

## Review

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# Transcranial Electromagnetic Treatment Against Alzheimer's Disease: Why it has the Potential to Trump Alzheimer's Disease Drug Development

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**Abstract.** The universal failure of pharmacologic interventions against Alzheimer's disease (AD) appears largely due to their inability to get into neurons and the fact that most have a single mechanism-of-action. A non-invasive, neuromodulatory approach against AD has consequently emerged: transcranial electromagnetic treatment (TEMT). In AD transgenic mice, long-term TEMT prevents and reverses both cognitive impairment and brain amyloid- $\beta$  (A $\beta$ ) deposition, while TEMT even improves cognitive performance in normal mice. Three disease-modifying and inter-related mechanisms of TEMT action have been identified in the brain: 1) anti-A $\beta$  aggregation, both intraneuronally and extracellularly; 2) mitochondrial enhancement; and 3) increased neuronal activity. Long-term TEMT appears safe in that it does not impact brain temperature or oxidative stress levels, nor does it induce any abnormal histologic/anatomic changes in the brain or peripheral tissues. Future TEMT development in both AD mice and normal mice should involve head-only treatment to discover the most efficacious set of parameters for achieving faster and even greater cognitive benefit. Given the already extensive animal work completed, translational development of TEMT could occur relatively quickly to "proof of concept" AD clinical trials. TEMT's mechanisms of action provide extraordinary therapeutic potential against other neurologic disorders/injuries, such as Parkinson's disease, traumatic brain injury, and stroke.

**Keywords:** AD transgenic mice, amyloid- $\beta$ , cognitive benefits, electromagnetic treatment, mitochondrial function, neuronal activity, transcranial

## WHY ALZHEIMER'S DISEASE DRUGS HAVE FAILED AND WHY THEY WILL CONTINUE TO BE PROBLEMATIC

For well over a decade, researchers in both academia and the pharmaceutical industry have been searching for a "disease-modifying" therapeutic that could arrest

or reverse memory-robbing Alzheimer's disease (AD) [1]. Unfortunately, these noble efforts have been universally unsuccessful, and for a variety of reasons. First, the pathogenesis of AD itself remains unsettled, although most AD researchers agree that amyloid- $\beta$  (A $\beta$ ) dyshomeostasis is central for precipitating the disease [2]. Aside from this, a general problem independent of drug design is that most AD clinical trials have been carried out in patients already diagnosed with AD, wherein the disease process is already well-established and difficult to stabilize or reverse. However, the brain pathogenesis of AD begins 1–2

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decades before AD diagnosis, as we first suggested in 1987 [3], with newly-established pre-symptomatic and mild cognitive impairment (MCI) phases now defined that precede overt AD [4]. As such, therapeutic intervention as early as possible (at least by the MCI phase) should enhance the chances of cognitive benefit, especially if interventions are carried out for periods longer than the typical 2–12 month clinical trials that have thus far characterized AD trials.

Aside from the issues of unsettled AD pathogenesis and clinical trials starting too late, the central reason for universal failure of AD therapeutics to date has involved the prior clinical testing of “flawed” drugs (see below). Even with potentially less-flawed drugs on the horizon, two substantial barriers on the road to therapeutic efficacy will continue to plague AD drugs in translational development. What are these two barriers to efficacy of systemically-delivered AD drugs?

*1) The necessity of AD drug up-take not only into the brain, but also into neurons*

The vast majority of systemically-administered AD drugs do not effectively cross the blood-brain barrier (BBB). Unfortunately, very limited resources have been expended by academia and the pharmaceutical industry to enhance CNS drug delivery once AD candidates are identified [5]. In general, for a systemically-administered drug to have a chance at crossing the BBB, it should have a molecular weight of less than 400 Da and less than 8 hydrogen bonds. Regarding the latter, most  $\gamma$ -secretase inhibitors and essentially all  $\beta$ -secretase inhibitors form at least 8 hydrogen bonds, suggesting limited BBB transport [5]. A litany of failed clinical AD trials can be ascribed directly to poor or no BBB penetration. For example, the  $\gamma$ -secretase inhibitor Flurbiprofen failed Phase III clinical trials and was later found to not get into the brain very well. Another example is tramiprosate (Alzhemed), which was purported to be a brain anti-A $\beta$  aggregation compound. Aside from the fact that this mechanism was never proven for Alzhemed, it also failed to cross the BBB to enter the brain – a necessary condition for Alzhemed to physically bind to parenchymal A $\beta$  aggregates in order to disaggregate them. Not surprisingly, Alzhemed failed in Phase III clinical trials.

Even if an AD therapeutic agent does effectively cross the BBB to get into the brain parenchyma, brain penetration alone is probably not sufficient for AD therapeutic efficacy because the agent will almost certainly need to then gain access to the inside of neurons. Why

this second hurdle? Because of the high levels of “intra-neuronal” A $\beta$  (in oligomeric form) that are associated with synaptic mitochondrial membranes. It is important to recognize that A $\beta$  is produced intra-neuronally as a result of the amyloid- $\beta$  protein precursor (A $\beta$ PP) in pre-synaptic neuronal membranes being internalized via endocytosis; only then does ensuing secretase-induced A $\beta$ PP cleavage occur (Fig. 1A). Resulting intraneuronal A $\beta$  then aggregates into toxic oligomers that have a high affinity for synaptic mitochondrial membranes, resulting in suppression of mitochondrial function/ATP production [6–9]. This A $\beta$ -induced mitochondrial dysfunction appears not only to be central to AD pathogenesis, but also an early event therein [7, 9, 10–14]. Consistent with mitochondrial dysfunction/ATP deficits early in AD pathogenesis, fluorodeoxyglucose ( $^{18}$ F)-positron emission tomography (FDG-PET) scans reveal reduced brain glucose utilization in MCI that correlates with cognitive decline [15]. In view of the above findings, *any effective AD therapeutic will need to get into neurons in order to address the toxic A $\beta$  oligomerization causative to mitochondrial dysfunction*. One purported mitochondrial-enhancing drug called Dimebon (latrepiridine) was advanced to Phase III clinical trials in AD patients before it was found not to provide any cognitive benefits (NCT00838110, CONNECTION trial). It was later determined that the concentrations of Dimebon needed to affect mitochondrial function *in vitro* were far above the levels present physiologically in mitochondria [16].

A somewhat more promising AD drug is a metal chelator/metal-protein attenuating compound called PBT2 that appears to suppress metal-induced A $\beta$  oligomerization. Although PBT2 decreases soluble/oligomeric A $\beta$  levels extracellularly in AD transgenic (Tg) mouse brains [17], there is no indication that it gets into neurons to decrease A $\beta$  oligomerization associated with mitochondrial dysfunction there. Not surprisingly then, Phase IIa clinical trials have reported that PBT2 administration to mild AD patients does not improve performance on the ADAS-Cog or MMSE tests of cognitive function [18], though some benefit was seen in two alternative measures of executive function. Finally, immunotherapy against AD has been investigated for over a decade now in both animal models and human studies. In contrast to the problem of limited or no BBB transport that many AD drugs have faced, both active and passive immunotherapy appear to have the opposite problem of actually inducing damage to the BBB. This unacceptable BBB disruption (apparently induced by high

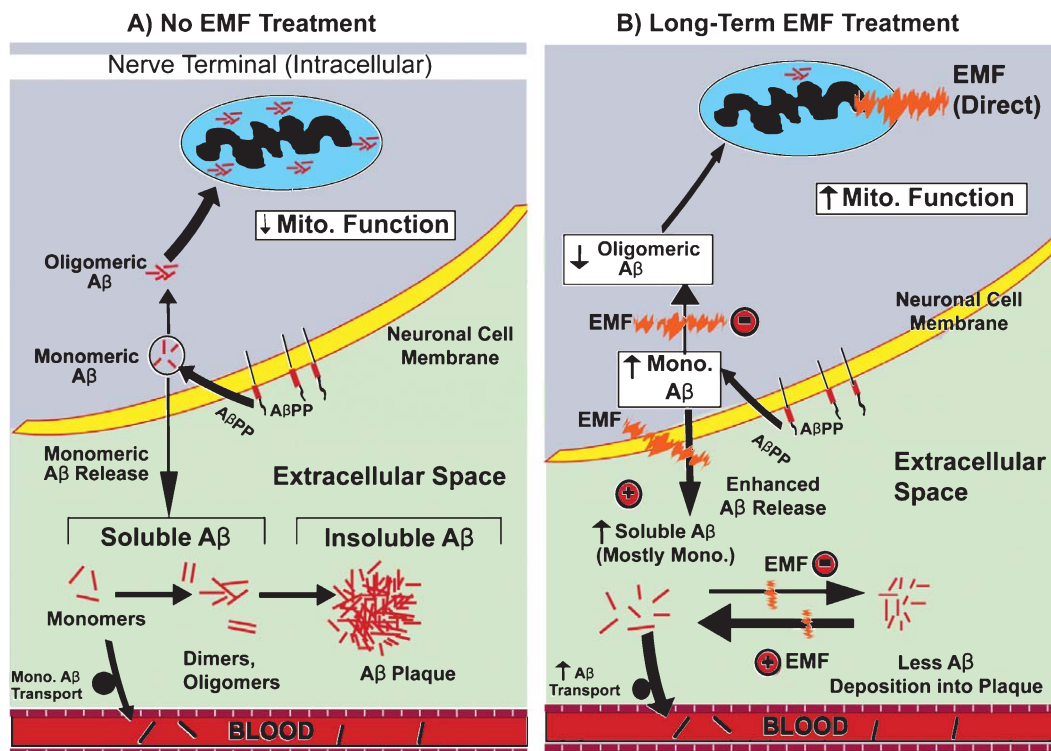


Fig. 1. Diagrams showing brain A $\beta$  processing/aggregation without EMF treatment (A) and with EMF treatment (B). A) Following internalization of amyloid- $\beta$  protein precursor (A $\beta$ PP) into vesicles, enzymatic cleavage by  $\beta$ - and  $\gamma$ -secretase produce monomeric A $\beta$ , which then has two fates: 1) formation of intraneuronal A $\beta$  oligomers, which then have a high affinity for mitochondrial membranes to disrupt mitochondrial function; or 2) vesicular release from neuronal terminals during neuronal activity. Extracellular monomeric A $\beta$  can either aggregate into dimers, oligomers, and large A $\beta$  deposits or it can be cleared from the brain by transport into the blood. B) Long-term EMF treatment affects brain A $\beta$  processing/aggregation at multiple sites and provides general actions by: 1) limiting or reversing intraneuronal A $\beta$  oligomer formation, resulting in enhanced mitochondrial function; 2) direct enhancement of mitochondrial membranes to remove intraneuronal monomeric A $\beta$ ; and 3) increasing neuronal activity to remove intraneuronal monomeric A $\beta$ ; and 4) limiting or reversing extracellular A $\beta$  aggregation into oligomers/plaques to result in more monomeric A $\beta$ , which is then transported across capillary endothelial cells and into the blood for degradation. The collective effect of all four EMF actions is to remove aggregated A $\beta$  from the brain, while enhancing mitochondrial function and neuronal activity in general.

plasma A $\beta$  levels) results in abnormal IgG uptake from blood to brain, cerebral hemorrhage, and possibly even the viral encephalitis present in some subjects [5, 19]. Indeed, the efficacy of immunotherapy to reduce brain A $\beta$  aggregation appears to require a disrupted BBB in order to allow circulating anti-A $\beta$  antibodies access to the brain parenchyma [5].

