frontiers | Frontiers in Endocrinology

TYPE Mini Review
PUBLISHED 25 September 2024
DOI 10.3389/fendo.2024.1406455

OPEN ACCESS

EDITED BY
Arthur David Conigrave,
The University of Sydney, Australia

REVIEWED BY
Enikö Kallay,
Medical University of Vienna, Austria
Kunjbihari Sulakhiya,
Indira Gandhi National Tribal University, India

*CORRESPONDENCE
Mohammed S. Razzaque
mohammed.razzaque@utrgv.edu;
msr.nagasaki@gmail.com

RECEIVED 25 March 2024
ACCEPTED 28 August 2024
PUBLISHED 25 September 2024

CITATION
Patel V, Akimbekov NS, Grant WB, Dean C, Fang X and Razzaque MS (2024) Neuroprotective effects of magnesium: implications for neuroinflammation and cognitive decline.
Front. Endocrinol. 15:1406455.
doi: 10.3389/fendo.2024.1406455

Neuroprotective effects of magnesium: implications for neuroinflammation and cognitive decline

Veer Patel[1], Nuraly S. Akimbekov[2,3], William B. Grant[4], Carolyn Dean[5], Xiaoqian Fang[6] and Mohammed S. Razzaque[1,7]*

[1]Department of Pathology, Lake Erie College of Osteopathic Medicine, Erie, PA, United States, [2]Scientific-Practical Center, West Kazakhstan Marat Ospanov Medical University, Aktobe, Kazakhstan, [3]Sustainability of Ecology and Bioresources, Al-Farabi Kazakh National University, Almaty, Kazakhstan, [4]Sunlight, Nutrition, and Health Research Center, San Francisco, CA, United States, [5]New Capstone, Inc., Mooresville, NC, United States, [6]Department of Neuroscience, School of Medicine, University of Texas Rio Grande Valley (UTRGV), Edinburg, TX, United States, [7]Department of Medical Education, School of Medicine, University of Texas Rio Grande Valley (UTRGV), Edinburg, TX, United States

Neurodegenerative diseases, which are characterized by progressive neuronal loss and cognitive decline, are a significant concern for the aging population. Neuroinflammation, a shared characteristic of these diseases, is implicated in their pathogenesis. This article briefly summarizes the role of magnesium, an essential mineral involved in numerous enzymatic reactions and critical for neuronal bioactivity, in the context of neuroinflammation and cognitive decline. The potential neuroprotective effects of magnesium, including the mechanisms of neuroprotection by magnesium through maintaining neuronal ion homeostasis, reducing inflammation, and preventing excitotoxicity, are also described. Additionally, we discuss the impact of inadequate magnesium on neuroinflammation and its potential as a therapeutic agent for attenuating cognitive decline to improve neurodegenerative conditions.

KEYWORDS

magnesium, neuroinflammation, neurodegenerative disease, cognitive decline, neuroprotection

Introduction

As the global population ages, neurodegenerative diseases, which are characterized by an ongoing loss of neuron structure and function, are becoming increasingly public health burdens. These disorders, including dementia (along with vascular dementia), amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Parkinson's disease, and Huntington's disease, often result in cognitive decline, which severely impacts the quality of life of affected individuals. The insidious nature of these diseases and the lack of curative interventions highlight the need for novel therapeutic strategies. The global prevalence of dementia,

primarily Alzheimer's disease, is expected to double every 20 years, reaching 81.1 million by 2040 (1, 2). Similarly, Parkinson's disease, the second most common neurodegenerative disorder, affects 2-3% of the population aged ≥65 years (3, 4). These statistics highlight the escalating public health challenge of neurodegenerative diseases.

Neuroinflammation, a common feature of neurodegenerative diseases, is recognized as a critical player in the pathogenesis of these disorders (5, 6). The inflammatory response in the brain is a double-edged sword. Whereas acute inflammation can be beneficial for neuronal repair and recovery, chronic inflammation can lead to persistent neuronal damage and eventually to neurodegeneration (7). Inflammatory processes in the brain are primarily mediated by microglia shown in Figure 1. Upon activation, microglia release proinflammatory cytokines, including interleukin 1β (IL-1β), tumor necrosis factor -α (TNF-α), IL-6, IL-18, and IL-12, reactive oxygen species (ROS), and other neurotoxic substances, including nitric oxide, glutamate, and prostaglandins, as well as enzymes such as matrix metalloproteinases (MMPs) (7). Given the critical role of neuroinflammation in neurodegeneration, modulating the inflammatory response could reduce disease progression and is likely to improve clinical outcomes.

We conducted a literature search using PubMed, Google Scholar, and Scopus databases. The search was performed using the keywords: "neuroinflammation", "magnesium", "cognitive function" and "neurodegenerative diseases". We included peer-reviewed articles in English published between 2000 and 2023.

Neuroinflammation and neurodegeneration

Neuroinflammation is partly mediated by the activation of glial cells and the release of proinflammatory mediators in the brain (8). It plays a crucial role in the pathogenesis of neurodegenerative diseases.

