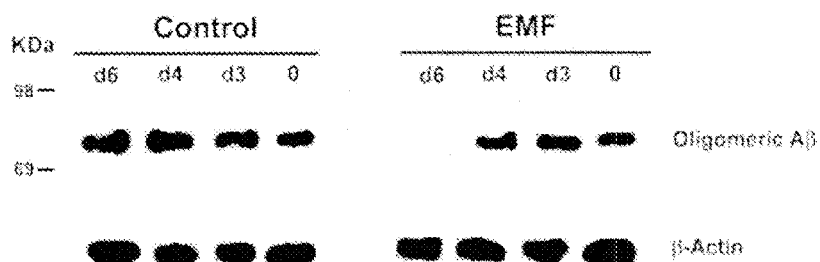


Figure 8A

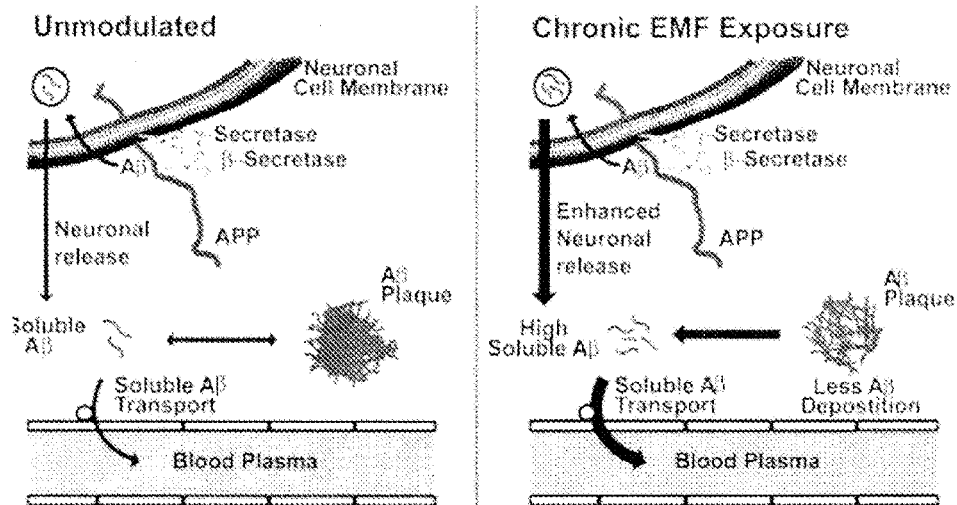


Figure 8B

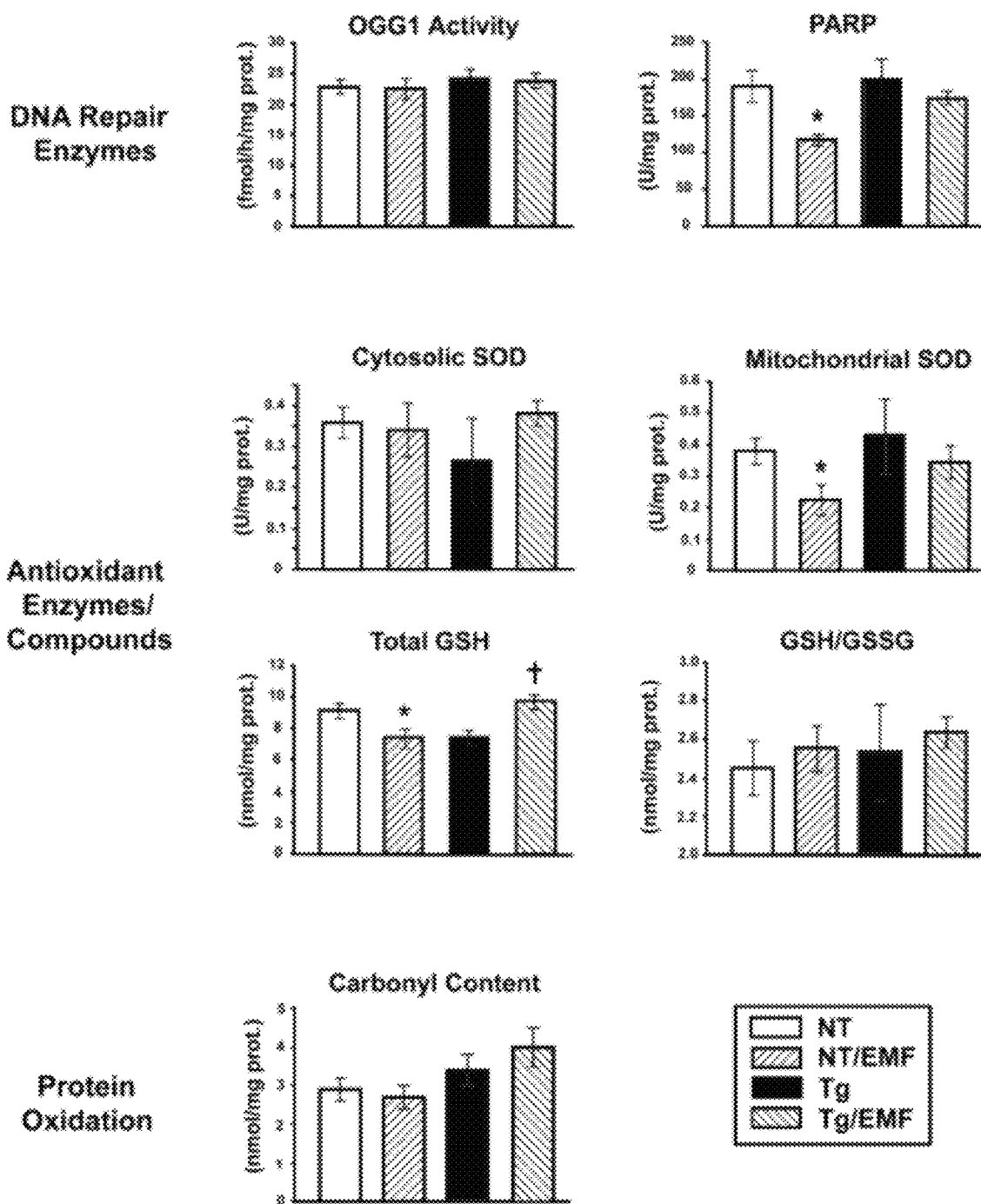


Figure 9

PREVENTION AND TREATMENT OF ALZHEIMER'S DISEASE THROUGH ELECTROMAGNETIC FIELD EXPOSURE

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of prior filed International Application, Serial Number PCT/US2010/026952, entitled "Prevention, Treatment, and Diagnosis of Alzheimer's Disease Through Electromagnetic Field Exposure", filed Mar. 11, 2010, which claims priority to U.S. Provisional Application No. 61/159,251, entitled "Electromagnetic Field Treatment Protects Against and Reverses Cognitive Impairment in Alzheimer's Mice", filed Mar. 11, 2009, which is incorporated herein by reference.