In view of the above findings, the ability (or inability) of AD drugs to both penetrate the BBB and then gain access to the inside of neurons would appear critical and precedent to any cognitive benefit.

2) *The necessity for more than a "single" mechanism of AD drug action and without deleterious side effects*

Recitation of the known mechanism(s) of action for each past and currently-being-developed drug against

AD reveals the stark reality that each of these drugs basically has a single mechanism of action [1]. To use an old adage, most AD drugs appear to be 'one pony shows' in trying to address primarily a single aspect of AD pathogenesis. For example,  $\beta$ - and  $\gamma$ -secretase inhibitors target A $\beta$  production and NSAIDs are directed to brain inflammation, while other drugs target only A $\beta$  aggregation (PBT2) or neurofibrillary tangle aggregation (Rember/methylene blue). Aside from their uni-dimensional actions, these AD drugs can have undesirable or unacceptable side-effects. For example, the  $\gamma$ -secretase inhibitor Semagacetat suppressed the processing on Notch and other normal  $\gamma$ -secretase substrates in mid/moderate AD subjects during Phase III clinical trials [2], as evidenced by skin cancer and GI symptoms. Some Semagacetat-treated patients actually showing enhanced cognitive decline in those trials. Another example is the previously-mentioned BBB

disruption and cerebral hemorrhage induced by AD immunotherapy. Clearly, what is needed is a therapeutic intervention that attacks AD at multiple points in its pathogenesis without induction of undesirable side-effects. Just as a cocktail of therapeutics is currently being successfully utilized to treat AIDS, so must a similar cocktail of therapeutic compounds (or single therapeutic with multiple disease-targeting properties) be utilized against AD.

In summary, essentially all clinically-tested AD drugs have been flawed in having low brain/neuronal availability and/or a single mechanism of action. Moreover, future drugs currently in translational development are unlikely to have the “intra-neuronal” presence necessary to decrease mitochondrial-associated A $\beta$  oligomerization and most will continue to be uni-dimensional in their mechanistic action. These continuing barriers to efficacy of systemically-delivered AD drugs have awakened the emerging view that it is time for AD researchers to think outside of the “drug development” box and to open their minds to possible non-pharmacologic, neuromodulatory interventions against the disease.

#### **TRANSCRANIAL ELECTROMAGNETIC FIELD TREATMENT AS A VIABLE NON-PHARMACOLOGIC ALTERNATIVE**

Since conventional pharmacotherapy has thus far failed to slow or reverse the AD disease process, investigation of non-pharmacologic approaches is not only warranted, but necessary. In that context, the field of bioelectromagnetics could offer a surprising and unexplored therapeutic intervention against AD and generalized memory impairment: high frequency transcranial electromagnetic treatment (TEMT). TEMT treatment is very different from other ‘neuromodulatory’ approaches such as electroconvulsive therapy or the more recent transcranial direct current stimulation (tDCS), which involve brief alternating or direct current application, respectively, through contact electrodes on the head. TEMT is also quite different from transcranial magnetic stimulation (TMS), wherein strong magnetic pulses are administered in trains of constant frequency/intensity for 15–30 min sessions administered acutely or daily over weeks/months. Improved cognitive performance of AD subjects during tDCS and TMS have been reported [20], although these improvements appear transient and are most likely due to a generalized excitation of cortical activity. More importantly, there is no evidence that tDCS

or TMS are disease-modifying against AD, no AD animal work supports their use against AD, and they both lack brain penetration power beneath the cerebral cortex. Therefore, the only way these neuromodulatory approaches could impact sub-cortical structures is indirectly, through descending cortical pathways. By contrast, this paper will show that TEMT appears to be disease-modifying, is supported by a strong foundation of AD animal work, and can penetrate deep into the brain to impact the multiple brain areas devastated by AD. Unlike deep brain electrical stimulation, TEMT is non-invasive and is capable of treating all AD-diseased brain areas/systems (not just a single focal brain region/system). Although ultrasound has recently been shown to acutely excite neural circuits in the brain [21], this neuromodulatory approach is still in its infancy, having not been shown to impact cognitive function in any animal and only having been administered acutely. Thus, TEMT has distinct therapeutic advantages against AD compared to all other neuromodulatory approaches (i.e., tDCS, TMS, ultrasound, and deep brain stimulation).

Among TEMT’s attributes that are not shared by other neuromodulatory approaches is its high frequency oscillating properties. Electromagnetic field (EMF) treatment/exposure involves the principle that charged particles in motion (an electric current) produce both electric and magnetic fields. These electric and magnetic fields are two parts of a greater whole, the EMF. Once an EMF has been produced, other charged objects in this field are induced to move, thus creating a dynamic entity. In biologic applications and occupational exposure, EMFs are typically generated by alternating current, with the frequency of this alternating current typically ranging from very low (<60 Hz) to very high (>3000 MHz) levels and in either a pulsed or continuous fashion. EMF frequency is the critical parameter for safety considerations because either extreme of the EMF frequency spectrum (e.g., very low or very high frequencies) can induce deleterious biologic effects. In sharp contrast, “high frequency” EMFs (in the 300–1900 MHz range) have not been shown to be harmful and can actually provide beneficial biologic actions, as this paper will underscore. Parenthetically, millions of people self-administer a degree of high-frequency EMF treatment to their head daily through use of their cell phones.

At this juncture, it may be useful to define use of TEMT versus EMF treatment, which is being used interchangeably throughout the text. The term TEMT is meant to indicate application of EMF treatment to the head/brain. Although any clinical neurologic

application of TEMT will likely be restricted to head-only treatment, almost all experimental EMF treatment work in animals (including all of our work) has involved full body EMF treatment/exposure. Such EMF exposure is clearly transcranial in nature, but is obviously not restricted to the cranium. Therefore, the term TEMT will be used to encompass either head-only or full body EMF treatment, recognizing that full body EMF exposure incurs both cranial and peripheral EMF exposure.

In the first long-term TEMT study comprehensively evaluating cognitive endpoints, we have reported that full body EMF exposure (at cell phone levels of  $\approx 900$  MHz) over a 7–9 month period prevented or reversed cognitive impairment in AD mice, while even providing cognitive enhancement to normal mice [22]. Further, we have identified three complimentary mechanisms of TEMT action in the brain: 1) disruption of aggregation of the abnormal protein A $\beta$ , the production/aggregation of which is thought to initiate AD; 2) mitochondrial enhancement; and 3) increased neuronal activity [22–25]. Prior to this recent work, there had been little data concerning the long-term effects of high frequency (300–1900 MHz) EMF exposure on brain physiology or cognitive function. Some human studies had investigated behavioral effects of *acute* unilateral exposure to high frequency EMFs, such as those associated with cell phone use. A number of these studies reported small beneficial effects of a single brief (30–120 min) EMF exposure on attention and/or working memory in normal individuals. However, it is important to underscore that no controlled long-term studies with EMF treatment have been done in humans, particularly related to cognitive performance. Nonetheless, there is already indirect evidence that long-term EMF exposure has beneficial effects on human cognitive function. First, a recent epidemiological study reported that heavy cell phone use over several years resulted in better performance on a word interference test [26]. Second, another epidemiological study reported that long-term cell phone users have a 30–40% decreased risk of hospitalization for AD and dementia in general [27]. Certainly, physiologic mechanisms (i.e., increased neuronal activity, disruption of A $\beta$  aggregation) could be involved in the cognitive benefits reported in these two studies. Alternative non-physiologic explanations could also be involved (i.e., cell phone-induced cognitive training/stimulation to enhance attention, prodromal symptoms of AD reduce cell phone use). Only prospective/controlled studies investigating multiple endpoints will resolve which mechanisms are most likely.

The aforementioned animal and human findings warrant a serious look at the therapeutic potential for high frequency TEMT against AD and cerebral insufficiency in general. Thus, the purposes of this paper are to: 1) document the safety of this approach; 2) document the evidence for TEMT-induced cognitive benefits; 3) indicate evidence for the probable mechanisms involved in these cognitive benefits; and 4) provide a blueprint for translational studies that are now needed to aggressively determine the efficacy of TEMT against AD and other brain disorders/injuries. There are two psychological obstacles against this new TEMT approach for cognitive enhancement. *First* is educating skeptics that TEMT intervention is almost certainly safer for human treatment in comparison to AD drugs being developed. *Second* is getting investigators weaned off the to-this-point failed concept that only a synthetic drug will be successful against AD.

#### WHY HIGH FREQUENCY TEMT IS ALMOST CERTAINLY SAFE

When we began our EMF studies in 2007, we were like most researchers and much of the lay public in believing that, if there was an EMF exposure effect on health or cognitive function, it would be a negative effect. Indeed, several epidemiologic studies had reported that occupational exposure to very low EMFs (50–60 Hz), such as those typically present for electricians or welders, was associated with an increased risk of AD ([28], see [29] for review). As well, a group of Swedish investigators had repeatedly reported that high frequency (i.e., 900 MHz) EMF exposure due to cell phone use was associated with an increased risk of brain tumors [30, 31]. Moreover, some early animal studies had also found EMF exposure to increase the incidence of various cancers and DNA damage, albeit usually at very high “microwave oven” frequencies (i.e., 2450 MHz) and with acute exposure or cell culture endpoints [32–34].

Given the above background of scientific literature in 2007, we embarked on our initial EMF studies with the erroneous hypothesis that high frequency EMF treatment (at cell phone levels) might precipitate AD pathology/cognitive impairment in AD Tg mice and possibly induce cognitive impairment in normal mice as well. The basic tenets for this hypothesis were flawed, however. Since the intervening years between 2006 and the present, better-designed human/animal studies have concluded time and time again that long-term exposure to high frequency EMFs of around

900 MHz (GSM) and 1850–1950 MHz (digital) that typify cell phones in the U.S. and Europe, respectively, have no negative impact on health, particularly on incidence of brain tumors [35–38]. Regarding the later, the 13-nation INTERPHONE Study Group recently published its analysis of long-term ( $\geq 10$  years) cell phone use in over 5,000 brain cancer patients, concluding there is no increased risk of brain cancer associated with long-term cell phone use in adults [39] – a conclusion supported through analysis by the National Institute of Environmental Health Sciences [40] and very recently extended to include children/adolescents as well [41]. In the largest cohort study published to date, Frei et al. [42] followed 358,000 cell phone subscribers in Denmark for up to 17 years (1990–2007) in reporting no increased incidence of brain tumors or evidence for a dose-response relationship therein. Brain tumors affect less than 1% of any population and are not increased by cell phones and their high frequency EMF exposure. The impossibility of high frequency EMFs to induce cancer is supported by the research of none other than Albert Einstein, who won the 1905 Nobel Prize in Physics for establishing that much higher EMF frequencies are required (UV, x-rays, gamma rays) to break covalent bonds in molecules and, thus, to increase cancer risk.