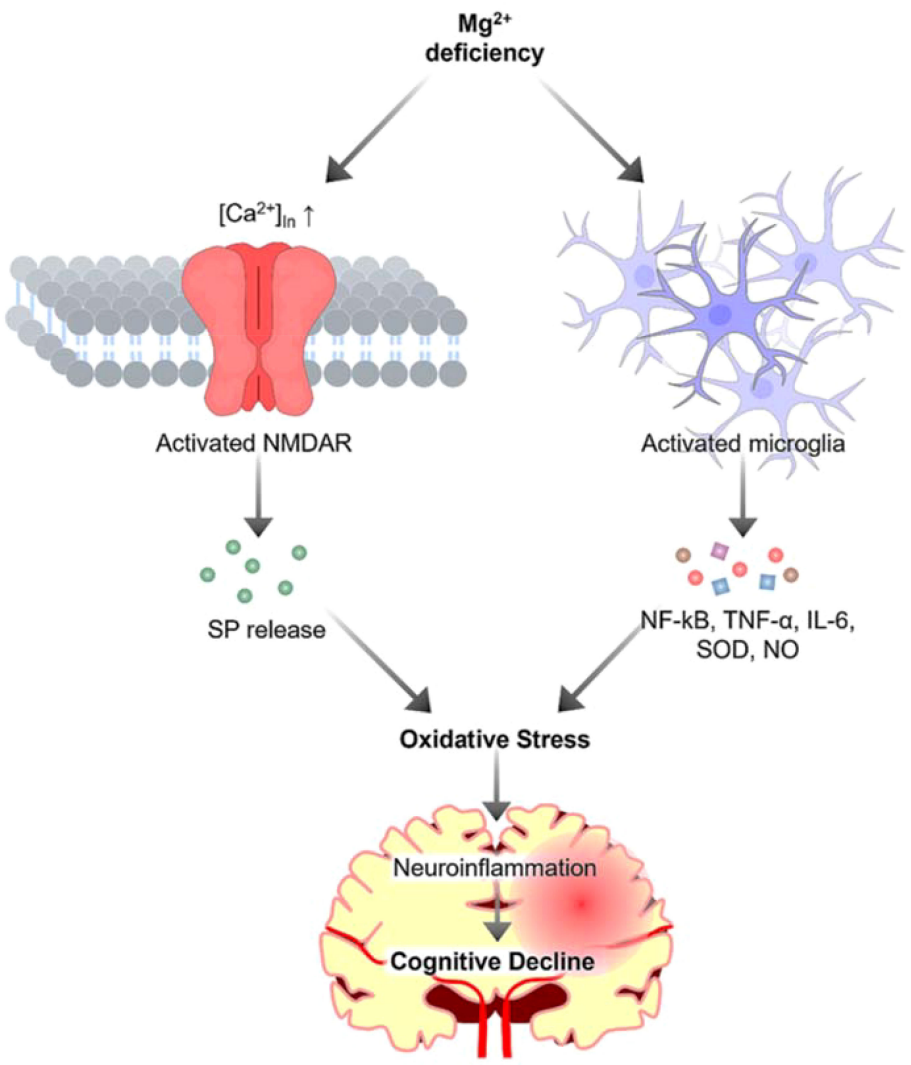

FIGURE 1
The role of magnesium in neuroinflammation. Magnesium deficiency activates microglia, resulting in the release of proinflammatory cytokines and toxic substances, which contribute to oxidative stress. Additionally, magnesium deficiency triggers calcium influx, inducing the release of substance P (SP), further exacerbating oxidative stress to increase neuroinflammation and ultimately contributes to cognitive decline.

Magnesium has been shown to modulate neuroinflammation (9, 10). It is recognized for its diverse roles in maintaining human health, specifically in modulating inflammatory signaling pathways within the neurological landscape. Magnesium plays a crucial role in over 600 enzymatic reactions in the human body (11). According to Workinger, "Magnesium is a critical mineral in the human body and is involved in ~80% of known metabolic functions" (12). The concentrations of magnesium in serum and cerebrospinal fluid (CSF) are regulated to maintain normal physiological function. Normal serum magnesium levels typically range from 0.75 to 0.95 mmol/L (13), while in CSF, they range between 0.77 and 1.17 mmol/L (14). Magnesium levels are generally higher in CSF as compared to the serum levels, perhaps due to the active transport of magnesium across the blood-brain barrier (15); the blood-brain barrier and the choroid plexus help regulate magnesium levels in the CSF. In magnesium deficiency state, CSF concentrations decline, although such reduction lags behind and is usually less pronounced than the changes noted in plasma levels of magnesium (15). Serum magnesium levels are crucial for neuromuscular function, enzyme activity, and bone structure (16). Magnesium in CSF plays a vital role in supporting various functions of the central nervous system. Decreased CSF magnesium levels correspond with reduced concentrations of extracellular brain magnesium and have been associated with epilepsy (14). Additionally, magnesium is well known for its implication in multiple neurological disorders (17). For instance, magnesium sulfate supplementation has been associated with reduced neuroinflammation in a rat model of Alzheimer's disease (10). Studies involving animal models suggest that magnesium deficiency may trigger greater recruitment of phagocytic cells (18). These cells could lead to generation of more ROS, leading to the production of various cytokines, such as TNF-α, which are key players in the inflammatory response (18). In Alzheimer's disease, neuroinflammation is a pathological feature exacerbated by the accumulation of amyloid-beta plaques through the activation of inflammatory proteins including IL-1, IL-6, and TNF-α. Interestingly, magnesium supplementation has been shown to reduce the levels of these proinflammatory cytokines and increase the levels of anti-inflammatory mediators in the hippocampus of a rat model of Alzheimer's disease, suggesting modulation of an inflammatory responses (19). However, due to the complexity of the immune system in the brain, with the involvement of microglia, astrocytes, and various cytokines and chemokines, dampening inflammation alone might not be sufficient. Chronic neuroinflammation results in an adverse cascade of events, causing neuronal damage, disrupting synaptic functionality, and leading to cognitive impairment. When this inflammatory response is sustained, it results in the overproduction of proinflammatory cytokines. This hyperreactive state disrupts the delicate balance of synaptic plasticity (the ability of synapses to strengthen or weaken over time) thereby diminishing key cognitive functions like memory retention and learning (20).

Furthermore, prolonged inflammation triggers oxidative stress, wherein excess free radicals lead to neurotoxicity and cellular damage (9). This accelerates the progression of neurodegenerative processes, as observed in diseases such as Alzheimer's disease and Parkinson's disease, which are characterized by the accumulation of disease-specific proteins in the brain, amyloid-beta and alpha-synuclein, respectively (21). Additionally, inflammation-induced oxidative stress and resultant neuronal damage have been identified as significant contributors to cognitive decline following traumatic brain injury. These findings illustrate the detrimental link between chronic neuroinflammation and cognitive decline.

Magnesium deficiency syndromes

Hypomagnesemia (typically below 0.61 mmol/L) can cause a wide range of disorders and has significant neurological consequences. The causes of hypomagnesemia can be related to gastrointestinal disorders, including chronic diarrhea, malabsorption syndromes (e.g., celiac disease, inflammatory bowel disease), chronic pancreatitis, and excessive vomiting. Similarly, renal disorders, including tubular dysfunction, diabetic nephropathy (leading to increased urinary magnesium loss), and the use of certain medications (e.g., diuretics, proton pump inhibitors, and some antibiotics), can cause hypomagnesemia. Alcoholism, severe burns, chronic stress, hyperaldosteronism, and prolonged parenteral fluid administration without magnesium supplementation can also lead to hypomagnesemia. Magnesium plays a key role in neural function, and its inadequacy can lead to various neurological symptoms and complications, including neuromuscular hyperexcitability, muscle twitches and cramps, tremors, and seizures. Of clinical importance, the severity of neurological symptoms often correlates with the severity of magnesium deficiency. Patients with mild hypomagnesia (below 0.61 mmol/L) may cause subtle symptoms, while severe hypomagnesia (below 0.49 mmol/L) can lead to more pronounced neurological manifestations. Severe magnesium deficiency syndromes can be associated with cognitive and mood disturbances, headaches, migraines, and neuropathy (numbness and tingling sensations, particularly in the extremities). The long-term complications of severe magnesium deficiency have also been linked to nystagmus (involuntary eye movements) and neurodegenerative diseases, possibly mediated by neuroinflammation. It is essential to maintain an optimal balance of magnesium, along with other minerals and vitamins, throughout life to support normal physiologic functions, including neurological health (22–25).

Role of magnesium in neuroinflammation

In the nervous system, magnesium is essential for maintaining neuronal ion homeostasis, modulating synaptic plasticity, and regulating neurotransmitter release (26). Kang et al. highlighted the integral role of this mineral in managing the activity of N-methyl-D-aspartate (NMDA) receptors (27). Their findings emphasize the significance of this interaction in maintaining the balance of glutamate, an excitatory neurotransmitter. If left unchecked, glutamate can potentially tip the scale toward inflammation. Kramer et al. suggested the aftereffects of magnesium deficiency (28). According to their findings, insufficient magnesium can trigger an increase in substance P, a neuropeptide that propagates inflammatory pain (28).