GOVERNMENT SUPPORT

This invention was made with government support under Grant No. AG025711 awarded by the National Institutes of Health. The government therefore has rights in the invention.

BACKGROUND OF THE INVENTION

After reviewing an extensive literature, the World Health Organization and other health councils/organizations have concluded that there are no adverse health risks to adults or children associated with electromagnetic fields (EMFs) generated by cell phone use. However, there is little data concerning the long-term effects of EMFs on brain physiology and function. Epidemiologic studies have suggested that occupational (low frequency) EMF exposure may increase risk of Alzheimer's Disease (AD), while other studies have found that acute exposure to cell phone (high frequency) EMF has essentially no effect on cognitive function in normal individuals. To date, no controlled long-term studies of EMF effects on cognitive function have been done in humans, mice, or animal models for AD.

Another amyloid-related neurological disorder is traumatic brain injury (TBI). The primary/initial injury induced by TBI is largely unavoidable, but triggers secondary brain injury over the hours/days following injury that may be readily treatable. In both humans and animals, a key component to this secondary injury is rapid brain accumulation of the protein β -amyloid ($A\beta$) in as little as one day after injury. The two enzymes (β - and γ -secretase) responsible for $A\beta$ production from amyloid precursor protein (APP) are also increased in brain following TBI. Not surprisingly then, many TBI fatalities have brain $A\beta$ aggregations (deposits) at autopsy. This secondary production and accumulation of brain $A\beta$ after TBI activates inflammatory pathways, induces oxidative damage/apoptosis, and causes neuronal loss. Since $A\beta$ production and ensuing $A\beta$ aggregation following TBI appear to be key mediators of brain tissue loss and resulting cognitive dysfunction, therapeutics aimed at post-TBI suppression of one or both of these processes could greatly limit secondary TBI injury and provide substantial functional recovery. In mice following experimental TBI, suppression of $A\beta$ production (by reducing β - or γ -secretase activity) lessens cognitive deficits, as well as reduces hippocampal neuronal loss. This work demonstrated that brain $A\beta$ accumulation should be a primary therapeutic target against TBI. Unfortunately, these reductions in secretase activity were achieved through genetic manipulation and pharmacologic-level inhibition—neither of which is practical for human therapeutic intervention.

SUMMARY OF INVENTION

This invention is the first to use long-term EMF exposure, directly associated with cell phone use, to provide treatment for neurological disorders as well as preventative cognitive benefits. Both cognitive-protective and cognitive-treatment effects of EMF exposure are demonstrated herein for young adult Alzheimer's transgenic (Tg) mice and older Tg mice, respectively. Even normal mice were shown to receive cognitive benefits from EMF treatment. In Alzheimer's Tg mice, long-term EMF treatment reduced brain β -amyloid ($A\beta$) deposition through $A\beta$ anti-aggregation actions that were confirmed in brain homogenate studies. Several inter-related mechanisms of EMF action are proposed, including increased $A\beta$ clearance from the brains of AD mice, increased neuronal activity, and. Accordingly, the invention includes the use of EMF exposure as a non-invasive, non-pharmacologic therapeutic against AD and an effective memory-enhancing approach in general.

According to a first embodiment the invention includes a method of treating a neurological disorder, such as Alzheimer's Disease, in a subject in need thereof by positioning an electromagnetic field emitting source proximal to the subject and exposing the subject to an electromagnetic field having a predetermined frequency for a predetermined absorption period. Preferably, each individual treatment (comprising exposure to the predetermined frequency for the predetermined absorption period) is continued at a predetermined schedule (preferably daily) for a predetermined treatment period.

The predetermined frequency, according to a preferred embodiment, is about 918 MGH with a specific absorption rate (SAR) of about 0.25 W/kg+/-2 dB. The predetermined absorption period of this preferred embodiment is about one hour. The treatment period is long-term, being greater than about 6 months and preferably between about 7 and 9 months.

The invention also includes, in a second embodiment, a method of preventing, in a subject at risk, an amyloid-related neurological disorder by positioning an electromagnetic field emitting source proximal to the subject and exposing the subject to an electromagnetic field having a predetermined frequency for a predetermined absorption period. Preferably, each individual treatment (comprising exposure to the predetermined frequency for the predetermined absorption period) is continued at a predetermined schedule (preferably daily) for a predetermined treatment period.

The predetermined frequency, according to a preferred embodiment, is about 918 MHz with a specific absorption rate (SAR) of about 0.25 W/kg+/-2 dB. The predetermined absorption period of this preferred embodiment is about one hour. The treatment period is long-term, being greater than about 6 months and preferably between about 7 and 9 months.

BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the invention, reference should be made to the following detailed description, taken in connection with the accompanying drawings, in which:

FIG. 1. EMF exposure, begun in young adulthood, protects Alzheimer's Tg mice from cognitive impairment and improves basic memory of normal mice. Cognitive interference testing at 4-5 months (A) and 6-7 months (B) into EMF exposure revealed overall cognitive benefits at the initial test point and cognitive protection of Tg mice at the later test point during Block 1. (C) Proactive interference testing during Block 2 revealed both overall benefit (at 4-5M) and cognitive protection of Tg mice (at 6-7M). *P<0.05 vs. other group(s) at

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same timepoint; $\dagger P < 0.05$ vs. Tg/EMF group. (D) Normal mice at 6-7 months into EMF exposure showed superior Y-maze spontaneous alternation. $*P < 0.05$ vs. all other groups.

FIG. 2. (Aged Adult Long-term Study) Tg mice are impaired in cognitive function prior to EMF exposure. RAWM working memory performance during the last of three 2-day test blocks (left) and over all 3 blocks (right), is shown for NT and Tg mice tested at 4 months of age. Left graph: $*P < 0.0005$ for T1 vs. T5; $\dagger P < 0.005$ vs. NT group. Right graph: $*P < 0.05$ or higher level of significance vs. NT group.

FIG. 3. (Aged Adult Long-term Study) The first two months of EMF exposure does not result in cognitive benefit to impaired Tg mice. EMF exposure had no effect on RAWM working memory performance of either NT or Tg mice over all 14 days of testing, with both groups of Tg mice being impaired on working memory trials T4 and T5 vs. both groups of NT mice. $*P < 0.02$ or higher level of significance for both Tg groups vs. both NT groups.