Importantly, no studies have ever shown even an association (much less causality) between exposure to cell phone-level EMFs and increased risk of AD or precipitation of AD. Indeed, our work in AD Tg mice (to be detailed in a later section) consistently suggests that long-term TEMT protects against and reverses AD-like pathology and associated cognitive impairment [22, 23]. Moreover, this long-term TEMT (daily for up to 9 months) was found to be very safe in having no deleterious effects on a variety of health endpoints evaluated, specifically, no effects on brain oxidative stress or abnormal brain histology, no significant brain heating, no damage to DNA in circulating blood cells, and no gross changes to peripheral tissues [22, 23, 25].

What is the conclusion that can be reached regarding the safety of TEMT as a therapeutic? It is nearly impossible to prove a negative statement in science such as “*EMF exposure does not cause health concerns or cancer*”. Accordingly, there will always be individuals who believe high frequency EMFs cause health concerns/cancer irrespective of the evidence to the contrary. Nonetheless, based on a large body of both human- and animal-based scientific evidence, long-term TEMT would appear to be at least as safe (and

probably safer) than AD therapeutic drugs currently being administered and developed.

### **PRIOR WORK INVESTIGATING COGNITIVE EFFECTS OF EMF TREATMENT**

Given the ubiquitous use of cell phones in present-day society and the associated interest in cognitive/neurologic impacts that chronic cell phone use may entail, both human and animal studies have focused on cell phone level EMF treatment/exposure (i.e.,  $\approx 900$  MHz in U.S. and 1850–1950 MHz in Europe) and in GSM, CW, or UMTS modality. Human and animal studies related to cognitive effects of such EMF exposure are considered separately below.

#### *Human studies*

To date, all controlled human studies investigating cognitive effects of EMF exposure have been single exposure (3–120 min) studies [38, 43], with the exception of two studies involving daily EMF exposure for 6–27 days [44, 45]. All of these studies were exclusively in normal individuals (no AD or other neurologically-diseased subjects) and all of them involved unilateral EMF exposure to only one hemisphere via a cell phone held next to the head. In view of this non-chronic and unilateral EMF exposure, it is not surprising that a recent meta-analysis of these controlled human studies found no collective beneficial or impairing effects on cognitive performance [43]. Nonetheless, several PET studies have reported that unilateral, acute EMF exposure (via cell phone) can affect regional cerebral blood flow (rCBF) [46, 47] and increase brain glucose utilization [48]. Thus, even acute high frequency EMF treatment can affect brain physiology and neuronal activity, as will be discussed in a later section.

Results from acute, single EMF treatment/exposure studies are probably not indicative of physiologic and cognitive effects being provided by long-term/daily EMF exposure – in other words, the exposure typical of chronic cell phone users or the repeated EMF treatments almost certainly required for any clinical EMF applications. In this context, no controlled human studies have investigated the long-term effects of high frequency EMF treatment in normal or AD subjects over weeks, months, or years. As mentioned earlier, however, two epidemiologic-based human studies have already provided indirect evidence that years of high frequency EMF exposure (via cell phone use)

is associated with enhanced cognitive performance in normal subjects [26] and a much reduced risk of hospitalization due to AD and vascular dementia [27].

#### *Animal studies*

The complete lack of long-term EMF treatment studies in humans to investigate cognitive effects is at least partially alleviated by our long-term TEMT studies at high frequency (918 MHz) in AD Tg mice and normal mice [22, 25], detailed in the next section. A number of earlier studies had investigated cognitive effects of full body EMF treatment at much lower (25–50 Hz) and much higher (2450 Hz) frequencies in rodents. Impairments in radial arm/Morris maze performance first reported by Lai and colleagues following a single 2450 MHz EMF exposure [49, 50] could not be repeated in multiple follow-up studies [51–53]. Studies involving 1–28 days of EMF exposure at 25–50 Hz have largely reported an impairing effect on Morris maze/Y-maze performance, although one study found enhanced Morris maze performance following 4 weeks of daily EMF treatment at 50 Hz [54].

The above inconsistent cognitive effects at very high (i.e., microwave oven) and very low (i.e., electrical appliance) EMF frequencies are irrelevant to the high frequency ( $\approx 900$  MHz) that we have found to consistently provide cognitive benefit when administered long-term over months through full body exposure [22, 25]. Earlier cognitive-based EMF studies at 900 MHz (GSM) either involved such full body EMF treatment to freely-moving rodents or head-only treatment to restrained rodents. Three full body exposure studies in adult rodents have all involved 900 MHz treatment for only a few days [55, 56] or only once weekly over months [57]. Although Sienkiewicz et al. [55] reported no effects of a very low EMF power level (0.05 W/kg SAR) on radial maze performance, Fragopoulou et al. [56] and Nittby et al. [57] reported cognitive impairment. It is important to underscore, however, that these latter two studies purporting cognitive impairment following only a few days (or intermittent) EMF exposure has methodological drawbacks. For example, one study did not control for stressful background radio noise in their EMF treatment group during the four days of EMF treatment/Morris maze testing [56], while the other study evaluated cognitive performance in only a one-day object recognition task and at 3–7 weeks *after* EMF treatment had been completed [57]. Another study involved adolescent, immature rats being given full body EMF treatment daily for

5 weeks [58]. Although cognitive assessment in Morris maze began after completion of EMF treatments, a beneficial increase in the rate of learning (acquisition) and memory retention was evident in these juvenile rats – the first demonstration of EMF-induced cognitive benefit in animals of any age. Cognitive-based studies administering head only 900 MHz EMF treatment have involved daily EMF treatment for 7–14 days [59, 60] and for 2 or 6 months [61], with all three studies reporting no treatment effects on cognitive performance in Morris maze, 8-arm maze, or recognition tasks.

With the exception of immature rats showing cognitive benefit from long-term EMF treatment at 900 MHz [58], why had all prior 900 MHz studies involving normal adult rodents failed to find the EMF-induced cognitive benefits that we have more-recently reported in normal mice [22, 25]? First, many of these prior studies involved only short-term/intermittent EMF exposure [55–57, 59, 60], which our work shows is usually not sufficient for cognitive benefit [22]. Second, the cognitive tasks selected have often been tasks that are relatively insensitive to various cognitive domains and not directly relevant to humans. Third, given the premise that daily cognitive testing is best performed following daily EMF treatment, cognitive testing has sometimes occurred in the weeks following completion of all EMF treatments for unknown reasons. In all animal studies involving 900 MHz EMF treatment, the *strength* of EMF exposure (as indexed by SAR level) has generally been similar and near cell phone levels; SAR levels across these studies were typically no higher than 3 W/kg, and sometimes much lower (Note: peak SAR levels from cell phone EMF exposure are limited to 2 W/kg).

To summarize the prior work investigating cognitive effects of high frequency (900 MHz) EMF treatment, all of the controlled human studies have involved *normal* individuals being given only acute/non-chronic EMF exposure unilaterally, resulting in no positive or negative cognitive impact. Adult animal studies have involved *normal* rodents being given either acute or longer-term EMF exposure, and generally without cognitive impact in the better-designed studies.

#### **COGNITIVE EFFECTS OF LONG-TERM EMF TREATMENT IN AD MICE AND NORMAL MICE**

In our own EMF studies begun in 2007, we wished to determine the cognitive effects of daily, long-term

EMF treatment at GSM cell phone levels (918 MHz, 0.25–1.05 W/kg, pulsed/modulated) in AD Tg mice [22, 23]. Although there are now numerous Tg mouse models for AD based on genetic insertion of mutant human A $\beta$ PP and/or tau genes, all of them are partial AD models in only re-capitulating some aspects of the disease. In this regard, the A $\beta$ PPsw and A $\beta$ PPsw + PS1 Tg models (carrying the human mutant A $\beta$ PP K670N,M671L gene and/or the mutant Presenilin M146L gene) are the most widely utilized A $\beta$ -generating models and, thus, were the choice for our EMF studies. For A $\beta$ PPsw and A $\beta$ PPsw + PS1 mice, human A $\beta$  production begins in the brain at several months of age, with associated cognitive impairment present by 10–11 months and 5–6 months of age, respectively [22, 62–66]. Though only partial AD models, A $\beta$ PPsw and A $\beta$ PPsw + PS1 mice do faithfully re-capitulate what is believed to be the precipitating neuropathologic event in AD, namely, brain production and aggregation of A $\beta$ .

Aside from our choice of AD models, we wanted EMF treatment to begin at several ages in brain A $\beta$  pathogenesis and associated cognitive impairment, and thus performed three separate behavioral studies, as summarized in Table 1: Study I in young adulthood (prior to cognitive impairment), Study II in mature adulthood (cognitively impaired), and Study III in advanced old age (very cognitively impaired). Moreover, we had just designed and successfully utilized a cognitive interference (CI) task of short-term memory in mice [67] – a task based measure-for-measure on an analogous semantic task used clinically in humans to distinguish AD, MCI, and normal aged humans from one another [68]. The human version of the CI task involves the four measures diagramed in Fig. 2 (upper). In the first measure (3-Trial recall), the subject is presented with 10 familiar objects (Bag A) and asked to recall the objects following a brief distraction task, repeated three times. In the second measure (proactive interference), the subject is presented with 10 novel objects (Bag B) and asked to recall them; this, to determine whether previous learning (Bag A objects) intrudes upon present learning (Bag B objects). The third measure, 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, Delayed Recall is evaluated by asking the subject to recall the original set of ten items (Bag A) after a 20-min delay.

In the mouse version of the CI task (Fig. 2, lower), two different radial arm water mazes (RAWM A and

B) are employed in two separate rooms with different visual cues, and with different goal arms changed daily for both mazes. Following three successive trials in RAWM A (with intermittent Y-maze distraction), mice are tested in RAWM B, then in RAWM A twice again (total of 6 trials daily). As the most human-relevant cognitive task yet designed for rodents, this CI mouse task consequently became the primary (though not exclusive) task for our below studies [22, 23] evaluating cognitive impact of EMF treatment in AD Tg mice and normal mice. It is important to underscore that the specific examples of EMF-induced cognitive improvement given below are representative of multiple cognitive benefits we observed in several tasks taken from the three independent behavioral studies mentioned above (Studies I, II, and III) – as such, these EMF-induced cognitive benefits are real and cannot be dismissed as spurious. Cognitive effects of EMF treatment in these three behavioral studies are summarized in Table 1.

#### *AD Tg mice*

For Tg mice started on EMF treatment in young adulthood (2 months old) for Study I, mice were unimpaired in the CI task at 4.5 months into daily EMF treatment (Fig. 3A; Test 1). However, by 6–7 months into EMF treatment, control Tg mice became impaired in CI performance while EMF-treated Tg mice remained normal in maintaining their excellent performance level (Fig. 3A; Test 2). This protection-based study clearly showed EMF treatment as being prophylactic against otherwise inevitable memory impairment in AD Tg mice. In treatment-based Study II, mature cognitively-impaired AD mice did not show cognitive benefit in the CI task at 5 months into daily EMF treatment. However, by 8 months into EMF treatment, these Tg mice exhibited clearly-improved performance in several CI task measures (Fig. 3B). In Study III, we administered daily EMF treatment for a relatively short two-month period to Tg mice in advanced old age (21–27 months) and found no beneficial effects in the CI task or other complex tasks between 1–2 months into treatment [23]; the treatment period was probably too short for adequately countering the massive brain A $\beta$  pathology built up by that old age in Tg mice (see next section on mechanisms).