Other researchers have highlighted the complex interplay between magnesium and calcium within neurons (9). By restraining calcium influx into neurons, magnesium helps prevent events that could otherwise lead to intensified inflammation and neuronal injury. Whereas low levels of this mineral are associated with chronic inflammation, restoring magnesium balance has been shown to potentially counteract this condition (29). Apart from managing neurotransmitter activity, magnesium has been found to play a crucial role in modulating immune responses, particularly by interacting with nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (19). This research provides compelling evidence of the role of magnesium as an NF-κB inhibitor, a transcription factor that regulates the expression of pro-inflammatory cytokines, including TNF-α and IL-6 (19). By inhibiting NF-κB activation, magnesium can dampen the resultant proinflammatory gene expression, thereby reducing the overall inflammatory response within the brain (19). A meta-analysis by Veronese et al. revealed magnesium's anti-inflammatory effects, marked by reductions in plasma fibrinogen and other markers, such as tartrate-resistant acid phosphatase type 5 (TRACP 5) and tumor necrosis factor-ligand superfamily member 13B (TNFSF13B) (30). Additionally, it was also noticed that ST2 protein and IL-1 levels went down. However, the study revealed no significant changes in IL-6 or total antioxidant capacity levels, indicating a selective impact of magnesium on various inflammatory markers (30). Of clinical significance, measuring circulating ionized magnesium appears to be a more accurate indicator of magnesium supplement bioavailability compared to assessing total magnesium levels in plasma (31). Although the role of magnesium in regulating neurotransmission and immune responses is well established, it also plays a crucial role in maintaining brain health by acting as an antioxidant. Research findings suggest that magnesium may contribute to neutralizing ROS to delay the progression of neurodegenerative disorders (32).

Although magnesium is not considered a component of the antioxidant defense system, research indicates that magnesium deficiency may increase oxidative stress markers. These markers encompass oxidative modification products of lipids, proteins, and DNA. Furthermore, a significant association was observed between magnesium deficiency and weakened antioxidant defense mechanisms. This relationship between magnesium deficiency and oxidative stress involves multifaceted mechanisms at both the systemic and cellular levels, including inflammation, endothelial dysfunction, mitochondrial dysfunction, and excessive fatty acid production (32). The studies suggest that magnesium may possess inherent antioxidant properties, although not as a conventional antioxidant molecule such as vitamin C or vitamin E. One notable mechanism highlighted is magnesium's role in stabilizing the critical antioxidant enzyme superoxide dismutase (SOD) (32). SOD substantially mitigates oxidative damage by converting harmful superoxide radicals into less reactive molecular species. This stabilization of SOD by magnesium provides a unique and essential link between magnesium and the antioxidant defense system (32).

Magnesium and neuroprotection

Neuroprotective agents are substances that can potentially preserve neuronal structure and function. These substances help prevent or slow the progression of neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. These agents work through various mechanisms, including reducing neuroinflammation, shielding against oxidative stress, and modulating neurotransmission (33).

Many preclinical and clinical studies have suggested the potential of magnesium as a neuroprotective agent (Figure 2). Magnesium is present both intracellularly and extracellularly, with its intracellular presence in compartments such as the nuclei, mitochondria, and endoplasmic reticulum being crucial for central nervous system functions, including synaptic connectivity (34). Intracellular magnesium can modify synaptic properties, influencing various neuronal processes. For instance, recent research by Liu's group reported that presynaptic intracellular magnesium plays a crucial role in mediating the transition between two synaptic configurations: one involved in information encoding and learning, and the other in information storage and memorization (35). Research has demonstrated that magnesium can enhance cognitive function and synaptic plasticity in animal models of Alzheimer's disease, offering optimism for addressing cognitive decline (10). Additionally, a study conducted on a rat model of Alzheimer's disease demonstrated that magnesium sulfate supplementation improved cognitive function, synaptic plasticity, and dendritic spine morphology (10). Moreover, intracellular magnesium levels have been shown to correlate with Parkinson's disease activity. In 1-methyl-4-phenylpyridinium (MPP+) model of Parkinson's disease, the application of MPP+ induced an increase in intracellular magnesium concentration, which inhibited cellular ROS production, maintained ATP generation, and preserved cell viability, thereby protecting neurons from MPP+ toxicity (36). In demyelination rat models, a mutation in the mitochondrial magnesium uptake gene disrupted magnesium homeostasis in oligodendrocytes, affecting ATP production and leading to axonal demyelination (37). Besides supporting myelin formation, intracellular magnesium also enhanced oligodendrocytes' tolerance against cellular stress, increasing resistance to a hypoxic-ischemic injury (38). Although preclinical studies suggest that magnesium has potential neuroprotective effects, translating these findings to humans presents numerous challenges. Differences in metabolism, blood–brain barrier permeability, and magnesium bioavailability between humans and animal models may affect its efficacy in clinical settings. Additionally, the optimal dosage, duration of treatment, and form of magnesium (e.g., magnesium sulfate, magnesium citrate, etc.) that are both effective and safe for humans require rigorous clinical trials. A gap exists between demonstrating neuroprotection under controlled laboratory conditions and achieving measurable, meaningful outcomes in diverse human populations with varying stages of neurological conditions. Supplementation with magnesium sulfate increased brain magnesium contents and attenuated memory deficits induced by intracerebroventricular administration of streptozocin (ICV-STZ). Furthermore, magnesium reduces tau hyperphosphorylation, a hallmark of Alzheimer's disease, and modulates the PI3K/Akt signaling pathway (10). Additionally,

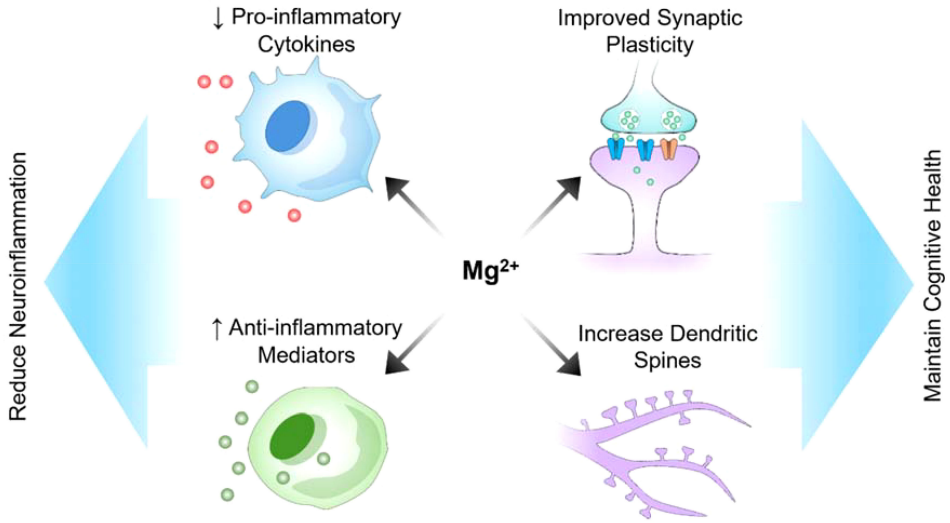

FIGURE 2
Potential role of magnesium in reducing neuroinflammation and maintaining cognitive health.