FIG. 4. (Aged Adult Long-term Study) At five months into long-term EMF exposure, no deleterious or beneficial effects were evident in cognitive interference testing of Tg mice, although normal (NT) mice showed EMF-induced cognitive benefits in some measures. Data for the final two-day block of testing are presented. $*P < 0.05$ vs. NT control

FIG. 5. At 8 months into EMF exposure, cognitively-impaired Alzheimer's Tg mice exhibited cognitive benefits and reduced brain A β deposition. (A) Cognitive interference testing revealed Tg/EMF mice as vastly superior to Tg controls in 3-trial recall and retroactive interference performance. Even NT mice receiving EMF exposure showed better recall performance than NT controls, particular early in recall testing. The final 2-day block of testing is shown from four days of testing. Upper graph: $*P < 0.025$ vs control; Lower graph: $*P < 0.05$ or higher level of significance vs. control. (B) During both 1-hour EMF exposure periods in a given day at 8 months into exposure, Tg mice exhibited significantly higher body temperatures compared to mice in all other groups. NT/EMF exhibited marginally-higher body temperatures during the morning exposure. $*P < 0.05$ or higher levels of significance vs. all other groups; $\dagger P < 0.05$ vs. NT group. (C) Long-term EMF exposure significantly reduced total A β deposition in entorhinal cortex and hippocampus of Tg mice. Photomicrographic examples of typical A β immunostained plaques from Tg and Tg/EMF are provided. $*P < 0.02$ vs. Tg control group. Scale bar=50 μ M. (D) Long-term EMF exposure nearly increased soluble A β levels in brain, while decreasing A β levels in plasma. Percent change in plasma A β measures involved comparison between pre-treatment blood A β levels at 5 months of age vs. A β levels attained at euthanasia (e.g., following 8+ months of EMF exposure).

FIG. 6. (Aged Adult Long-term Study) Robust cognitive benefits of 8-months EMF exposure were evident in cognitive interference testing, irrespective of genotype (combined NT and Tg groups). Depicted are individual recall trials and the average of all 3 recall trials for the final 2-day block of testing. $*P < 0.02$ vs control group; $*P < 0.005$ vs control group.

FIG. 7. Brain temperature (as measured by temporal muscle probe) before, during, and between EMF exposure/non-exposure in naïve mice of various genotypes and ages. Measurements were all recorded during a single day, with identical results attained on several other single day EMF exposures (not shown), all done within a two-week period. Each measurement represents the mean of 4-5 mice per group.

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FIG. 8. (A) In vitro EMF exposure of hippocampal homogenates from Tg mice results in progressively decreased A β aggregation (oligomerization) between 3 and 6 days into exposure. Western blots display the 80 kDa A β oligomer on top and the β -Actin protein control on bottom. Left panel shows non-treated Tg controls of A β aggregation, while right panel shows the same homogenates exposed to EMF treatment through 6 days. (B) Diagrams depicting the proposed inhibitory actions of long-term EMF exposure on A β aggregation and stimulation of neuronal A β release in aged Tg mice, resulting in higher interstitial fluid A β levels and increased brain clearance of A β .

FIG. 9. (Young Adult Long-term Study) Markers of oxidative damage and antioxidant enzymes/compounds in hippocampus were largely unaffected by long-term EMF exposure in Tg mice. NT/EMF mice exhibited a decreased level of the DNA repair enzyme PARP and suppressions in some antioxidant enzymes/compounds, but no changes in brain oxidative damage. This constellation of EMF effects in NT mice can actually be interpreted as a decrease in oxidative stress. $*P < 0.05$ vs. NT controls. Abbreviations: GSH, reduced glutathione; GSH/GSSG, ratio of reduced to oxidized glutathione; OGG1, 8-oxoguanine glycosylase; PARP, poly ADP-ribose polymerase; SOD, superoxide dismutase.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The invention includes, in a general embodiment, a method of treating and/or preventing an amyloid-related neurological disorder (such as AD and/or TBI) by exposing a subject to EMF for a predetermined treatment period. An amyloid-related neurological disorder is any disorder which is characterized by aberrant beta-amyloid activity. In addition to AD and TBI, examples of amyloid-related neurological disorders include Lewy body dementia, inclusion body myositis and cerebral amyloid angiopathy. The term "preventing" includes both total prevention of the onset of the amyloid-related neurological disorder as well as reducing the degree of the effects of such disorder.

In a preferred embodiment the treatment period is long term, spanning months. The predetermined frequency, according to a preferred embodiment, is about 918 MHz with a specific absorption rate (SAR) of about 0.25 W/kg \pm 2 dB. The predetermined absorption period of this preferred embodiment is about one hour. The treatment period is long-term, being greater than about 6 months and preferably between about 7 and 9 months. The term "about" is not meant to limit the invention to a strict numerical interpretation and includes ranges of the relevant parameter which does not materially affect the basic and novel characteristics of the invention.

To elucidate the effects of long-term (7-9 months) EMF exposure on AD-like cognitive impairment and neuropathology, the inventors exposed Alzheimer's transgenic (Tg) mice and littermate non-transgenic (NT) mice to the exact EMF level that the human head is exposed to during cell phone use (918 MHz, 0.25 W/kg). Here the inventors show that such long-term intermittent EMF exposure: 1) protects young adult Tg mice from later cognitive impairment, 2) reverses cognitive impairment and AD-like brain pathology older Tg mice, and 3) increases cognitive performance of normal NT mice. The novel behavioral task utilized to reveal these cognitive benefits was designed and implemented to closely mimic (measure-for-measure) a human "cognitive interference" task, which very effectively discriminates AD, mild cognitive impairment (MCI), and non-demented individuals.

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In an initial “young adult” long-term study, 2-month old Tg and NT mice were started on daily EMF exposure for the next 7 months, with cognitive testing performed at 4-5 and 6-7 months into EMF exposure (see Examples 1, 2 and 5 below). During the first test period, there were no effects of transgenicity on cognitive interference performance. Therefore, NT and Tg groups were combined into EMF treatment and non-treatment groups, which elucidated significantly better performance of EMF-exposed mice during 3-trial recall testing compared to non-exposed mice (FIG. 1A). By the second test period (2 months later), Tg control mice were impaired in not only 3-trial recall, but also in retroactive interference compared to the excellent performance of both NT groups (FIG. 1B). By contrast, Tg mice that had been receiving chronic EMF exposure for 7 months showed significantly better performance than Tg controls—not only at the end of recall (A3), but also for “overall” 3-trial recall (A1-A3) and retroactive interference (A4). Although all 4 groups performed at the same mediocre level during early proactive interference testing (FIG. 1B), later proactive interference testing at both 4-5 month and 6-7 month time points revealed overall protection for EMF-exposed mice and strikingly better performance of Tg/EMF mice compared to Tg controls, respectively (FIG. 1C). In a final task of general mnemonic function prior to euthanasia, normal NT mice that had been given chronic EMF exposure for 7 months showed much higher Y-maze spontaneous alternation than control NT mice, which performed similar to Tg mice (FIG. 1D). Thus, EMF exposure begun in young adulthood completely protected Tg mice from certain cognitive impairment and even enhanced cognitive performance of normal NT mice. Although animals were euthanized prior to overt A β deposition, near significant increases in levels of soluble A β were evident in the hippocampus and frontal cortex of EMF-exposed Tg mice (see Table 1 and Example 7).