#### *Normal mice*

In each of the above three behavioral studies involving AD Tg mice, normal mice of the same age were



Table 1  
Summary of long-term EMF treatment effects reported by Arendash and colleagues

	Animals/Treatment*	Cognitive Effects	A $\beta$ Effects	Effects on Other Measures	Reference
Study I (Young adulthood)	A $\beta$ PPsw mice; 2 M old 7.5 M of EMF treatment	Tg: $\uparrow$ CI at 6–7 M NT: $\uparrow$ Y-maze at 7.5 M	No A $\beta$ deposition Trend for $\uparrow$ soluble A $\beta$ in brain & blood	Tg & NT: Minimal or no effects on brain DNA repair enzymes, antioxidant enzymes or protein oxidation	[22]
Study II (Mature adulthood)	A $\beta$ PPsw mice; 5 M old 8.5 M of EMF treatment	Tg: $\uparrow$ CI at 8 M NT: $\uparrow$ CI at 5 M	A $\beta$ deposition $\downarrow$ 32–35% Trends for $\uparrow$ soluble A $\beta$ in brain & $\downarrow$ soluble A $\beta$ in blood	Tg, NT: No effect on blood cell DNA damage; $\uparrow$ body temp (1°C) during ON periods at 8.5 M	[22]
Study III (Advanced old age)	A $\beta$ PPsw mice; 21–26 M old 2 M of EMF treatment	NT: $\uparrow$ Y-maze at 1 M NT+Tg: $\uparrow$ Y-maze	A $\beta$ deposition $\downarrow$ 24–30% in entorhinal cortex	Tg + NT: $\uparrow$ neuronal activity Tg, NT: At 1–6 weeks, $\uparrow$ body temp ( $\approx$ 0.5°C) and stable brain temp ( $<$ 0.5°C increase) Tg: $\downarrow$ CBF during ON Tg+NT: $\downarrow$ CBF during ON/OFF	[23, 25]
Study IV (Old age)	A $\beta$ PPsw + PS1; 15–17 M old 1 M of EMF treatment	Not evaluated	Mitochondrial soluble A $\beta$ $\uparrow$ 5–10 fold	Tg: Brain mito function $\uparrow$ 50–150% on 6 measures NT: Brain mito function $\uparrow$ 9–12% on 3 of 6 measures; Tg, NT: $\uparrow$ body temp (1°C) and stable or $\downarrow$ brain temp	[24]
Study V (Advanced old age)	A $\beta$ PPsw + PS1; 22 M old 12 days of treatment	Not evaluated	Not evaluated	Tg: No change in brain or body temps. at 5 & 12 days Tg: Near sign. $\downarrow$ (19%) in CBF during ON at 12 days	[23]

\*EMF Treatment = Daily, full-body for two sessions (early morning/late afternoon) at GSM 918 MHz; 0.25–1.05 W/kg, pulsed and modulated; CBF, cerebral blood flow; CI, cognitive interference task; NT, non-transgenic (normal) mice; Tg, transgenic mice (either A $\beta$ PPsw or A $\beta$ PPsw + PS1); Tg+NT, both groups combined.

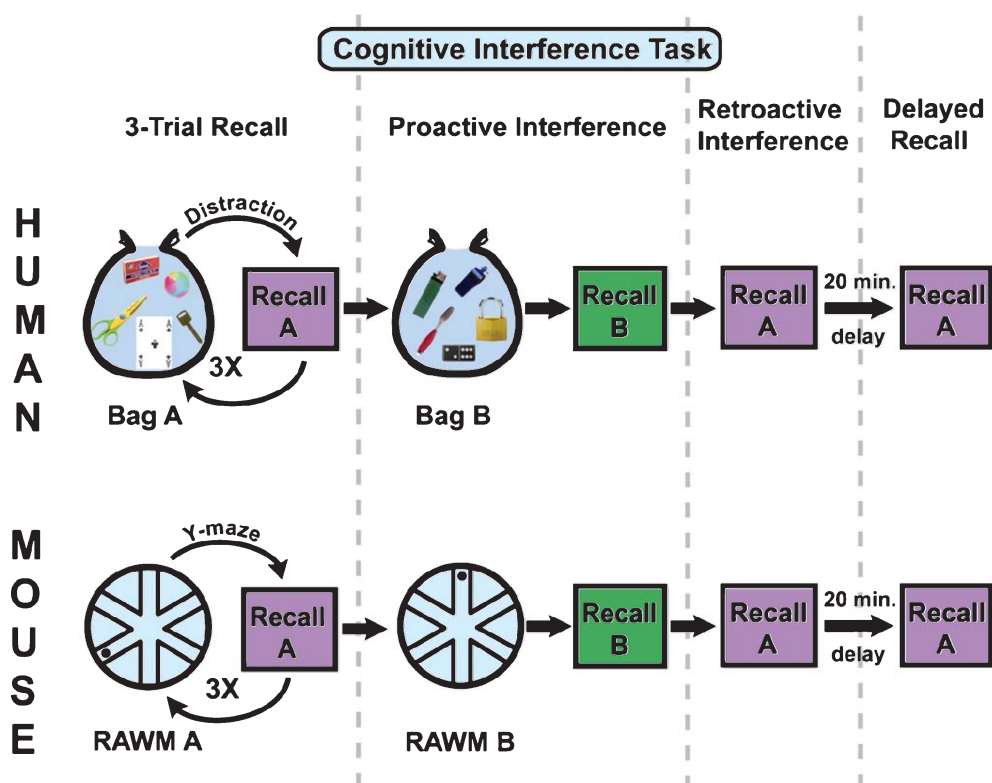


Fig. 2. The four analogous measures in the Cognitive Interference task, as administered to humans (upper) and mice (lower). In humans, the task is extraordinarily sensitive in distinguishing AD, MCI, and aged normal subjects from one another, while in mice it is similarly sensitive in identifying A $\beta$ -induced and age-related impairments in cognitive performance. Therapeutics against AD should be evaluated for their efficacy in such a task – a task that spans the translational bridge.

concurrently given EMF or sham treatment. All young adult normal mice in the protection-based Study I performed well in the CI task at 4.5 and 6–7 months into EMF treatment, so there was no EMF benefit. However, normal mice in treatment-based Study II showed early cognitive benefit in the CI task at 5 months into treatment (Fig. 3C). EMF-induced cognitive benefit to normal mice extended beyond the CI task, as evidenced by the Y-maze alternation task of general mnemonic function (Fig. 3D). At 7.5 months into EMF treatment, normal mice of young adult Study I showed significantly better performance in this task compared to untreated control mice. This beneficial effect of EMF treatment on Y-maze alternation was also found to be present in normal mice started on EMF treatment in advanced old age (Study III) after only one month into EMF treatment (Fig. 3D).

To summarize our three separate behavioral studies carried out thus far, long-term EMF treatment can protect against cognitive impairment in young adult AD Tg mice, and can even reverse pre-existing

cognitive impairment in older Tg mice if administered for a long enough period (i.e., a number of months). Long-term EMF treatment can also improve cognitive performance in normal mice, even as soon as one month into treatment. However, for most cognitive measures that EMF treatment improved in Tg or normal mice, anywhere from 5–8 months of daily treatment was required. Parenthetically, no deleterious cognitive effects of EMF treatment were ever observed, nor were any sensorimotor/anxiety effects that could have impacted cognitive performance. Moreover, our analysis of oxidative markers from brains of both Tg and normal mice given long-term EMF treatment revealed: 1) minimal or no effects on DNA repair enzymes, antioxidant enzymes, or extent of protein oxidative damage (Table 1; Study I); and 2) no histologic or gross changes to brain and peripheral organs. Even after 8+ months of daily EMF treatment, both Tg and normal mice in Study II showed no increase in DNA damage to blood cells, indicative that peripheral oxidative stress levels were not impacted. Thus,

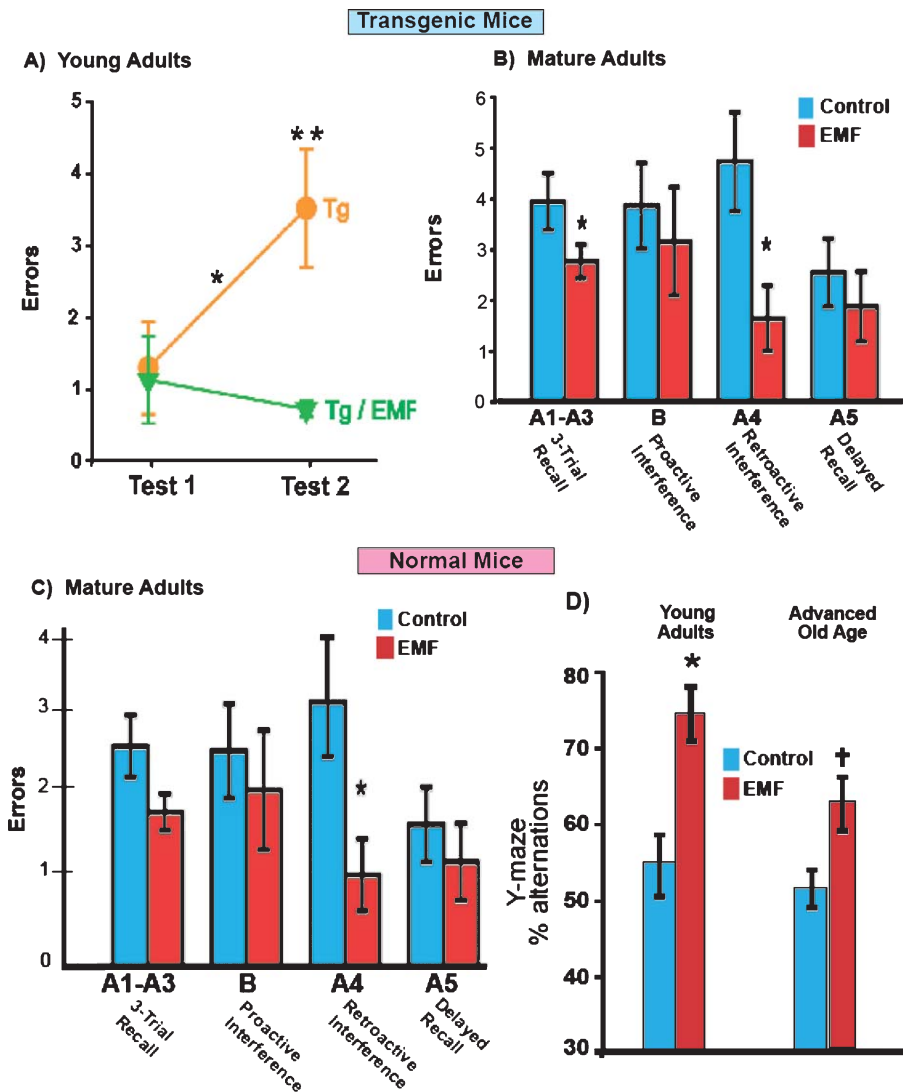


Fig. 3. Long-term EMF treatment protects against and reverses cognitive impairment in AD Tg mice (A and B), as well as provides supra-normal cognition to normal mice (C and D). A) Young adult Tg mice being given EMF exposure exhibit stable performance across two cognitive interference (CI) test periods separated by months, while performance of control Tg mice worsened substantially (retroactive interference measure is depicted).  $*p < 0.05$  by paired *t*-test;  $**p < 0.01$  for Tg versus Tg/EMF. B) At 8 months into EMF treatment, mature Tg mice were superior to Tg controls in both 3-trial recall and retroactive interference measures of the CI task.  $*p < 0.025$  versus control. C) Normal mature mice at 5 months into EMF treatment showed significantly improved retroactive interference in the CI task.  $*p < 0.05$  versus normal control. D) Y-maze alternation performance was enhanced at 7.5 months (left) and 1 month (right) into EMF treatment of normal mice in the young adult and advanced age studies, respectively.  $*p < 0.05$  versus control,  $†p = 0.06$  versus control. A–C reprinted with permission from [22].

every brain and body index we have evaluated thus far in mice indicates high frequency EMF treatment to be completely safe for long-term therapy.