magnesium supplementation has been associated with improved neurological outcomes in models of acute brain injury, demonstrating its relevance in central nervous system injuries (39). Moreover, in an experimental setting involving a rat model of sciatic nerve injury, a diet rich in magnesium was found to stimulate neurological function recovery and enhance nerve regeneration, revealing its potential in the treatment of peripheral nerve disorders (39). The neuroprotective effects of magnesium are believed to stem from its capacity to regulate neuronal calcium homeostasis, thus reducing excitotoxicity, and its ability to modulate neuroinflammatory processes (10). The mechanisms by which magnesium exerts its effects (e.g., calcium homeostasis regulation, reduction in excitotoxicity, anti-inflammatory actions) suggest that its neuroprotective properties could be applicable to a wide range of neurological conditions. However, this also raises questions about specificity and targeted therapy. For instance, although reducing tau hyperphosphorylation is promising for treating Alzheimer's disease, it is unclear how these mechanisms interact in the presence of other neurodegenerative disorders or comorbidities. The multifunctional nature of magnesium might mean that its efficacy could vary greatly depending on the specific pathological context. Additionally, magnesium appears to influence nitric oxide production; nitric oxide is a molecule critical for regulating cerebral blood flow and neuronal damage.

Between 2002 and 2008, several randomized clinical trials explored the potential of magnesium sulfate for neuroprotection in preterm births and its effects on cerebral palsy (40). Although these studies did not consistently achieve statistical significance for their primary outcomes, they indicated that magnesium sulfate exposure significantly reduced the likelihood of cerebral palsy in preterm infants. A similar clinical study by Temkin et al., 2007 involving 499 participants aimed to test whether magnesium treatment favorably affects outcomes in head-injured patients (41). The results show that participants who were randomly assigned to the lower dose magnesium group performed significantly worse than those in the placebo group. Therefore, there was greater mortality with the magnesium dose than with the placebo. These findings highlight a discrepancy between preclinical expectations and clinical observations, suggesting that the magnesium infusions given to patients within 8 hours of traumatic brain injury did not have a neuroprotective effect on traumatic brain injury (41). However, other studies have claimed that intravenous magnesium infusion and hyperbaric oxygen therapy could reduce the clinical symptoms of brain injury (42–44). Therefore, additional pre-clinical and clinical research is needed to provide stronger scientific validation.

Another study investigated the combined effects of magnesium supplementation and treadmill exercise on memory deficits in aged rats (45); combined approach led to improved memory function in the aged rats. In the context of central nervous system injury, a comprehensive review highlighted the significant decrease in blood and brain (free) magnesium concentrations following both direct and indirect neurotrauma (46). A decrease in magnesium was associated with neurological deficits and oxidative stress, emphasizing the importance of magnesium homeostasis in central nervous system injury. The administration of magnesium salts, such as magnesium sulfate and magnesium chloride, increased brain (free) magnesium concentrations and improved functional outcomes (46).

The cognitive lifeline: magnesium supplementation

Research has demonstrated that magnesium supplementation can effectively increase extracellular magnesium levels, particularly in the serum, which may help inhibit the aggregation of calciprotein particles and reduce vascular calcifications, helping manage conditions such as chronic kidney disease (47). However, the effects on intracellular magnesium levels are more complex and require a long-term, consistent approach to supplementation. This slow adjustment is necessary because of the body's regulatory

mechanisms, which ensure that cellular functions remain stable and effective.

In neurological disorders such as Alzheimer's disease and Parkinson's disease, the neurodegenerative process has occurred for many years, potentially reducing the responsiveness of these disorders to the benefits of magnesium. Magnesium impacts calcium regulation and neurotransmitter functions, which are implicated in the pathophysiology of these diseases. In Parkinson's disease, abnormal magnesium levels are linked to transporter dysfunctions, suggesting that supplementation could stabilize these transport mechanisms and potentially slow disease progression (48).

Conversely, in acute neurological conditions such as stroke or traumatic brain injury, rapid onset and progression do not allow magnesium levels to be corrected in a timeframe that influences immediate outcomes. In these cases, emergency treatments focus on restoring blood flow or reducing inflammation rather than correcting metabolic imbalances. The slow cellular uptake and regulatory effects of magnesium are less practical here because the therapeutic window is very narrow, and the rapid physiological changes postinjury require immediate interventions that go beyond magnesium supplementation. Therefore, while chronic neurological disorders could benefit from sustained magnesium research owing to their slow progression, acute disorders would receive minimal benefit from such research. This is due to need for immediate and aggressive treatment in acute conditions, where the timing and rapid action are critical.

Magnesium supplementation varies significantly in form and administration, each tailored for specific clinical scenarios. Oral magnesium, available in forms such as oxide, citrate, and glycinate, is commonly used for long-term management of conditions such as cardiovascular health and migraine prophylaxis. These forms are preferred for their high bioavailability and ease of administration, making them ideal for ongoing, nonemergency supplementation. Conversely, intravenous magnesium, primarily known as magnesium sulfate, is used in emergency settings where rapid correction of magnesium levels is critical. This form is used in acute medical conditions such as severe asthma, eclampsia, or life-2threatening arrhythmias. Direct administration into the bloodstream provides an immediate therapeutic effect, which is crucial in life-saving interventions. Topical magnesium, often in the form of oils or gels, is used for local applications such as muscle soreness and cramps. While it offers the advantages of bypassing the gastrointestinal system and avoiding some side effects associated with oral forms, its systemic absorption and overall efficacy are less documented.

The relationship between magnesium intake and cognitive function is a promising research area. A study from the National Health and Nutrition Examination Survey (NHANES) 2011 to 2014 investigated the associations of vitamin D status and magnesium with cognitive status in older adults (49). The study found that higher serum 25-hydroxyvitamin D [25(OH)D] levels, linked with magnesium metabolism, were associated with reduced risk of declining cognitive function. Specifically, an inverse association of higher serum 25(OH)D levels with cognitive function was observed primarily among participants with a daily total magnesium intake of <254 mg or ≤375 mg. Essential roles of magnesium in the activation of vitamin D has been explained in various research publications (49–53). The associations between serum 25(OH)D and risk of mortality may be modified by the intake level of magnesium (49). Nevertheless, some studies reportered that there were no clear associations for cognitive function with overall magnesium intake (54). Although not directly focused on magnesium, research has highlighted the potential cognitive benefits of other dietary components. For instance, a study conducted in Qatar revealed that habitual consumption of nuts (almonds, cashews, Brazil nuts, and walnuts), which are rich in magnesium, is positively associated with cognitive function, especially among older adults (55).