To determine if EMF treatment could reverse cognitive impairment and brain β -amyloid (A β) pathology in older Alzheimer’s Tg mice, the inventors exposed 5-month old mice to daily EMF treatment for the following 8 months. In an “aged adult” long-term study, cognitive testing was performed before the start of EMF treatment, as well as at 2 months, 5 months, and 8 months into EMF treatment (Examples 1, 3 and 5). During pre-exposure cognitive testing at 4 months of age, naïve Tg mice were clearly impaired in the radial arm water maze (RAWM) task of working memory (FIG. 2). Re-testing of mice in this same RAWM task at 2 months into EMF treatment (FIG. 3), as well as in the cognitive interference task at 5 months into EMF treatment (FIG. 4), showed no beneficial effects of EMF on cognitive performance of Tg mice. In NT mice, however, cognitive interference performance was significantly improved at 5 months into exposure (FIG. 4).

After 8 months of EMF treatment, all mice were re-evaluated in the cognitive interference task of working memory (FIG. 5). At this 13 month age, non-treated Tg control mice were noticeably impaired while the cognitive performance of Tg mice receiving EMF exposure was strikingly better (FIG. 5, lower). On 3-trial recall, Tg/EMF mice performed significantly better than Tg controls overall and even on the initial recall trials. In addition, Tg/EMF mice showed vastly superior retroactive interference performance compared to Tg controls. Even NT mice continued to show cognitive benefits from ongoing EMF exposure through 8 months (FIG. 5A, upper). In fact, profound beneficial effects of EMF exposure on 3-trial recall were evident irrespective of genotype (FIG. 6).

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Because it is well-known that EMF exposure can increase body/tissue temperature, the inventors monitored body temperature via rectal probe during a single day of EMF exposure just prior to euthanasia of mice in the adult long-term study (i.e., at 8½ months into EMF exposure). Compared to animals in all other groups, Tg mice being given EMF exposure had significantly higher body temperature (over 1° C. higher) for both early morning and late afternoon EMF exposures (FIG. 5B). During the “off” period between the two EMF exposures, no group differences in body temperature were observed. Thus, body temperatures of Tg mice were elevated only during “on” periods of EMF exposure. To determine if this hyperthermic response to long-term EMF exposure is also induced by acute EMF exposure and if body temperature was accurately reflecting brain temperature in the inventor’s mice, an additional study was performed over a single day in naïve aged mice (Example 4). No effects of acute EMF exposure on brain or body temperature were evident for Tg or NT mice of several ages (FIG. 7), indicating that long-term EMF exposure was required for the increased body temperature seen in Tg mice during “on” periods.]

After euthanasia at 13½ months of age, A β immunostaining (Example 7) from Tg mice revealed substantially lower A β burdens in both hippocampus (135%) and entorhinal cortex (432%) of EMF-exposed Tg mice compared to Tg controls (FIG. 5C). These same EMF-exposed Tg mice exhibited nearly significant increases in hippocampal and cortical levels of soluble A β (FIG. 5D; Example 7). Long-term EMF exposure in Tg mice concurrently induced nearly-significant decreases in plasma A β 1-40 and A β 1-42 levels compared to Tg controls (FIG. 5D).

There are several mechanisms, separately or in combination, that are most likely involved in the beneficial impact of EMF exposure on AD-like cognitive impairment/A β neuropathology in Tg mice and on cognitive performance in normal mice. A first mechanism would be an ability of EMF exposure to suppress A β aggregation and/or to disaggregate pre-existing A β associated with neuritic plaques in Tg mice. Consistent with this mechanism is the presently-reported EMF-induced decrease in brain A β deposition in aged Tg mice, along with their nearly significant increases in soluble brain A β levels. To explore the anti-A β aggregating potential of EMFs, the inventors sonicated hippocampal homogenates from aged (14 month old) Tg mice (to disaggregate their A β). They then subjected these homogenates to the same EMF strength/parameters as in the inventor’s in vivo studies (Example 8). By four days into EMF treatment, substantially less aggregated (oligomeric) A β was evident by Western blots compared to non-exposed hippocampal homogenates (FIG. 8A). This is the first demonstration that cell phone-level EMF treatment can decrease brain A β aggregation.

The delayed ability of EMF treatment to benefit cognitive performance in adult Tg mice (e.g., manifesting itself at 8 months into exposure), likely reflects the time required for the currently-used EMF parameters to significantly impact the dynamic equilibrium between deposited/insoluble and soluble A β in the brain (FIG. 8B), such that A β could be cleared from the brain.

A second mechanism of action involves the ability of EMF exposure to increase neuronal/EEG activity. This ability is underscored by studies showing that cell phone-level EMF exposure increases cortical PET signaling. With regard to A β and AD Tg mice, amyloid precursor protein (APP) in pre-synaptic neuronal cell membranes is internalized via endocytosis, after which A β is cleaved and available for release during neuronal activity (FIG. 8B). Increased neuronal activity has been shown to result in greater synaptic release of this

intracellular A β into brain ISF, which would make it available for transcytotic transport out of the brain.

The inventors began these studies with the hypothesis that long-term EMF exposure would be deleterious to cognitive function in Tg and/or NT mice, most probably through increased oxidative stress. Indeed, previous *in vitro* and “acute” (hours, days) *in vivo* studies had found EMF exposure to increase oxidative stress/damage in various organ systems/animals. However, the inventor’s analysis of oxidative markers from brains of mice in the young adult long-term study (exposed to EMFs for 6-7 months) revealed minimal or no EMF-induced effects on DNA repair enzymes, antioxidant enzymes, or extent of protein oxidative damage (FIG. 9). These results are consistent with a prior study involving cell phone EMF exposure to rabbits for 7 days, wherein no effects on brain oxidative markers were seen (see M. Kemal-Irmak et al., *Cell Biochem. and Function* 20, 279 (2002)). The inventors infer that minimal/no brain oxidative damage results from chronic cell phone-level EMF exposure or that compensatory mechanisms come into play during long-term EMF exposure that largely negate any acute EMF-induced increases in oxidative stress/damage.

The presently reported beneficial effects of EMF exposure in both NT and Tg mice were observed after months of exposure to cell phone-level EMFs. Whether or not more acute exposure would have provided similar cognitive benefits is not known. In contrast to the cognitive improvement shown by EMF-exposed “normal” NT mice in the inventor’s study, prior studies involving acute (7-14 days) EMF exposure to normal rodents failed to show any effects on cognitive performance (see Z. Sienkiewicz et al., *Bioelectromagnetics* 21, 151 (2000) and D. Dubreuil, T. Jay, J-M Edeline, *Behav. Brain Res.* 145, 51 (2003)). A limited daily (15-45 minute/day) and total EMF exposure length, different SAR levels, or use of different cognitive assessments could have been confounding factors in these earlier studies. In normal humans, short-term EMF exposure studies have also failed to demonstrate enhanced cognitive performance (Kwon and Hamalainen, *Bioelectromagnetics* 32, 253 (2011)). In view of these and the inventor’s present findings, the inventors propose that only long-term EMF exposure may provide cognitive benefits to humans at cell phone-level EMF strengths. Second, the inventors propose that such EMF exposure has the capacity to enhance cognitive function in normal, non-demented individuals.