Given the ability of long-term EMF treatment to benefit both A $\beta$ -generating Tg mice and normal mice, there must be both A $\beta$ -dependent and generalized mechanisms of EMF action occurring. Our discovery of such beneficial mechanisms of EMF action is discussed in the following section.

### THREE COMPLIMENTARY MECHANISMS OF EMF ACTION HAVE BEEN IDENTIFIED

Prior to our recent studies [22, 23, 25], there was no evidence from controlled adult studies that high frequency EMF exposure had cognitive benefit. As such, no plausible biologic mechanism(s) had ever been presented to explain such EMF-induced cognitive enhancement. Our work has, however, recently

revealed three complimentary, inter-related mechanisms of long-term EMF action that have broad implication not only for AD therapeutics, but other neurologic conditions as well. Although prior studies had reported high frequency (cell phone level) EMF effects on functionality of various transmitter and signal transduction systems in the brain, these studies universally involved acute EMF exposure and/or cell culture systems [37, 69]. However, only through *long-term* EMF exposure in *living* subjects can physiologically- and therapeutically-relevant mechanisms be revealed and verified. In this context, we have identified the first non-thermal mechanisms of long-term EMF action in the living brain as: 1) anti-A $\beta$  aggregation; 2) mitochondrial enhancement; and 3) increased neuronal activity.

#### *Anti-A $\beta$ aggregation*

Aside from cognitive effects, the major therapeutic endpoints evaluated in AD transgenic models involve effects on monomeric A $\beta$  production and its ensuing aggregation first into soluble oligomers, then into insoluble A $\beta$  aggregate cores of extracellular neuritic plaques. Thus neurochemical measurement of brain soluble/insoluble A $\beta$  levels and immunohistologic quantification of brain A $\beta$  deposits/burden are standard endpoints for disease-modifying therapeutic efficacy. In both of our behavioral studies wherein AD mice were euthanized at an age when robust extracellular A $\beta$  deposition was present (Studies II and III), long-term EMF treatment suppressed or reversed that deposition (Fig. 4). In Study II, adult A $\beta$ PPsw transgenic mice were started on daily EMF treatment at 5 months of age (before any brain A $\beta$  deposition). At 8.5 months into EMF treatment, brain A $\beta$  deposition was 32–35% lower in EMF-treated mice compared to controls, indicating a significant prevention of A $\beta$  deposition (Fig. 4A). We then further demonstrated the anti-A $\beta$  aggregation ability of EMF treatment by sonicating hippocampal homogenates from aged A $\beta$ PPsw mice (to disaggregate their A $\beta$  deposits), then subjecting the homogenates to the same daily EMF protocol as in our *in vivo* studies. By four days into EMF treatment, substantially less aggregated oligomeric A $\beta$  was evident in Western blots compared to non-treated homogenates (Fig. 4B). In Study III, very old A $\beta$ PPsw + PS1 mice bearing huge brain burdens of deposited A $\beta$  exhibited an impressive 24–30% removal of deposited brain A $\beta$  following two months of daily EMF treatment, indicating a “disaggregation” of pre-existing A $\beta$  deposits had occurred

(Fig. 4C). Even a single month of daily EMF treatment to aged AP $\beta$ Psw + PS1 transgenic mice (Study IV; see Table 1) apparently results in disaggregation of toxic A $\beta$  oligomers located “intraneuronally” within mitochondria. EMF-treated AD mice in that study had dramatically higher (5–10 $\times$ ) soluble A $\beta$  levels in their mitochondria from both cortex and hippocampus, most probably indicative of a huge increase in disaggregated monomeric A $\beta$  (Fig. 4D). Collectively, these multiple studies provide clear and compelling evidence that high frequency EMF treatment can decrease brain A $\beta$  aggregation both extracellularly and intraneuronally – a feature unmatched by any AD drug currently under translational development.

How might EMF treatment prevent or reverse the process of brain A $\beta$  aggregation/deposition? First, repeated EMF treatment to cell cultures (albeit at a low 50 MHz level) induces upregulation of heat shock transcription factor 1 (HSF-1) [70], which has been shown to disaggregate A $\beta$  *in vivo* [71]. Secondly, greater cell phone use (i.e., longer EMF exposure) in humans is associated with higher blood levels of the A $\beta$ -binding protein transthyretin [72], which may facilitate brain A $\beta$  removal by sequestering plasma A $\beta$  to enhance further A $\beta$  transport out of the brain (see below). In this regard, over-expression of the human transthyretin gene in AD transgenic mice reduces brain A $\beta$  deposition and enhances cognitive performance [73]. Moreover, human AD patients have much lower transthyretin levels in their plasma [74] and CSF [75] compared to non-demented controls. Thirdly, EMF treatment may directly impact the A $\beta$  aggregation process biophysically through some as yet unidentified mechanism that may, in fact, counter abnormal protein aggregation in general. Of these mechanisms against A $\beta$  aggregation, a direct (biophysical) impact of EMF treatment on A $\beta$  aggregation seems most likely because the ability of EMF treatment to prevent re-aggregation of A $\beta$  over days *in vitro* (Fig. 4B) presumably could not have involved induction of either heat shock proteins or transthyretin. Nonetheless, all three of the aforementioned processes are viable possibilities to explain the important ability of EMF to act as a powerful inhibitor of A $\beta$  aggregation in our *in vivo* studies.

In contrast to the reduction in brain A $\beta$  aggregation/deposition induced by long-term EMF treatment, soluble levels of A $\beta$  in brain and plasma are not significantly affected. Indeed, for both the Young Adult and Mature Adult studies (Studies I and II), strong trends for EMF-induced increases in brain levels of soluble A $\beta$  were evident, as exemplified by soluble A $\beta$ <sub>1-40</sub>

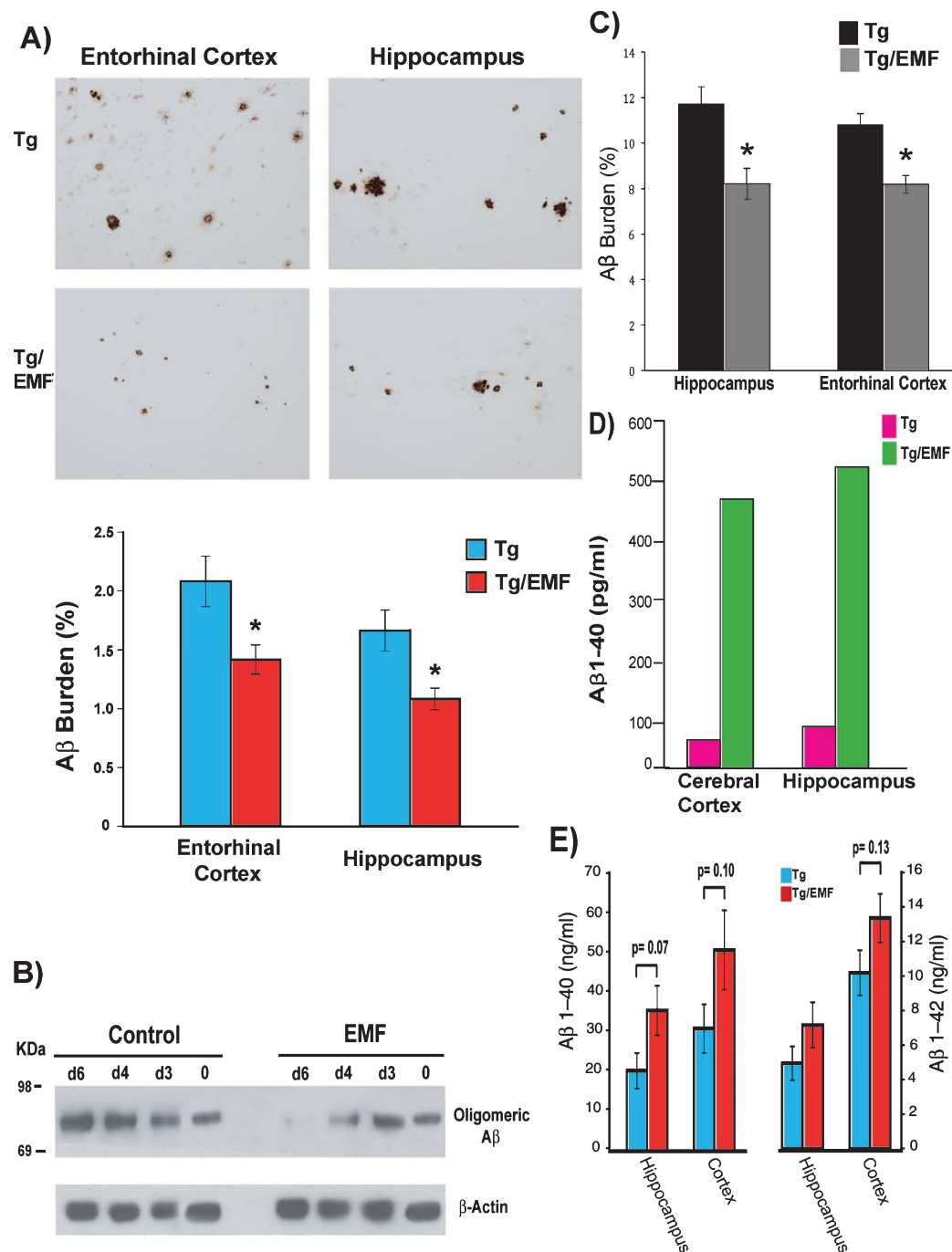


Fig. 4. Effects of EMF treatment on brain A $\beta$  aggregation/deposition and brain soluble A $\beta$  levels. A) In Study II, 8.5 months of EMF treatment to mature Tg mice substantially reduced development of brain A $\beta$  deposition/burden, both from direct visual observation of brain sections (upper) and quantification of A $\beta$  deposition/percent burden in hippocampus and entorhinal cortex (lower). Micrometer bar = 50  $\mu$ m; \* $p$  < 0.02 versus Tg control group. B) Western blots showing that *in vitro* EMF exposure of sonicated hippocampal homogenates from 14 M old Tg mice results in progressively decreased A $\beta$  re-aggregation (oligomerization) between 3 and 6 days into treatment. C) In Study III, two months of EMF treatment to Tg mice in advanced old age resulted in reversal of their very extensive A $\beta$  deposition, as indexed by quantification of A $\beta$  deposition/percent burden in hippocampus and entorhinal cortex. \* $p$  < 0.005 versus Tg control group. D) In Study IV, one month of EMF treatment to aged Tg mice greatly increased mitochondrial soluble A $\beta$ <sub>1-40</sub> levels in cerebral cortex and hippocampus. E) In Study II, 8.5 months of EMF treatment to mature Tg mice nearly increased soluble A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> levels in hippocampus and cerebral cortex. A, B, and E reproduced with permission from [22].