Furthermore, a multicenter study of hemodialysis patients revealed a U-shaped association between serum magnesium levels and mild cognitive impairment. Both lower and higher serum magnesium levels were observed to increase the risk of mild cognitive impairment in this specific population. The optimal range of magnesium levels for the lowest risk of mild cognitive impairment was identified as 1.12–1.24 mmol/L (56). This discrepancy suggests that while the current reference range for serum magnesium (0.75–0.95 mmol/L) may be adequate for typical physiological functions, higher levels would be necessary for optimal cognitive health. This indicates that standard ranges might not fully address the specific needs of the brain and neurological health. Therefore, maintaining serum magnesium levels at the higher end of the range could provide potential neuroprotective benefits. The empirical data from specialized populations like hemodialysis patients delineate magnesium's potential as a cognitive lifeline. The observed associations between magnesium levels and cognitive outcomes highlight the significance of this mineral and raise questions about optimal intake levels for cognitive preservation.

Magnesium glycinate, known for its high bioavailability, ensures that magnesium is efficiently absorbed into the bloodstream and, consequently, available to the body and brain (57). Although direct studies on the impact of magnesium glycinate on cognitive function are limited, its role in enhancing sleep quality and reducing anxiety could indirectly support cognitive health by promoting restorative sleep and lowering stress levels, both of which are beneficial for cognitive performance and neuroprotection (58). Magnesium L-threonate has been specifically studied for its unique ability to increase magnesium concentrations in the brain, thus directly influencing cognitive functions. Rats supplemented with magnesium L-threonate showed a significant increase in synaptic density in regions of the brain associated with memory and learning, translating to a 15% improvement in maze navigation tasks compared to controls. This study demonstrated that this form of magnesium could reverse certain aspects of brain aging and improve synaptic density, suggesting that magnesium has promising implications for delaying and treating cognitive decline associated with aging and neurodegenerative diseases (59).

The existing body of research underscores the need for more rigorous, long-term clinical trials to provide conclusive evidence. A study by Nosheny et al. emphasized the role of dyadic cognitive reports and subjective cognitive decline in early Alzheimer's disease

research and trials (60). Although this study did not focus on magnesium directly, it highlighted the importance of long-term monitoring and the complexities in data interpretation, suggesting that similar rigorous methodologies should be applied to studies on magnesium. Furthermore, research by Planche et al. on brain atrophy subtypes during aging indicated that certain atrophy patterns might predict long-term cognitive decline and future Alzheimer's disease (61).

Conclusion

The role of magnesium in cognitive health and neuroprotection is both compelling and complex, a testament to the sophisticated nature of the nervous system and its interplay with essential nutrients. Research has revealed that magnesium is a critical player in maintaining and regulating neurobiological behaviors. In fact, its ability to mediate inflammatory signaling pathways and inhibit the activation of NF-κB provides a basis for its potent anti-inflammatory effects. By reducing oxidative burden and inflammation (two phenomena significantly contributing to cognitive decline), magnesium helps to preserve neuronal integrity. Epidemiological and clinical research consistently stresses the importance of adequate magnesium levels for improving cognitive health. Studies have shown a direct correlation between magnesium intake and cognitive function in healthy individuals. Although existing studies have laid a substantial foundation, they also highlight the need for further in-depth research, including more comprehensive, long-term clinical trials to determine the therapeutic potency of magnesium in improving cognitive health to provide safe and compassionate patient care (62), to reduce the burden of neurodegenerative diseases.

Author contributions

MR: Conceptualization, Supervision, Writing – review & editing. VP: Writing – original draft. NA: Visualization, Writing – review & editing. WG: Writing – review & editing. CD: Writing – review & editing. XF: Visualization, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors thank Dr. Peace Uwambaye for providing useful suggestions. Additional information has been collected from online sources, including ChatGPT and Google Scholar. VP is a former student of Master of Medical Science at the Lake Erie College of Osteopathic Medicine (LECOM), Erie (USA).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. GBD 2019 Dementia Forecasting Collaborators. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *Lancet Public Health*. (2022) 7: e105–25.

2. Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, et al. Global prevalence of dementia: A delphi consensus study. *Lancet*. (2005) 366:2112–7.

3. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, et al. Parkinson disease. *Nat Rev Dis Primers*. (2017) 3:17013.

4. Willis AW, Roberts E, Beck JC, Fiske B, Ross W, Savica R, et al. Incidence of parkinson disease in North America. *NPJ Parkinsons Dis*. (2022) 8:170.

5. Risen SJ, Boland SW, Sharma S, Weisman GM, Shirley PM, Latham AS, et al. Targeting neuroinflammation by pharmacologic downregulation of inflammatory pathways is neuroprotective in protein misfolding disorders. *ACS Chem Neurosci*. (2024) 5:1533–47.

6. Wyss-Coray T, Mucke L. Inflammation in neurodegenerative disease–a double-edged sword. *Neuron*. (2002) 35:419–32.

7. Block ML, Zecca L, Hong J-S. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci*. (2007) 8:57–69.

8. Yang QQ, Zhou JW. Neuroinflammation in the central nervous system: Symphony of glial cells. *Glia*. (2019) 67:1017–35.

9. Lingam I, Robertson NJ. Magnesium as a neuroprotective agent: A review of its use in the fetus, term infant with neonatal encephalopathy, and the adult stroke patient. *Dev Neurosci*. (2018) 40:1–12.

10. Xu Z-P, Li L, Bao J, Wang Z-H, Zeng J, Liu E-J, Li X-G, et al. Magnesium protects cognitive functions and synaptic plasticity in streptozotocin-induced sporadic alzheimer's model. *PLoS One*. (2014) 9:e108645.

11. Gerry K. Schwalfenberg SJG. The importance of magnesium in clinical healthcare. *Scientifica*. (2017) 2017:4179326.

12. Workinger JL, Doyle RP, Bortz J. Challenges in the diagnosis of magnesium status. *Nutrients*. (2018) 10:1202.

13. Elin RJ. Assessment of magnesium status for diagnosis and therapy. *Magnes Res*. (2010) 23:S194–198.

14. Haensch CA. Cerebrospinal fluid magnesium level in different neurological disorders. *Neurosci Med*. (2010) 1:60–3.

15. Morris ME. Brain and CSF magnesium concentrations during magnesium deficit in animals and humans: neurological symptoms. *Magnes Res*. (1992) 5:303–13.

16. Fiorentini D, Cappadone C, Farruggia G, Prata C. Magnesium: biochemistry, nutrition, detection, and social impact of diseases linked to its deficiency. *Nutrients*. (2021) 13:1136.