To date, there is no evidence that high frequency EMFs affect the risk of AD. Indeed, the present study provides striking evidence for both protective and disease-reversing effects of long-term EMF exposure, and at cell phone-level intensities in an established transgenic model for AD.

Example 1

Animals

A total of 96 mice, derived from the Florida Alzheimer’s Disease Research Center’s colony, were included in these studies. Each mouse had a mixed background of 56.25% C57, 12.5% B6, 18.75% SJL, and 12.5% Swiss-Webster. All mice were derived from a cross between heterozygous mice carrying the mutant APPK670N, M671L gene (APPsw) with heterozygous PS1 (Tg line 6.2) mice, which derived off-spring consisting of APP/PS1, APPsw, PS1, and non-transgenic (NT) genotypes. After weaning and genotyping, APPsw and NT mice were selected for behavioral studies, while temperature-monitoring studies also included APP/PS1 mice. All mice were maintained on a 12-hour dark and 12-hour light

cycle with *ad libitum* access to rodent chow and water. All animal procedures were performed in AAALAC-certified facilities under protocols approved by the USF Institutional Animal Care and Use Committees.

Example 2

Young Adult Long-Term Study

A total of 24 APPsw (Tg) mice and non-transgenic (NT) littermates, aged 2-2½ months, were divided into the following four groups: Tg controls, Tg+EMF, NT controls, NT+EMF (n=6 per group). All APPsw mice were screened for plasma A β levels and both Tg groups were balanced in terms of plasma A β levels. Tg and NT mice exposed to EMFs were housed in cages within a large Faraday cage, which also housed the antenna of an EMF generator providing two 1-hour periods of electromagnetic waves per day (early morning and late afternoon) at standard cell phone levels/frequencies (918 MHz, 0.25 W/kg, 2 dB). At 6½ and at 9 months of age (4-5 and 6-7 months into EMF exposure), all mice were evaluated in a cognitive interference task (see Behavioral Test Protocols below) that closely parallels, and was designed from, a cognitive interference task utilized in humans to differentiate aged non-demented, MCI, and AD patients from one another (1). Behavioral testing always occurred during “OFF” periods of EMF exposure cyclicity (e.g., during the lights on period between any two exposure periods). After cognitive interference testing at 9 months of age, all animals were tested for general mnemonic function in the Y-maze task of spontaneous alternation, as well as for sensorimotor function and anxiety (see Behavioral Testing section for descriptions of all tasks). Following completion of all behavioral testing at 9½ months of age, all mice were euthanatized and perfused with physiologic saline. The rostral hippocampus and posterior cortex were dissected out bilaterally, quick frozen, and stored at -80° C. for later neurochemical analysis of A β and antioxidant enzyme levels (see Example 7).

TABLE 1

Effects of chronic EMF exposure on brain A β levels (pg/ml)				
	Tg	Tg/EMF	% Change	“p” value
Hippocampus				
A β 1-40	4022 \pm 359	4750 \pm 208	+18%	0.11
A β 1-42	808 \pm 116	1000 \pm 40	+24%	0.15
Frontal Cortex				
A β 1-40	2785 \pm 245	4241 \pm 743	+52%	0.09
A β 1-42	751 \pm 88	1107 \pm 281	+47%	0.26

It is important to indicate that the described beneficial cognitive effects of chronic EMF exposure to both Tg and NT mice in the “young adult” study (FIG. 1) did not occur through non-cognitive effects on sensorimotor function or anxiety. Just prior to euthanasia at 9½ months of age, all mice were tested in a battery of sensorimotor/anxiety tasks (open field activity, balance beam, string agility, and elevated plus-maze). Compared to NT and Tg controls, there were no differences in performance of NT/EMF or Tg/EMF mice, respectively. Thus, non-cognitive effects of EMF exposure can be ruled out for significantly contributing to the beneficial cognitive effects provided by long-term EMF exposure.

Following euthanasia, brain tissues were analyzed for oxidative markers to determine any effects of long-term EMF

exposure on oxidative stress (FIG. 9). For Tg mice, EMF exposure had essentially no effect on hippocampal DNA repair enzymes (OGG1, oxoguanine glycosylase; PARP, poly ADP ribose polymerase), antioxidant enzyme markers (cytosolic and mitochondrial SOD, GSH/GSSH), or protein oxidative damage (protein carbonyl content). Although NT mice exposed to EMFs exhibited decreased PARP, SOD, and glutathione levels in hippocampus (FIG. 9), their cerebral cortex tissue (and that of Tg mice) revealed no effects of EMF exposure on any oxidative markers analyzed (data not presented). Additionally, no group differences in DNA oxidation (8-hydroxyguanine) were seen in striatal tissues from all four groups.

Example 3

Aged Adult Long-Term Study

At 4 months of age, Tg mice (n=12) and NT littermates (n=16) were first evaluated in the radial arm water maze (RAWM) task of working memory (see behavioral methodology) to establish that Tg mice were cognitively impaired prior to EMF exposure. Based on pre-treatment performance in the RAWM task and pre-treatment blood A β levels, Tg and NT groups were each divided into two balanced sub-groups as follows: Tg controls, Tg+EMF, NT controls, NT+EMF (n=5-8 mice/group). At 5 months of age, Tg and NT mice to be exposed to EMFs had their cages placed within a large Faraday cage, which contained an EMF generator antenna providing the same exposure of two 1-hour periods of electromagnetic waves per day at standard cell phone levels (918 MHz, 0.25 W/kg, 2 dB) as in the Young Adult study. At 7 months of age (2 months into EMF exposure), all mice were re-tested in the RAWM task. Then at 10 and 13 months of age (5 and 8 months into EMF exposure), all mice were evaluated in the same cognitive interference task that was utilized in the Young Adult study (see Behavioral Testing Protocols), with all behavioral testing being performed during "OFF" periods in EMF exposure cyclicity.

A few days prior to euthanasia at 13½ months of age (8½ months into EMF exposure), body temperature measurements were taken on a single day with a rectal probe during both early morning and late afternoon EMF exposures, as well as at 2 hour intervals between those exposures. At euthanasia, a terminal blood sample was taken from all mice, then brains were perfused with isotonic PBS. The caudal forebrain was paraffin-embedded and processed for A β immunohistochemical staining, while the remaining forebrain was sagittally bisected and dissected into hippocampus and cortical areas that were quick-frozen for neurochemical analyses.