and A $\beta$ <sub>1-42</sub> levels in both cortex and hippocampus of EMF-treated Tg mice in Study II (Fig. 4E). How is it that, in Tg mice that show clear cognitive benefit from long-term EMF treatment, decreases are observed in brain A $\beta$  deposition while nearly significant increases in brain soluble A $\beta$  are concurrently evident? Although these two EMF effects on deposited versus soluble forms of A $\beta$  seem incongruous, they are in fact totally consistent with what is expected when A $\beta$  aggregation is prevented or reversed to result in cognitive benefit. This is because it is the dynamic equilibrium between soluble monomeric A $\beta$  and oligomeric/deposited A $\beta$  (not one or the other in isolation) that is pivotal to whether cognitive dysfunction or benefit occurs (Fig. 1A). Stated another way, total amounts of soluble A $\beta$  are not critical to cognitive function; but levels of aggregated A $\beta$  forms (i.e., oligomeric, fibrillar) are important. EMF treatment appears to induce disaggregation of oligomeric  $\rightarrow$  monomeric A $\beta$  within the brain's soluble A $\beta$  pool, resulting in elevated/nearly elevated soluble A $\beta$  levels. Underscoring this point, we have found the EMF-induced combination of cognitive benefits, decreased brain A $\beta$  aggregation, and unchanged/elevated brain soluble A $\beta$  levels is also observed in Tg mice given other suppressors of A $\beta$  aggregation, such as melatonin [76] and the nicotinic metabolite cotinine [77].

By preventing A $\beta$  aggregation or disaggregating already-formed A $\beta$  oligomers/aggregates in and outside neurons, EMF treatment creates a flux to soluble monomeric A $\beta$ ; it is this monomeric A $\beta$  form that is capable of being transported out of neurons and across the BBB [78] to eventually be degraded in the blood (Fig. 1B). The delayed ability of EMF treatment to provide cognitive benefit in complex, A $\beta$ -sensitive tasks (e.g., manifesting itself at 6–8 months into treatment of Tg mice) probably reflects the time required for our currently-used EMF parameters to substantially decrease the pool of aggregated/deposited A $\beta$  by chronically decreasing (or reversing) flux of monomeric  $\rightarrow$  oligomeric/deposited A $\beta$  (Fig. 1B). The resultant increase in monomeric A $\beta$  in brain parenchyma then results in its enhanced transport and clearance from the brain. As shown in Fig. 1B, the anti-A $\beta$  aggregation ability of EMF treatment is complimented by two other EMF mechanisms of action (i.e., mitochondrial enhancement and neuronal activity enhancement), both of which will be discussed below.

Unlike various secretase inhibitors that are currently in translational development to treat AD, EMF treatment does not suppress or inhibit brain A $\beta$  production or inhibit A $\beta$ PP cleavage enzymes (i.e.,

$\beta$ - and  $\gamma$ -secretase). These secretases and their product (monomeric A $\beta$ ) likely have some beneficial physiologic effects, such as the important processing of Notch protein by  $\gamma$ -secretase [79], and should not be greatly suppressed by therapeutics. Rather EMF treatment prevents/reverses abnormal A $\beta$  aggregation and thus encourages A $\beta$  clearance out of neurons and out of the brain (Fig. 1B). In the absence of such EMF treatment, A $\beta$  aggregates located intraneuronally disrupt mitochondrial function (see below), while extracellular A $\beta$  aggregates/deposition in neuritic plaques disrupt neuronal function by: 1) harboring A $\beta$  oligomers that can diffuse away from plaques to induce neuronal damage [2]; and 2) acting as physical obstacles that compromise axons-of-passage and that cause abnormal swelling/dystrophy of nerve terminals in their vicinity [80]. Thus, the multiple mechanisms of EMF action are ideally suited to remove/clear A $\beta$  from the brain.

#### *Mitochondrial enhancement*

As shown in Fig. 1A, newly-formed monomeric A $\beta$  is created internally by neurons, where it aggregates into A $\beta$  oligomers that have a high affinity for mitochondrial membranes [6–9]. Binding of such A $\beta$  oligomers to mitochondrial membranes greatly impairs mitochondrial function, resulting in insufficient mitochondrial ATP production and ensuing neuronal dysfunction/degeneration [6]. This process of intraneuronal A $\beta$ -induced mitochondrial dysfunction is an early and central event in AD pathogenesis [7, 9, 10, 13], occurring well before A $\beta$  begins to aggregate extracellularly to form the core of neuritic plaques in AD Tg mice [12, 81]. Indeed, we have found the degree of cognitive impairment in AD Tg mice (i.e., A $\beta$ PPsw and A $\beta$ PPsw + PS1 models) is linked to the extent of their synaptic mitochondrial dysfunction and mitochondrial A $\beta$  levels [82]. Given all of the above, a therapeutic that can provide enhancement of mitochondrial function could provide substantial cognitive benefits against AD.

In the first study to investigate high frequency EMF effects on brain mitochondrial function in any animal [24], we provided our standard twice-daily EMF treatment for one month to aged (15–17 month old) A $\beta$ PPsw + PS1 mice and littermate normal mice (See Table 1; Study IV). In the cognitively-important cerebral cortex and hippocampus, EMF treatment greatly enhanced the impaired mitochondrial function of these Tg mice by 50–150% across six well-established measures (Fig. 5). This EMF-induced

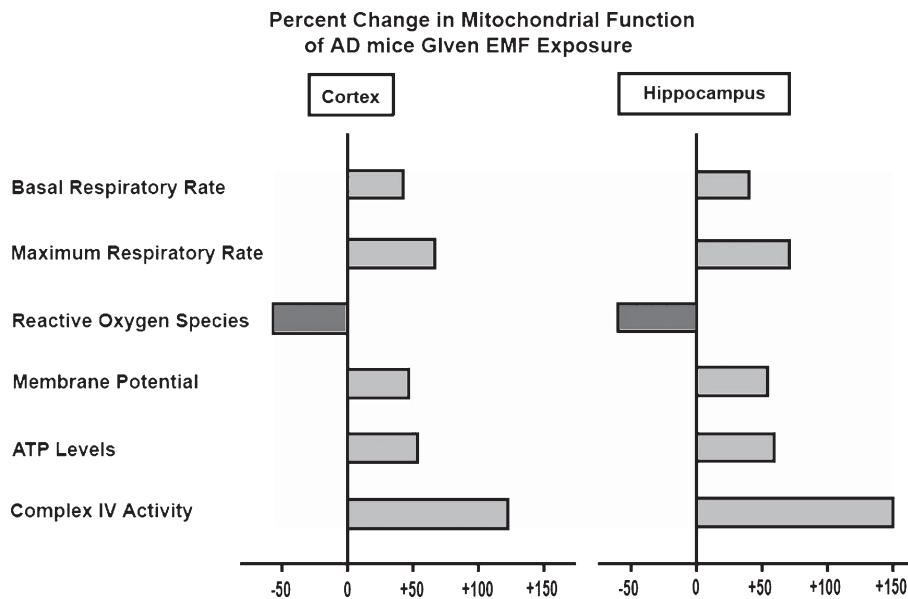


Fig. 5. Within the cerebral cortex and hippocampus, the percent change in mitochondrial function induced by 1 month of daily EMF treatment to aged A $\beta$ PPsw + PS1 mice. Striking 50–150% enhancements were induced by EMF treatment in both brain areas and across all six measures of mitochondrial function. Reproduced with permission from [24].

mitochondrial enhancement in Tg mice was linked to 5–10 fold increases in soluble A $\beta$  within the same brain mitochondria (see Fig. 4D), which we believe to be indicative that EMFs disaggregated toxic A $\beta$  oligomers associated with mitochondria into innocuous A $\beta$  monomers. The across-the-board enhancement in all mitochondrial measures provided by EMF treatment argues that some central mechanism of dysfunction was removed; we believe toxic A $\beta$  oligomers in mitochondrial membranes were removed through their disaggregation.

In the same mitochondrial function study (Study IV), one-month of EMF treatment also enhanced brain mitochondrial function in normal mice (over-and-above already excellent levels). This EMF-induced enhancement was not as robust as in Tg mice, significantly increasing (by 9–12%) three of the 6 mitochondrial function measures (basal and maximum respiratory rates, Complex IV activity) in cerebral cortex and hippocampus. Thus, a direct (A $\beta$  independent) and generalized enhancement of mitochondrial function could also be occurring with EMF treatment, especially in explaining A $\beta$ -independent cognitive benefits to normal mice as early as 1 month into treatment (Fig. 3D and Table I; Study III). We are unaware of any mechanisms that would link high frequency EMF treatment to generalized mitochondrial function in normal subjects, so their discovery awaits further study. This early and direct enhancement of

mitochondrial function at 1 month is nonetheless insufficient for providing cognitive benefit in complex, A $\beta$ -dependent tasks, as discussed previously. We also know that non-thermal mechanisms are involved in all the EMF-induced mitochondrial enhancements seen in normal mice and A $\beta$ -bearing Tg mice because there were no increases in brain temperature during or after EMF treatments [24]. The just-described mitochondrial function study (Study IV) is significant in being the first to demonstrate beneficial *in vivo* effects of EMF exposure on brain mitochondrial function in any animal.

#### Neuronal activity enhancement

An early characteristic of AD is progressive reduction in the brain's neuronal activity, as indexed by FDG-PET scan analysis. This progressive decline in PET-analyzed neuronal activity correlates well with cognitive decline and is highly predictive of conversion from MCI to AD [15, 83]. As such, therapeutics that enhance neuronal activity could provide substantial cognitive benefit in subjects being impacted by AD. Although high frequency EMF treatment has been reported to increase both neuronal activity (indexed by FDG-PET scan analysis) and EEG alpha-wave activity in brains of normal adults during a single EMF exposure [38, 48], no long-term daily EMF treatment studies have been done in normal or AD subjects.