17. Kirkland AE, Sarlo GL, Holton KF. The role of magnesium in neurological disorders. *Nutrients*. (2018) 10:730.

18. Patrycja L, Wojciech N, Edmond R, Yves R, Andrzej M. Phagocyte priming by low magnesium status: input to the enhanced inflammatory and oxidative stress responses. *Magnesium Res*. (2010) 23:1–4.

19. Sugimoto J, Romani AM, Valentin-Torres AM, Luciano AA, Ramirez Kitchen CM, Funderburg N, et al. Magnesium decreases inflammatory cytokine production: A novel innate immunomodulatory mechanism. *Am Assoc Immunologists*. (2012) 188:6338–46.

20. Citri A, Malenka RC. Synaptic plasticity: multiple forms, functions, and mechanisms. *Neuropsychopharmacology*. (2008) 33:18–41.

21. Irwin DJ, Lee VMY, Trojanowski JQ. Parkinson's disease dementia: convergence of α-synuclein, tau and amyloid-β pathologies. *Nat Rev Neurosci*. (2013) 14:626–36.

22. Akimbekov NS, Coban SO, Atfi A, Razzaque MS. The role of magnesium in pancreatic beta-cell function and homeostasis. *Front Nutr*. (2024) 11. doi: 10.3389/fnut.2024.1458700

23. Ahmad R, Shaju R, Atfi A, Razzaque MS. Zinc and diabetes: A connection between micronutrient and metabolism. *Cells*. (2024) 13:1359.

24. Anne Marie U, Murererehe J, Rehman M, Chittilla M, Uwambaye P, Razzaque MS. Oral manifestations of iron imbalance. *Front Nutr*. (2023) 10:1272902.

25. Acquaviva J, Abdelhady HG, Razzaque MS. Phosphate dysregulation and neurocognitive sequelae. *Adv Exp Med Biol*. (2022) 1362:151–60.

26. Maier JAM LL, Fedele G, Cazzaniga A, Mazur A. Magnesium and the brain: A focus on neuroinflammation and neurodegeneration. *Int J Mol Sci*. (2023) 24:233. Magnesium.

27. Kang SW, Choi S-K, Park E, Chae S-J, Choi S, Jin Joo H, et al. Neuroprotective effects of magnesium-sulfate on ischemic injury mediated by modulating the release of glutamate and reduced of hyperreperfusion. *Brain Res*. (2011) 1371:121–8.

28. Kramer JH, Mak IT, Chmielinska JJ, Spurney CF, Phillips TM, Weglicki WB. Experimental hypomagnesemia induces neurogenic inflammation and cardiac dysfunction. *Hearts*. (2020) 1:99–116.

29. Kostov K HL. Role of magnesium deficiency in promoting atherosclerosis, endothelial dysfunction, and arterial stiffening as risk factors for hypertension. *Int J Mol Sci*. (2018) 19:1724.

30. Veronese N, Pizzol D, Smith L, Dominguez LJ, Barbagallo M. Effect of magnesium supplementation on inflammatory parameters: A meta-analysis of randomized controlled trials. *Nutrients*. (2022) 14:679.

31. Zhan J, Wallace TC, Butts SJ, Cao S, Ansu V, Spence LA, et al. Circulating ionized magnesium as a measure of supplement bioavailability: results from a pilot study for randomized clinical trial. *Nutrients*. (2020) 12:1245.

32. Zheltova AA, Kharitonova MV, Iezhitsa IN, Spasov AA. Magnesium deficiency and oxidative stress: an update. *BioMedicine*. (2016) 6:20.

33. Karvandi MS, Sheikhzadeh Hesari F, Aref AR, Mahdavi M. The neuroprotective effects of targeting key factors of neuronal cell death in neurodegenerative diseases: The role of ER stress, oxidative stress, and neuroinflammation. *Front Cell Neurosci*. (2023) 17:1105247.

34. Romani A. Regulation of magnesium homeostasis and transport in mammalian cells. *Arch Biochem Biophys*. (2007) 458:90–102.

35. Zhou H, Bi GQ, Liu G. Intracellular magnesium optimizes transmission efficiency and plasticity of hippocampal synapses by reconfiguring their connectivity. *Nat Commun*. (2024) 15:3406.

36. Shindo Y, Yamanaka R, Suzuki K, Hotta K, Oka K. Intracellular magnesium level determines cell viability in the MPP(+) model of Parkinson's disease. *Biochim Biophys Acta*. (2015) 1853:3182–91.

37. Kuramoto T, Kuwamura M, Tokuda S, Izawa T, Nakane Y, Kitada K, et al. A mutation in the gene encoding mitochondrial Mg^2+ channel MRS2 results in demyelination in the rat. *PLoS Genet*. (2011) 7:e1001262.

38. Itoh K, Maki T, Shindo A, Egawa N, Liang AC, Itoh N, et al. Magnesium sulfate protects oligodendrocyte lineage cells in a rat cell-culture model of hypoxic-ischemic injury. *Neurosci Res*. (2016) 106:66–9.

39. Pan HC, Sheu ML, Su HL, Chen YJ, Chen CJ, Yang DY, et al. Magnesium supplement promotes sciatic nerve regeneration and down-regulates inflammatory response. *Magnes Res*. (2011) 24:54–70.

40. Jameson RA, Bernstein HB. Magnesium sulfate and novel therapies to promote neuroprotection. *Clinics Perinatology*. (2019) 46:187–201.

41. Temkin NR, Anderson GD, Winn HR, Ellenbogen RG, Britz GW, Schuster J, et al. Magnesium sulfate for neuroprotection after traumatic brain injury: a randomised controlled trial. *Lancet Neurol*. (2007) 6:29–38.

42. Gonzales-Portillo B, Lippert T, Nguyen H, Lee JY, Borlongan CV. Hyperbaric oxygen therapy: A new look on treating stroke and traumatic brain injury. *Brain Circ*. (2019) 5:101–5.

43. Sen AP, Gulati A. Use of magnesium in traumatic brain injury. *Neurotherapeutics*. (2010) 7:91–9.

44. Ruiz E, Brunette DD, Robinson EP, Tomlinson MJ, Lange J, Wieland MJ, et al. Cerebral resuscitation after cardiac arrest using hetastarch hemodilution, hyperbaric oxygenation and magnesium ion. *Resuscitation*. (1986) 14:213–23.

45. El-Domiaty HF, El-Roghy ES, Salem HR. Combination of magnesium supplementation with treadmill exercise improves memory deficit in aged rats by enhancing hippocampal neurogenesis and plasticity: a functional and histological study. *Appl Physiology Nutrition Metab*. (2022) 47:296–308.

46. Vink R, Cernak I. Regulation of intracellular free magnesium in central nervous system injury. *FBL*. (2000) 5:656–65.

47. Silaghi CN, Ilyes T, Van Ballegooijen AJ, Craciun AM. Calciprotein particles and serum calcification propensity: hallmarks of vascular calcifications in patients with chronic kidney disease. *J Clin Med*. (2020) 9:1287.