During the last 2-day block of pre-treatment testing (FIG. 2), NT mice nicely reduced their errors between Trial 1 (T1; the naïve trial) and combined working memory Trials 4+5; however, Tg mice could not do so. Indeed, combined T4+5 errors during this block were much higher in Tg mice compared to NT mice. This cognitive impairment extended across all 6 days of RAWM pre-treatment testing, as evidenced by the substantially higher number of working memory errors by Tg mice on both T4 and T5 overall (FIG. 2). Thus, aged Tg mice were cognitively impaired prior to EMF exposure in this study.

Animals were re-evaluated in the RAWM task at two months into EMF exposure (at 7 months of age). As depicted in FIG. 3, EMF exposure had no positive or negative effects on working memory for either NT or Tg mice over all 14 days of testing. Indeed, Tg mice in both groups were near identical in continuing to be impaired during working memory Trials 4

and 5. Thus, the initial two months of EMF exposure did not provide cognitive benefits to impaired Tg mice. This was also the case for Tg mice during cognitive interference testing performed 3 months later (e.g., 5 months into EMF exposure, at 10 months of age). In this regard, FIG. 4 (lower) shows no difference between Tg and Tg/EMF mice on any measure of cognitive interference testing during the final two-day block of testing. By contrast, NT mice at 5 months into EMF exposure exhibited improved performance on several measures of cognitive interference testing (FIG. 4, upper), particularly on the retroactive interference trial. Thus, during initial cognitive testing performed at 2 and 5 months into EMF exposure, there were no deleterious or beneficial effects observed in Tg mice, while NT mice actually showed some cognitive benefit at 5 months into exposure.

In contrast to the aforementioned cognitive testing at 2 and 5 months into EMF exposure, cognitive interference testing at 8 months into exposure revealed clearly improved cognitive performance to Tg mice (FIG. 5). These beneficial cognitive effects at 8 months into EMF exposure even spanned genotypes. Thus, when animals irrespective of Tg or NT genotype were combined into EMF exposure and control groups, EMF-exposed mice had clearly superior 3-trial recall compared to control mice (FIG. 6). This was true for individual trials A1 and A2, as well as overall (A1-A3), wherein EMF mice averaged only 1 error compared to the 3 error average of control mice.

Parenthetically, Tg mice of this aged adult study were impaired in their initial testing, but not Tg mice in the young adult study at a similar age, because the former mice were naïve to behavioral testing whereas the later had some behavioral shaping experience prior to testing.

Example 4

Aged Adult Acute Study

Results from body temperature reading of animals in the Aged Adult Long-term Study at 8½ months into EMF exposure revealed significant increases in body temperature for Tg mice selectively during EMF "On" periods. To follow-up on this finding, an acute study was performed in naïve Tg and NT mice to monitor both body temperature (via rectal probe) and brain temperature (via temporalis muscle probe) during and between EMF exposures. Prior studies have demonstrated that temporalis muscle temperature very accurately reflects brain temperature. For the present acute study, 10 and 15 month old APPsw mice, 15 month old APP+PS1 mice, and 10-13 month old NT mice were derived from the Florida Alzheimer's Disease Research Center's colony, with each mouse having the same background as mice in both long-term EMF studies. Mice at each age and genotype were divided into two groups of 4-5 mice/group for acute EMF-exposed or non-exposed. On a single day, body and brain temperatures were taken simultaneously at the following timepoints: Pre-treatment, during first EMF exposure/sham, 2 hours and 4 hours following exposure/sham, and during a second EMF exposure/sham. The same EMF generator equipment and setting were utilized as for the long-term EMF studies.

Example 5

EMF Exposure Protocol

For long-term EMF exposure, the cages of single-housed mice were maintained within a Faraday cage (4 meter height×4 meter width×4 meter length) and arranged in a

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circular pattern, with each cage approximately 26 cm from a centrally-located EMF-emitting antenna. The antenna was connect to an Hewlett Packard ESG D4000A digital signal generator (Houston, Tex.) set to automatically provide two 1-hour exposures per day at 918 MHz and a whole body SAR (specific absorption rate) of 0.25 W/kg \pm 2 dB. The resulting EMF exposure to the mice of these studies is typical of standard cell phone use in humans. With a 12-hour light On/Off cycle, the 1-hour daily exposures occurred in early morning and late afternoon of the lights on period. For acute EMF exposure, mice were similarly placed into the Faraday cage and provide a single day's EMF exposure (e.g., two 1-hour EMF periods). Sham-treated animals were located in a completely separate room with identical room temperature as in the EMF exposure room.

Example 6

Behavioral Testing Protocols

Radial Arm Water Maze.

To assess working (short-term) memory, an aluminum insert was placed into a 100 cm circular pool to create 6 radially distributed swim arms emanating from a central circular swim area. An assortment of 2-D and 3-D visual cues surrounded the pool. The number of errors prior to locating which one of the 6 swim arms contained a submerged escape platform (9 cm diameter) was determined for five trials per day. There was a 30-minute time delay between the 4th trial (T4; final acquisition trial) and 5th trial (T5; memory retention trial). The platform location was changed daily to a different arm, with different start arms for each of the 5 trials semi-randomly selected from the remaining 5 swim arms. During each trial (60 seconds maximum), the mouse was returned to that trial's start arm upon swimming into an incorrect arm and the number of seconds required to locate the submerged platform was recorded. If the mouse did not find the platform within a 60-second trial, it was guided to the platform for the 30-second stay. The numbers of errors and escape latency during trials 4 and 5 are both considered indices of working memory and are temporally similar to the standard registration/recall testing of specific items used clinically in evaluating AD patients. T1 (naïve initial trial), T4, and T5 were statistically evaluated over all 14 days of testing, as well as during the final 2-day block.

Cognitive Interference Task.

The inventors designed this task measure-for-measure from a cognitive interference task used to discriminate normal aged, MCI, and AD patients from one another. The interference testing protocol in humans consists of four tasks. The first task, three-trial recall, is a modified version of the Fuld object memory examination, in which the subject is presented with ten familiar objects (Bag A) and asked to recall the objects following a brief distraction task, repeated three times. In the second task, proactive interference, the subject is presented with ten novel objects (Bag B) and asked to recall them, to determine whether previous learning (Bag A objects) intrudes upon present learning (Bag B objects). The third task, short-delay recall, wherein the subject is asked to recall the original set of ten items (Bag A), provides a measure of retroactive interference (difficulty recalling previous learning due to intrusion by present learning). Finally, long-delay recall is evaluated by asking the subject to recall the original set of ten items (Bag A) after a 20-minute delay. A verbal fluency task is used as a distractor between successive trials of the three-trial recall task, as well as immediately preceding the proactive interference task.