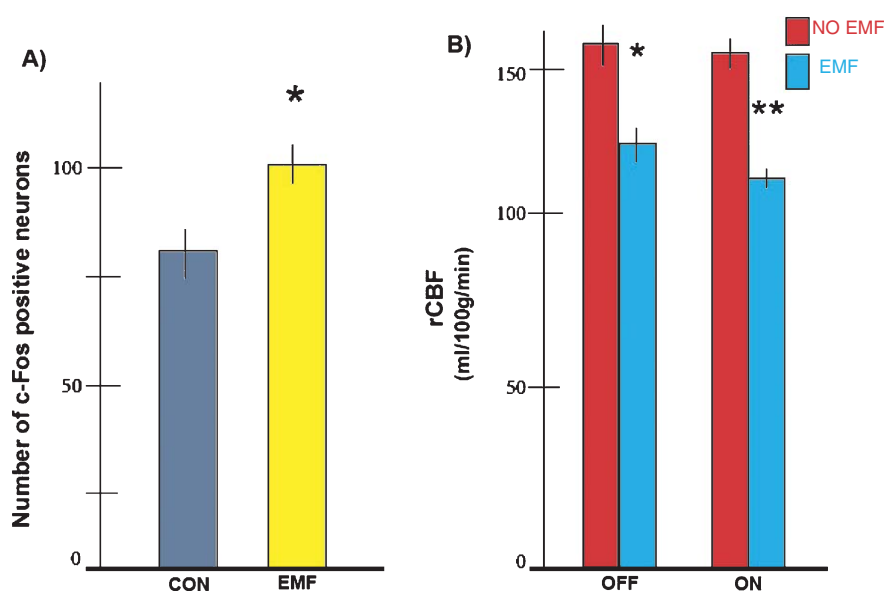


Fig. 6. Neuronal activity (as indexed by c-Fos positive neuronal numbers) in entorhinal cortex and cortical cerebral blood flow in very old (23–28 month old) Tg and NT mice collectively at two months after EMF treatment. A) Number of c-Fos positive neurons in entorhinal cortex for combined NT and Tg groups given EMF versus control/sham treatment.  $*p < 0.02$ . B) Regional cerebral blood flow (rCBF) in cerebral cortex of normal and Tg mice combined, with laser Doppler measurements being obtained after 2 months of daily EMF treatment. Irrespective of genotype, EMF-treated mice had significantly reduced rCBF during both ON and OFF periods.  $*p < 0.05$  versus No EMF;  $**p < 0.0001$  versus No EMF.

The lack of long-term EMF treatment studies in humans is at least partially negated by our recent study of long-term EMF effects on neuronal activity in mice (Table 1: Study III). In that study, we reported that daily EMF treatment for two months significantly enhances neuronal activity ( $\uparrow 21\%$ ) in entorhinal cortex of aged (23–28 month old) A $\beta$ PPsw mice and littermate normal mice, irrespective of genotype (Fig. 6A) [25]. Neuronal activity was evaluated mid-way between the two daily EMF treatments (at a time when behavioral testing would have normally occurred) and was indexed by neuronal expression of c-Fos, an established immunohistologic/indirect marker for neuronal activity [84]. Indeed, these same mice had been behaviorally evaluated (Y-maze task) at 1 month into EMF treatment, during the same temporal window that entorhinal cortex activity was evaluated (e.g., mid-way between daily EMF ON periods). A significant enhancement in Y-maze performance ( $\uparrow 26\%$ ) was observed, irrespective of genotype. Therefore, EMF treatment induced a generalized increase in both neuronal activity and Y-maze performance during the OFF period of EMF treatment. Because c-Fos is an immediate early gene, it is likely that EMF-induced increases in c-Fos neuronal staining within entorhinal cortex were even greater during ON periods, with a gradual attenuation of this effect during OFF periods.

The fact that long-term EMF treatment induces enhanced neuronal activity in entorhinal cortex has implications for a secondary mechanism of EMF action, namely, hippocampal neurogenesis. The entorhinal cortex sends a prominent stimulatory projection, the perforant pathway, to hippocampal neurons in the dentate gyrus. These hippocampal stem cells are continually dividing to produce new granular cells (neurons) in dentate gyrus. A very recent study has reported that electrical stimulation of the mouse entorhinal cortex enhances hippocampal neurogenesis weeks later [85], presumably by activating the perforant pathway. Moreover, mice given one week of daily EMF treatment at very low frequency (50 Hz) experience an increase in hippocampal neurogenesis, with resultant newly-born neurons becoming mature and integrated into hippocampal circuits [86]. Thus, it is entirely possible that our long-term EMF treatment, albeit at high frequencies, also induced hippocampal neurogenesis through perforant pathway and/or direct hippocampal stimulation.

Our finding that long-term EMF treatment enhances neuronal activity is consistent with a recent PET-based study in humans [48]. In that study, a significant 7% increase in neuronal activity occurred in cortical areas immediately beneath where a cell phone was being held for a single 50-min exposure. In the context that



toxic intraneuronal A $\beta$  is primarily removed/cleared from neurons through synaptic release at nerve terminals (see Fig. 1) [87], an EMF-induced enhancement of neuronal activity in cognitively-important brain areas (such as entorhinal cortex) should be of immense value to clear disaggregated A $\beta$  from neurons and from the AD brain. Given the progressive decline in neuronal activity that begins in MCI, years before diagnosis of AD [15, 83], early intervention with EMF treatment could increase neuronal activity to stabilize or improve cognitive function.

The above mechanisms of long-term EMF action thus far identified are intimately linked and complementary to one another, as depicted in Fig. 7. Both generalized (in normal subjects) and AD-specific actions exist in an interwoven network. The exact mechanism(s) of high frequency EMF action at the molecular level remain to be determined and will be an intense subject of future investigations.

### **POSSIBLE ROLE OF HORMESIS IN EMF ACTIONS**

A novel strategy for limiting cellular senescence involves the concept of hormesis, wherein repetitive mild stress (from otherwise damaging stimuli) provides beneficial anti-aging effects or protection from injury [88]. Notable examples of hormesis are: 1) repeated exposure to hypoxia enhances oxidative defenses to protect against later hypoxic exposure; and 2) repeated caloric/food restriction enhances longevity, cognitive function, and cardiovascular health. It is entirely possible that repeated/daily EMF treatment provides hormetic benefits by upregulating repair/maintenance systems. Very low frequency EMF treatment (generally <100 Hz) has already been shown to provide therapeutic effects such as bone tissue regeneration, osteogenesis, and immunologic enhancement [89]. Moreover, mouse/human cell cultures subjected to repeated EMF treatment at somewhat higher frequency (50 MHz) respond with delayed cellular senescence in young cells and reversal of senescence in aged cells [70].

It is important to note that, although hormetic effects are often mediated by constituents of the heat shock response (e.g., HSF-1, HSP70) [89, 90], EMF treatment at higher frequencies ( $\geq 50$  MHz) and modest SAR levels does not involve an increase in temperature, as the findings of Perez et al. [70] and our own work verifies. Thus, any hormetic mechanisms involved must be non-thermal and not due to thermal-induced cell injury.

Perhaps a good example of hormesis at the high EMF frequencies (900 MHz) emphasized in this paper are two similar studies by Salford and colleagues. In the first study, a single EMF treatment to rats was found to induce darkly-stained (damaged?) neurons and BBB breakdown in the brain some weeks later [91]. In the second study, the same EMF treatment given once weekly for 50 weeks resulted in no such darkly-stained neurons or BBB breakdown [92]. Thus, a single EMF exposure may have deleterious effects, but repeated long-term exposure probably negates any such effects, at least in part through hormesis. The extent to which hormetic mechanisms are involved in the EMF-induced cognitive and physiologic benefits we have identified will be important to determine in future studies.

### **EMF EFFECTS ON TEMPERATURE AND CEREBRAL BLOOD FLOW**

EMF-induced effects on body tissues can involve either thermal (heating) or non-thermal mechanisms [93], which may in turn be linked to changes in CBF. Before our own recent work [22–24], only one prior animal study investigated the effects of high frequency EMF exposure on brain/body temperature and/or CBF [94]. That study involved a single head-only GSM exposure for 18 min to anesthetized rats at very high frequency (2000 MHz) and very high SAR levels (10–263 W/kg). Not surprisingly, this acute EMF exposure increased brain temperature in a dose-dependent fashion (by 1–12°C) and increased cortical CBF (by 30–70%). In humans, no studies investigating EMF effects on brain temperature have apparently been done, while EMF effects on CBF have only involved a single cell phone-level EMF exposure resulting in inconsistent results (for review, see [38]). Thus, for both animals and humans, the impact of long-term EMF treatment on brain temperature and CBF had been unexplored prior to our recent work.

#### *Temperature*

Our recent studies [22–24] have investigated both acute and long-term body/brain temperature effects of EMF treatment, with the following findings during two EMF ON periods daily, as summarized in Table 1:

- 1) One day of EMF treatment has no effect on body or brain temperature of either Tg or normal mice [22].

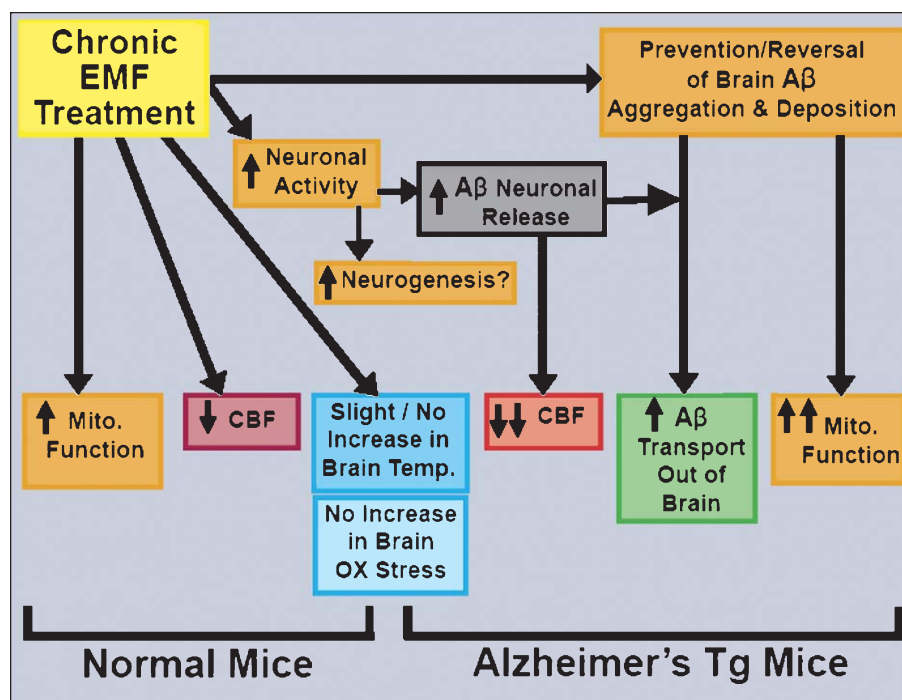


Fig. 7. Summary diagram depicting mechanisms of long-term EMF action in normal mice and AD transgenic (Tg) mice. Long-term EMF actions presumed to be beneficial to cognitive function include prevention/reversal of brain A $\beta$  aggregation, brain mitochondrial enhancement, and increased neuronal activity. The extent to which reduction in cortical cerebral blood flow (CBF) impacts cognitive function is unresolved. These long-term EMF actions occur through slight/no increase in brain temperature and without increasing brain oxidative stress/damage.