48. Ray S. The neuroprotective potential of magnesium in Parkinson's disease. *Magnes Res*. (2024) 36:69–81.

49. Deng X, Song Y, Manson JE, Signorello LB, Zhang SM, Shrubsole MJ, et al. Magnesium, vitamin D status and mortality: results from US National Health and Nutrition Examination Survey (NHANES) 2001 to 2006 and NHANES III. *BMC Med*. (2013) 11:187.

50. Erem S, Atfi A, Razzaque MS. Anabolic effects of vitamin D and magnesium in aging bone. *J Steroid Biochem Mol Biol*. (2019) 193:105400.

51. Razzaque MS. Magnesium: are we consuming enough? *Nutrients*. (2018) 10:1863.

52. Uwitonze AM, Rahman S, Ojeh N, Grant WB, Kaur H, Haq A, et al. Oral manifestations of magnesium and vitamin D inadequacy. *J Steroid Biochem Mol Biol*. (2020) 200:105636.

53. Uwitonze AM, Razzaque MS. Role of Magnesium in vitamin D activation and function. *J Am Osteopath Assoc*. (2018) 118:181–9.

54. Peeri NC, Egan KM, Chai W, Tao MH. Association of magnesium intake and vitamin D status with cognitive function in older adults: an analysis of US National Health and Nutrition Examination Survey (NHANES) 2011 to 2014. *Eur J Nutr*. (2021) 60:465–74.

55. Nafea H AO, Qaddourah S, Abdulwahab Z, Moawad J, Shi Z. Higher habitual nuts consumption is associated with better cognitive function among Qatari adults. *Nutrients*. (2021) 12:3580.

56. Yang Y, Long Y, Yuan J, Zha Y. U-shaped association of serum magnesium with mild cognitive impairment among hemodialysis patients: a multicenter study. *Ren Fail*. (2023) 45:2231084.

57. Cinar V. The effects of magnesium supplementation on thyroid hormones of sedentars and Tae-Kwon-Do sportsperson at resting and exhaustion. *Neuro Endocrinol Lett*. (2007) 28:708–12.

58. Nielsen FH, Johnson LK, Zeng H. Magnesium supplementation improves indicators of low magnesium status and inflammatory stress in adults older than 51 years with poor quality sleep. *Magnes Res*. (2010) 23:158–68.

59. Slutsky I, Abumaria N, Wu L-J, Huang C, Zhang L, Li B, et al. Enhancement of learning and memory by elevating brain magnesium. *Neuron*. (2010) 65:165–77.

60. Nosheny RL, Amariglio R, Sikkes SAM, Van Hulle C, Bicalho MAC, Dowling NM, et al. The role of dyadic cognitive report and subjective cognitive decline in early ADRD clinical research and trials: Current knowledge, gaps, and recommendations. *Alzheimers Dement (N Y)*. (2022) 8:e12357.

61. Planche V, Coupé P, Helmer C, Le Goff M, Amieva H, Tison F, et al. Evolution of brain atrophy subtypes during aging predicts long-term cognitive decline and future Alzheimer's clinical syndrome. *Neurobiol Aging*. (2019) 79:22–9.

62. Razi MO, Fouzia R, Razzaque MS. Decline of empathy among healthcare apprentices. *Int Med Edu*. (2023) 2:232–8.

nutrients

MDPI

Article

A Magtein®, Magnesium L-Threonate, -Based Formula Improves Brain Cognitive Functions in Healthy Chinese Adults

Chengxiang Zhang [1,2], Qi Hu [1,2], Shifen Li [1,2], Feifei Dai [1,2], Wen Qian [1,2], Susan Hewlings [3], Ting Yan [1,2] and Yubang Wang [1,2,*]

[1] The Key Laboratory of Modern Toxicology, Ministry of Education, Center for Global Health, School of Public Health, Nanjing Medical University, Nanjing 210029, China
[2] Safety Assessment and Research Center for Drug, Pesticide and Veterinary Drug of Jiangsu Province, Nanjing Medical University, Nanjing 210029, China
[3] Nutrasoource 120 Research La, Guelph, ON N1G 0B4, Canada
* Correspondence: ncsa@njmu.edu.cn; Tel.: +86-25-8686-8390; Fax: +86-25-8686-8318

Abstract: Magnesium is one of the most abundant essential minerals in the body. Magnesium supplements mostly have low bioavailability, except magnesium L-threonate. In 2010, a novel magnesium compound, magnesium L-threonate (Magtein®) was identified and was shown to raise the magnesium levels in the brain and neurons effectively. In this double-blind, placebo-controlled study, Magtein®PS, a magnesium L-threonate (Magtein®)- and phosphatidylserine-based formulation additionally containing vitamins C and D, was tested for its cognitive benefits in 109 healthy Chinese adults aged 18–65 years. Subjects were randomly assigned to receive either Magtein®PS or placebo (starch) capsules, at a dose of 2 g/day. "The Clinical Memory Test", the standard test commonly used in Chinese hospitals and academic institutes for cognitive evaluation, was administered before and 30 days after subjects received the supplement. Subjects receiving Magtein®PS showed significant improvements over the control group in all five subcategories of "The Clinical Memory Test" as well as the overall memory quotient scores. The older participants showed more improvement than younger participants. Results indicated significant benefits of Magtein®PS in improving memory and cognition in healthy Chinese adults.

Keywords: magnesium L-threonate; magnesium; Magtein®; Magtein®PS; memory; cognition

Citation: Zhang, C.; Hu, Q.; Li, S.; Dai, F.; Qian, W.; Hewlings, S.; Yan, T.; Wang, Y. A Magtein®, Magnesium L-Threonate, -Based Formula Improves Brain Cognitive Functions in Healthy Chinese Adults. *Nutrients* **2022**, *14*, 5235. https://doi.org/10.3390/nu14245235

Academic Editor: Patrizia Mecocci

Received: 16 September 2022
Accepted: 30 November 2022
Published: 8 December 2022

1. Introduction

Magnesium (Mg) is the second-most abundant intracellular mineral, and it is required as a cofactor for over 300 enzymatic reactions and is, thus, necessary for the biochemical functioning of numerous metabolic pathways in the body, including energy generation in every cell, protein production, gene regulation, bone and teeth maintenance, as well as the proper functioning of the brain and nervous systems. Mg is abundant in the food supply and can be found in foods such as grains, cereals and dark leaves, including spinach and cabbage [1]. Despite an abundance in the food supply, intake of Mg in the Chinese population has been shown to be below the 330 mg/day recommended by the China Nutrition Society [2]. For example, the average intake of dietary magnesium was reported to be 205 mg/day, in 2373 adults from Guangxi, China, of both sexes [3]. Therefore, supplementation may be warranted. Mg supplementation has been shown to improve symptoms of migraine headaches, Alzheimer's disease, stroke and to have a beneficial effect on subjective anxiety in subjects prone to mental stress [4,5]. Higher intake of Mg has been associated with lower depression symptoms [6]. A relationship between Mg and anxiety has also been identified. For example, test anxiety, related to exposure to stressful exam conditions, increases urinary Mg excretion, resulting in a partial reduction in serum Mg levels [7]. A study based on data from the National Health and Nutrition Survey (NHANES) between 2011 and 2014 included 2508 participants aged 60 years and older.