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The inventor's analogous interference task for mice involves two radial arm water maze set-ups in two different rooms, each with different sets of visual cues. The task requires animals to remember a set of visual cues, so that following interference with a different set of cues, the initial set of cues can be recalled to successfully solve the radial arm water maze task. A set of four behavioral measures were examined. Behavioral measures were: A1-A3 (Composite three-trial recall score from first 3 trials performed in RAWM "A"), "B" (proactive interference measure attained from a single trial in RAWM "B"), A4 (retroactive interference measure attained during a single trial in RAWM "A"), and "A5" (delayed-recall measure attained from a single trial in RAWM "A" following a 20 minute delay between A4 and A5). As a distractor between trials, animals are placed in a Y-maze and allowed to explore for 60 seconds between successive trials of the three-trial recall task, as well as immediately preceding the proactive interference task. As with the standard RAWM task, this interference task involves the platform location being changed daily to a different arm for both of the RAWM set-ups utilized, and different start arms for each day of testing for both RAWM set-ups. For A1 and B trials, the animal was initially allowed one minute to find the platform on their own before they were guided to the platform. Then the actual trial was performed in each case. As with the standard RAWM task, animals were given 60 seconds to find the escape platform for each trial, with the number of errors and escape latency recorded for each trial. Given the very close correspondence between error and latency scores in individual animals for both the RAWM and cognitive interference tasks, only error scores are presented in this report. Animals were tested for cognitive interference performance on four successive days, with statistical analysis performed for the two resultant 2-day blocks.

Y-Maze Alternation Task.

To measure basic memory function, mice were allowed 5 minutes to explore a black Y-maze with three arms, each measuring 21x4 cm. Basic mnemonic function was measured as a percentage of spontaneous alternation (the ratio of arm choices different from the previous two choices divided by the total number of entries)

Sensorimotor/Anxiety Tasks.

Open field activity, balance beam, string agility, and elevated plus maze anxiety were evaluated according to the methodology of Arendash et al. (G. W. Arendash et al., *Neuroscience* 149, 286 (2007)).

Example 7

Neurochemical and Immunohistochemical Analysis

A β ELISA Analysis.

The hippocampal and cerebral cortex tissues, as well as plasma samples, were processed for soluble A β 1-40 and A β 1-42 determinations by ELISA. For brain tissues, 30 mg samples were homogenized in 400 μ l RIPA buffer, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.2% SDS, and 1 tablet proteinase inhibitor per 100 ml (S8820, Sigma, St. Louis, Mo.), and sonicated for 20 seconds on ice. Samples were then centrifuged for 30 min at 27,000 g at 4° C., and supernatants were transferred into new screw cap tube. The supernatants obtained from this protocol were then stored at -80° C. for later determination of soluble A β levels using ELISA kits (KHB3482 for 40, KHB3442 for 42, Invitrogen, CA).

A β Immunohistochemistry and Image Analysis.

At the level of the hippocampus (bregma -2.92 mm to -3.64 mm), five 5- μ m sections (150 μ m apart) were made

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from each paraffin-embedded mouse brain using a sliding microtome. Immunohistochemical staining was performed following the manufacturer's protocol using a Vectastain ABC Elite kit (Vector Laboratories, Burlingame, Calif.) coupled with the diaminobenzidine reaction, except that the biotinylated secondary antibody step was omitted for A β immunohistochemical staining. The primary antibody was a biotinylated human A β monoclonal antibody (clone 4G8; 1:200, Covance Research Products, Emeryville, Calif.). Brain sections were treated with 70% formic acid prior to the pre-blocking step. Phosphate-buffered saline (0.1 mM, pH 7.4) or normal mouse serum (isotype control) was used instead of primary antibody or ABC reagent as a negative control. Quantitative image analysis was done based on previous methods with modifications. Images were acquired using an Olympus BX60 microscope with an attached digital camera system (DP-70, Olympus, Tokyo, Japan), and the digital image was routed into a Windows PC for quantitative analysis using SimplePCI software (Compix Inc., Imaging Systems, Cranberry Township, Pa.). Images of five 5- μ m sections (150 μ m apart) through both anatomic regions of interest (hippocampus and entorhinal cortex) were captured from each animal, and a threshold optical density was obtained that discriminated staining from background. Each region of interest was manually edited to eliminate artifacts. For A β burden analysis, data are reported as percentage of immunolabeled area captured (positive pixels) relative to the full area captured (total pixels). It should be noted that there was no evidence of histopathologic findings (e.g. neuronal degeneration, gliosis, subarachnoid hemorrhage, intra cerebral hemorrhage, perivascular micro-hemorrhage, or abnormal cell growth such as brain tumors) in any EMF-exposed mouse examined in these studies.

Oxidative Measurements.

For oxyguanosine glycosylase (OGG1) activity, the method for DNA glycosylase extraction by Cardozo-Pelaez et al. (F. Cardozo-Pelaez et al., *Free Rad. Biol. Med.* 28, 779 (2000)) was utilized, with slight modification. OGG1 activities in supernatants were determined using a duplex oligonucleotide containing 8-oxodG as the incision substrate. For preparation of the incision assay, twenty pmol of synthetic probe containing 8-oxodG (Trevigen, Gaithersburg, Md.) was labeled with P32 at the 5' end using polynucleotide T4 kinase (Boehringer Mannheim, Germany). Incision reactions were carried out in a mixture containing 40 mM HEPES (pH 7.6), 5 mM EDTA, 1 mM DTT, 75 mM KCl, purified bovine serum albumin, 100 fmol of 32P-labeled duplex oligonucleotide, and extracted guanosine glycosylase. The reaction mixture was incubated at 37° C. for 2 hours and products of the reaction were analyzed on denaturing 20% polyacrylamide gel. Pure OGG1 served as positive control and untreated duplex oligonucleotide was used for negative control. The gel was analyzed with a Biorad-363 Phosphorimager System. The incision activity of OGG1 was calculated as the amount of radioactivity in the band representing specific cleavage of the labeled oligonucleotide over the total radioactivity.

The colorimetric assay for PARP (poly ADP-ribose polymerase) activity was performed in 96-well plates (Trevigen, Inc., Gaithersburg, Md.) according to manufacturer protocol. In short, serial dilutions of PARP enzyme were distributed into wells to generate a standard curve. Clarified cell extracts (10 μ l/well) were added to triplicate wells for determining cellular PARP activity. The reactions were allowed to proceed for 1 hour at room temperature. The plate was washed 4 times with 1 \times PBS and then incubated for 20 minutes with 50 μ l/well Strep-HRP, diluted 1:500 with 1 \times Strep-Diluent (Trevigen). The plate was washed 4 times with 1 \times PBS in prepa-

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ration for the addition of the HRP substrate. For the colorimetric readout, 50 μ l of TACS-Sapphire (Trevigen) was added to each well and incubated in the dark at room temperature for 15 minutes. Development of the colorimetric reaction was stopped by the addition of an equal volume of 0.2 M HCl generating a yellow color that was read at 450 nm. Results were normalized to equal concentration of protein measured using the bicinchoninic acid assay.

Determination of superoxide dismutase (SOD) activity was based on the inhibition of nitrite formation that results from oxidation of hydroxylammonium by superoxide anion radical. All samples were divided into two sets for determination of total and cytosolic SOD. The mitochondrial SOD was inhibited by 10 mM of KCN. The activity of mitochondrial SOD was calculated as a difference between total and cytosolic SOD.