- 2) At both 5 days and 12 days into EMF treatment (Study V), very old Tg mice have no change in body or brain temperatures.
- 3) At 1 month into daily EMF treatment (Study IV), body temperature of aged Tg and normal mice is elevated by around 1°C, while brain temperature is either stable (NT mice) or decreased (Tg mice).
- 4) At 1, 3, and 6 weeks into EMF treatment (Study III), aged Tg and normal mice experience a minimal elevation in body temperature (<1°C) and either stable or slightly increased (<0.5°C) brain temperature.
- 5) At 8.5 months into daily EMF treatment (Study II), body temperature of both Tg and normal mice is elevated by approximately 1°C (brain temperature not determined).

For all of the above EMF studies, any minimal elevations in body or brain temperature that occurred during ON periods were far below what would be needed to incur brain/physiologic damage [93]; moreover, any temperature elevations always returned back down to normal levels during OFF periods (when behavioral testing was performed). It thus seems clear that

the EMF-induced cognitive benefits observed in these same mice are due to non-thermal brain mechanisms, the first three of which were identified and discussed in a previous section. Any slight EMF-induced increase in brain temperature is probably reflective of increased neuronal activity [25] and/or EMF-generated heat in the periphery. Parenthetically, such small EMF effects on brain temperature will probably be even less with future studies involving head-only EMF treatment, since there would presumably be no EMF-induced peripheral hyperthermia.

#### Cerebral blood flow

Prior human studies investigating EMF effects on CBF were all single exposure studies (<1 h), with several human PET studies reporting that rCBF in cerebral cortex is reduced during a single exposure EMF treatment [47, 95]. However, our own studies offer the first insight into effects of long-term daily EMF exposure on CBF [23]. As with PET-measured CBF in the acute human studies, our laser doppler-measured CBF for mice was regional (rCBF) in being limited to the cerebral cortex.

Tg (A $\beta$ PPsw) and normal mice in advanced old age had rCBF determined during and several hours following EMF treatment at 2 months into daily EMF treatment (Table 1; Study III). Tg mice (but not normal mice) exhibited a significant 13% decrease in rCBF during ON versus OFF periods. This EMF-induced reduction in rCBF was even greater ( $\downarrow$ 25%) compared to control Tg mice during sham ON periods. Obviously, the difference between Tg and normal mice is brain production and aggregation/deposition of A $\beta$  in Tg mice. Moreover, these same EMF-treated mice had increased brain neuronal activity at the same 2 month juncture into EMF treatment (Table 1: Study III). Since intraneuronal A $\beta$  is synaptically released in greater amounts during increased neuronal activity [87], there is presumably greater efflux/clearance of this soluble/monomeric A $\beta$  out of the brain and into blood during EMF exposure (see Fig. 1B). Inasmuch as vascular A $\beta$  is a well-known constrictor of smooth muscle in resistance vessels (e.g., arterioles), we believe this enhanced presence of cerebrovascular A $\beta$  due to EMF exposure induces cerebral vasoconstriction and the resulting decrease in rCBF that was observed in aged Tg mice. This reduction in rCBF observed in aged A $\beta$ PPsw mice induced by long-term EMF treatment (Table 1: Study III) was also seen in aged A $\beta$ PPsw + PS1 (Tg) mice at 12 days into EMF treatment (Table 1: Study V). A nearly significant 19% decrease in rCBF occurred during ON periods, with 4 of 5 Tg-treated mice exhibiting rCBF decreases of 7–46%. As with the long-term EMF treatment study (Study III), it is likely that EMF treatment at 12 days elevated vascular A $\beta$ , causing a modest vasoconstriction in the brain and the ensuing decrease in CBF that was observed.

In the long-term study (Study III), rCBF was reduced even during OFF periods in both Tg and normal mice being given EMF treatment. Indeed, when both genotypes were combined to investigate main effects of EMF treatment, rCBF was significantly decreased during both ON ( $\downarrow$ 23%) and OFF ( $\downarrow$ 16%) periods (Fig. 6B). Clearly, some non-specific EMF mechanism is reducing rCBF during OFF periods in both Tg and normal mice. For example, this may be a continuing auto-regulatory response to limit brain heating due to the slight body hyperthermia present during ON periods. Along this line, body hyperthermia (such as that induced by exercise) has been shown to decrease CBF in humans by 18% [96, 97]. rCBF would be further decreased in Tg mice during ON periods due to increased vascular A $\beta$  and resulting additional cerebrovascular constriction.

The fact that a reduction in rCBF accompanied the EMF-mediated therapeutic outcomes in Tg and normal mice represents a paradigm shift from the traditionally accepted neuroprotective approach of an increase in rCBF. These observations highlight the need for closely monitoring rCBF, plus other physiological parameters, in order to aid successful translation of EMF treatment studies in AD patients.

Figure 7 not only depicts the negligible effect of long-term EMF treatment on brain temperature, but also the decrease in rCBF induced by long-term EMF treatment in Tg mice (moderate reduction) and normal mice (small reduction).

#### **FUTURE TRANSLATIONAL DEVELOPMENT OF TEMT TO TREAT AD: WHAT NEEDS TO BE DONE NEXT?**

There is certainly a promising foundation of data that demonstrates an excellent cognitive-enhancing potential for high frequency EMF treatment (which was also referred to as TEMT). So where should the research in this new field of bioelectromagnetics go from here? Future experimental development clearly needs to involve both a continuation of basic science studies and initiation of clinical trials against AD.

##### *Basic science studies*

All of our EMF studies to the present point, and those of most other investigators, have involved full body EMF treatment/exposure. Although we have established that such treatment does not result in any remarkable changes in body physiology or any pathologic effects [22–24], the only way to eliminate the possibility that peripheral (non-CNS) mechanisms are involved in the cognitive benefits observed is to focus future studies on head-only EMF treatment; parenthetically, there should be no need to expose the entire body to EMF treatment. Though it may be technically challenging to fit mice with EMF-generating head units that allow free mobility in their cage and that would mimic EMF treatment in the much larger human head, this advance must and will be accomplished.

Secondly, the currently-utilized set of EMF parameters (at cell phone levels) requires five or more months of daily treatment for mice to exhibit cognitive benefit in most tasks. Modifying EMF parameters to attain a shorter treatment period for cognitive enhancement is therefore highly desirable. Experimenting with a

range of frequencies (e.g., 300–1900 MHz) is unlikely to decrease time-to-cognitive-benefit compared to our presently-utilized  $\approx 900$  MHz since lower frequencies within this range would go right through the brain and higher frequencies would not penetrate the brain sufficiently; thus, 900 MHz may be optimal for EMF frequency. Although EMF strength (SAR levels) could be safely increased to around 2 W/kg from our currently utilized 0.25–1.05 W/kg, even higher SAR levels may impose potentially undesirable effects. Rather, it would seem more prudent to compare continuous (unmodulated) EMF waves to various pulsed and amplitude-modulated signals. It is noteworthy that our cognitive-enhancing EMF treatments always involved pulsed, modulated GSM signal. A recent, comprehensive review concluded that acute EMF-induction of biologic effects (e.g., on EEG, CBF) occurs primarily with GSM-type modulation and a pulsed signal, not continuous wave or UMTS fields [37].

Importantly, occurrence of EMF-induced cognitive benefits specifically against AD may be accelerated by enhanced removal/clearance of monomeric A $\beta$  from the brain. Through its ability to prevent or reverse brain A $\beta$  aggregation, EMF treatment appears to make increased amounts of monomeric A $\beta$  available for transport out of the brain (Fig. 1B). Plasma A $\beta$ -binding proteins, such as transthyretin, could enhance that transport and might be given as an adjunct with EMF treatment. Care would need to be taken, however, to insure that such enhanced A $\beta$  brain clearance would not occur so rapidly as to result in the drawbacks (i.e., BBB breakdown and cerebral hemorrhage) that have plagued immunotherapeutic approaches against AD, as discussed to earlier.

#### *Clinical studies*

In view of the expanding foundation of basic science studies we and others have provided indicating high frequency TEMT as safe, disease-modifying, and cognitively beneficial in mouse models for AD, when might human clinical trials be initiated and in what study population? The fact that all prior clinical studies have involved only *acute* EMF treatment given *unilaterally* underscores the necessity of performing controlled long-term clinical trials with bilateral head-only EMF treatment. Although future basic research studies to be done in mice will probably identify a more efficacious set of EMF parameters, the currently-utilized cell phone level EMF parameters are safe and could be investigated in the near future for cognitive enhance in MCI and/or AD subjects.

### **EMF TREATMENT OF BRAIN INJURY AND OTHER NEUROLOGIC DISORDERS**

Given the three mechanisms of TEMT action that we have already identified, the potential of TEMT extends well beyond AD to other brain disorders and injuries. For example, the primary/initial injury induced by traumatic brain injury (TBI) is largely unavoidable, but triggers secondary brain injury over the hours and days thereafter. In both humans and animals, a key component to this secondary injury is rapid brain production and aggregation of A $\beta$  in as little as one day after TBI [98]. Since we have shown that TEMT prevents A $\beta$  aggregation over days (Fig. 4B), this therapeutic could be of immense value to quickly and effectively protect against the secondary damage incurred by TBI.

Because EMF treatment provides a “generalized” enhancement to brain mitochondrial function and neuronal activity (Fig. 1B), it could prove to be an effective therapeutic against cognitive impairment associated with cerebrovascular disease and stroke. Moreover, since Parkinson’s disease is characterized by brain mitochondrial hypofunction and brain aggregates of  $\alpha$ -synuclein protein, EMF treatment may enhance mitochondrial function and disaggregate  $\alpha$ -synuclein in PD patients to stabilize or reverse the motor dysfunction of PD. Thus, multiple forms of disease- and injury-related trauma to the brain are potentially addressable through TEMT.

### **CONCLUSION**

The adage “*Necessity is the mother of invention*” now rings true for AD therapeutic development. The universal failure of therapeutic drugs against AD as disease-modifiers to slow or alter AD pathogenesis has harbored in a novel non-pharmacologic approach to AD and perhaps to other cognitive-based neurologic conditions, namely TEMT. Indeed, a whole new and exciting field of cognitive research may have emerged with TEMT application for both cognitive insufficiency and cognitive enhancement. It would be a mistake to group TEMT with other non-invasive neuromodulatory approaches that seek to stimulate the brain, such as tDCS or TMS. Aside from their lack of deep brain penetration, neither of these approaches has the foundation of basic science/cognitive studies in AD animal models and normal animals that TEMT has—a foundation so necessary in being supportive and predictive of future efficacy in humans. Certainly,

achieving therapeutic success in AD animal models does not guarantee efficacy in human clinical trials (especially since all AD animal models are only partial models for the disease). However, pharmacologic or neuromodulatory therapeutics that have no or very limited success in animal studies are less likely to produce clinical efficacy.

As with any novel and unconventional therapeutic intervention, funding to aggressively pursue translational development of TEMT is critical and may be challenging. In that context, Big Pharma has no interest in developing a non-pharmacologic, neuromodulatory intervention against AD such as TEMT. Moreover, both federal funding agencies and cell phone companies have universally ignored the cognitive potential of TEMT. Such funding barriers may slow, but ultimately will not stop the development of TEMT intervention against AD and cognitive insufficiency in general, especially if conventional drug development against AD continues to generate failed compounds.

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