For determination of total and oxidized glutathione, tissue samples were homogenized in cold assay buffer (0.1 M monobasic sodium phosphate and 0.05 M EDTA, pH 7.5) and de-proteinized with 40% trichloroacetic acid. Supernatant obtained after centrifugation at 10,000 g for 10 min (4° C.) was assayed for total glutathione (GSH). The reaction was monitored by optical density at 405 nm every minute for 5 min with ELx800 microplate reader (Bio-Tek Instruments, Inc., Winooski, VE) using KCJunior software (Bio-Tek Instruments, Inc., Winooski, VE). For determination of glutathione, samples were mixed with 10 mM of 2-vinylpyridine as GSH scavenging agent and reaction was monitored after 1 h of incubation.

The procedure for determination of protein carbonyl content was similar to that described by Levine et al. (R. L. Levine et al., *Meth. Enzymol.* 186, 464 (1990)), with slight modifications. Briefly, two sets of 250 μ l samples homogenates were labeled as "test" and "reference". One mL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) prepared in 2.5 M HCl was added to test samples and 2.5 M HCl alone was added as "reference". The contents were mixed and incubated in the dark for 1 hr. Then 1 mL of 20% TCA was added to each tube. The tubes were centrifuged in 10 min at 3,500 rpm for 20 min and protein pellets that were washed with 1 mL of 10% TCA. Additionally, the precipitates were washed 3 times with 1 mL of mixture ethyl acetate:ethanol (1:1, v/v) to remove unreacted DNPH and lipids. Finally, each pellet was dissolved in 1 mL of 6M guanidine hydrochloride at 37 C during 10 min. The insoluble matter was removed by centrifugation. Carbonyl content was determined with Ultrospec III spectrophotometer (Pharmacia, LKB). Each test sample was read against the corresponding control at 370 nm using an absorption coefficient of 22,000 M⁻¹ cm⁻¹.

Example 8

In Vitro A β Aggregation Studies

Hippocampus tissue was isolated from 14 month old APPsw Tg mice and homogenized in RIPA buffer with sonication. Tissue homogenates were aliquoted at 42 μ g per vial in 30 μ l volume and stored at -80° C. For each time point, two vials were thawed, with one placed into a rotor for EMF treatment and the other put in a rotor in the same room without EMF treatment. Immediately following treatment, samples of 14 μ l were loaded onto 4-12% Bis-tris gel (Invitrogen, Carlsbad, Calif.) and probed with 6E10 detection after being transferred onto PVDF membranes. Membranes were then stripped with stripping buffer (Thermo Fisher) and re-probed with anti-mouse β -actin by following the standard western protocol.

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It will be seen that the advantages set forth above, and those made apparent from the foregoing description, are efficiently attained and since certain changes may be made in the above construction without departing from the scope of the invention, it is intended that all matters contained in the foregoing description or shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

It is also to be understood that the following claims are intended to cover all of the generic and specific features of the invention herein described, and all statements of the scope of the invention which, as a matter of language, might be said to fall therebetween. Now that the invention has been described, What is claimed is:

1. A method of treating and preventing a symptom associated with Alzheimer's Disease in a subject, wherein said symptom is associated with amyloid deposit or cognitive ability, comprising:

positioning an electromagnetic field emitting source proximal to the subject;

exposing the subject to electromagnetic treatment by an electromagnetic field generated by a digital signal generator, said electromagnetic field having a predetermined frequency for a predetermined absorption period, said predetermined frequency having a range of about 902 MHz to about 928 MHz;

continuing exposure to the subject for the predetermined absorption period for a predetermined treatment period greater than about three (3) days; and

reversing or reducing cognitive impairment or amyloid aggregation in said subject, decreasing a rate of amyloid deposition, or enhancing cognitive performance in said subject based on said step of continuing exposure for greater than about three (3) days.

2. The method of claim 1, wherein the predetermined frequency is about 918 MHz.

3. The method of claim 1, wherein the electromagnetic field has a specific absorption rate of about 0.25 W/kg to about 1.6 W/kg.

4. The method of claim 1, wherein the predetermined absorption period is about one hour twice daily.

5. The method of claim 1, wherein the predetermined treatment period is greater than about 4 months.

6. The method of claim 1, wherein the predetermined treatment period is between about 7 and 9 months.

7. The method of claim 1, wherein an enhancement of said cognitive performance in said subject is indicated by an enhancement of working or short-term memory performance of said subject.

8. A method of treating and preventing an amyloid-related neurological disorder in a subject thereof, comprising:

positioning an electromagnetic field emitting source proximal to the subject;

exposing the subject to electromagnetic treatment by an electromagnetic field generated by a digital signal generator, said electromagnetic field having a predetermined frequency for a predetermined absorption period,

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said predetermined frequency having a range of about 902 MHz to about 928 MHz;

continuing exposure to the subject for the predetermined absorption period for a predetermined treatment period greater than about three (3) days; and

reversing or reducing cognitive impairment or amyloid aggregation in said subject, decreasing a rate of amyloid deposition, or enhancing cognitive performance in said subject based on said step of continuing exposure for greater than about three (3) days.

9. The method of claim 8, wherein the predetermined frequency is about 918 MHz.

10. The method of claim 8, wherein the electromagnetic field has a specific absorption rate of about 0.25 W/kg to about 1.6 W/kg.

11. The method of claim 8, wherein the predetermined absorption period is about one hour twice daily.

12. The method of claim 8, wherein the predetermined treatment period is greater than about 4 months.

13. The method of claim 8, wherein the predetermined treatment period is between about 7 and 9 months.

14. The method of claim 8, wherein an enhancement of said cognitive performance in said subject is indicated by an enhancement of working or short-term memory performance of said subject.

15. A method of enhancing cognitive function in a normal, cognitively unimpaired subject, comprising:

positioning an electromagnetic field emitting source proximal to the subject;

exposing the subject to electromagnetic treatment by an electromagnetic field generated by a digital signal generator, said electromagnetic field having a predetermined frequency for a predetermined absorption period, said predetermined frequency having a range of about 902 MHz to about 928 MHz;

continuing exposure to the subject for the predetermined absorption period for a predetermined treatment period; and

enhancing cognitive performance of said subject based on said step of continuing exposure for said predetermined treatment period, wherein said enhancing cognitive performance of said subject includes enhancing working memory performance of said subject.

16. The method of claim 15, wherein the predetermined frequency is about 918 MHz.

17. The method of claim 15, wherein the electromagnetic field has a specific absorption rate of about 0.25 W/kg to about 1.6 W/kg.

18. The method of claim 15, wherein the predetermined absorption period is about one hour twice daily.

19. The method of claim 15, wherein the predetermined treatment period is greater than about three (3) days.

20. The method of claim 15, wherein the predetermined treatment period is greater than about four (4) months.

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