After adjusting for multiple confounding variables, they found that total magnesium was independently associated with significantly higher global cognitive scores [8]. Although there is an established need for supplementation, most magnesium compounds available on the market have low bioavailability and do not lead to increased magnesium levels in the brain because they cannot cross the blood–brain barrier easily [9,10]. Thus, it is of great interest to identify a Mg supplement that can elevate brain Mg levels.

In a 2010 publication in the journal *Neuron*, scientists from the Massachusetts Institute of Technology (MIT) reported their discovery of a magnesium compound called magnesium L-threonate (Magtein®) that can effectively deliver magnesium to brain cells [11]. According to the study, magnesium L-threonate (Magtein®) has greater bioavailability compared to other magnesium supplements. Indeed, when compared to other sources of magnesium, such as chloride, citrate, glycinate and gluconate, magnesium L-threonate (Magtein®) demonstrated higher absorption and higher retention [12,13]. In addition, magnesium L-threonate (Magtein®) was able to significantly elevate magnesium concentrations (7% to 15% of initial value in 24 days) in cerebrospinal fluid in rats when administered orally, while other magnesium compounds could not [11]. The increased brain levels are most likely due to the increased absorption and the related higher circulating levels of magnesium. In humans, L-threonic acid is an ascorbic acid metabolite [14], having been identified in plasma [15], in the aqueous humor [16], in the urine [17,18] and in the brain [13]. In addition to its endogenous occurrence, L-threonic acid can be found in a wide variety of foods, such as canned mushrooms, fruit juice and processed meats [19] as a major part of vitamin C metabolites.

In a rodent model, Slutsky and colleagues reported that after one month of magnesium L-threonate (Magtein®) supplementation, the concentration of magnesium in the brain increased, and there was a significant improvement in memory and learning in both young rats and in elderly rats. In addition, magnesium L-threonate improved memory recovery in elderly rats. Magnesium L-threonate supplementation did not influence body weight, motility or the amount of water and food intake. The possible mechanisms of action of magnesium L-threonate on cognitive functions is via the activation of the NMDA receptors, which leads to increased synaptic density and improved memory [11].

Based on these preclinical results, it would be of great interest to investigate the effects of magnesium L-threonate in a human population. A randomized, double-blind, placebo-controlled study in older American adults (between ages 50 and 70) was published in 2016 [20]; supplementation with magnesium L-threonate significantly improved overall cognitive scores as compared to placebo ($p = 0.003$; Cohen's d = 0.91). Cognitive fluctuation was also reduced. Aging is associated with magnesium deficiency. A previous study has shown a beneficial effect of magnesium in an older population [21].

Phosphatidylserine (PS) is a natural component of neuronal cell membranes and is required for healthy nerve cell membranes and myelin [22]. Exogenous PS (300–800 mg/day) is absorbed efficiently and crosses the blood–brain barrier and safely slows, halts or reverses biochemical alterations and structural deterioration in nerve cells. PS also supports human cognitive functions, including short-term memory, consolidation of long-term memory, the ability to create new memories, the ability to retrieve memories, the ability to learn and recall information, the ability to focus attention and concentrate, the ability to reason and solve problems, language skills and the ability to communicate [22].

Vitamin B6 deficiency can lead to negative magnesium balance due to increased magnesium excretion [23]. Additionally, vitamin B6 helps facilitate intestinal absorption of magnesium [24]. Therefore, vitamin B6 might have synergistic effects with Mg supplement for treating magnesium deficiency [25].

Vitamin C and D have been added to magnesium L-threonate in a clinical study in patients with mild to moderate dementia, which showed significant improvements in cognition [26]. Vitamin D has been shown to promote Mg reabsorption in the kidney [27], promote Mg absorption in the GI tract [28] and low vitamin D levels have been associated with increased risk of AD [29,30]. Thus, the ingredients in the Magtein®PS-based formula

could potentially provide synergistic effects by increasing magnesium absorption, increasing brain magnesium levels and increasing cognitive functions. It is the purpose of this study to assess changes in cognitive function with the supplementation of Magtein®PS in healthy adults.

2. Materials and Methods

Magtein®PS, a magnesium L-threonate (Magtein®) and phosphatidylserine-based formula was provided by Magceutics, Inc. (Fremont, CA, USA) with the following composition:

Magtein®PS (each capsule):
Magnesium L-threonate (Magtein®): 400 mg
Vitamin D3: 80 IU
Vitamin C: 12 mg
Vitamin B6: 4 mg
Phosphatidylserine 50 mg

Magtein®PS and placebo (starch) specification: 0.504 g/capsule, recommended dose was 2 g/person/day. Daily dosage was 2 capsules in the morning and 2 capsules in the evening before sleep.

2.1. Subject Selection

This study was approved by the Ethics Committee of Safety Assessment and Research Center for Drug, Pesticide and Veterinary Drug of Jiangsu Province (NO. GZ01020150029-5). It was conducted in the Center for Health Safety of Nanjing Medical University and its affiliated hospital located in Hefei, Anhui, China. Subjects were chosen from healthy volunteers (meaning they were free of any diseases as listed below in the exclusion criteria) aged 18 to 65 years old willing to participate by signing the consent form. All subjects were without previous experience participating in similar tests randomized and matched for education level and age. Subjects were screened for basic health parameters by interview and clinical examination. Subjects who reported the following diseases were excluded: heart disease; high blood pressure ($\geq$140/90 mmHg); renal or hepatic impairment/disease; diabetes; bipolar disorder; Parkinson's disease; Alzheimer's disease; dementia; thyroid disease; affective disorder or psychiatric disorder diagnosed clinically; immune disorder (such as HIV/AIDS); diagnosed cancer. In addition, the following subjects were excluded: taking drugs containing magnesium such as magnesium sulphate and magnesium chloride; planning a pregnancy or pregnant; values of clinical laboratory examination exceeded normal values.

Comparison analyses were performed in two aspects: before/after within each group, between groups. Experimental group and control group were randomized with age, gender, education balanced. Subjects were randomized based on cognitive function (MQ) using Block randomization. Results of 102 subjects were collected at the end of trial, 51 for Magtein®PS group, and 51 for control group. Double blind was achieved by making sure the shape and weight of the placebo and active product were the same in shape and weight and deidentified. One researcher who did not interact with the subject knew the identity of the products.

2.2. Testing Methods

The commonly used clinical cognition test, "The Clinical Memory Test" (CMT), published by the Institute of Psychology of the Chinese Academy of Science in 1996, was applied to 109 healthy volunteers. "The Clinical Memory Test" (CMT) is the standard test commonly used in Chinese hospitals and academic institutes for memory and cognitive evaluation. It consists of 5 subtests: directed memory (DM), paired-association learning (PAL), free recall of pictures (FRP), recognition of meaningless figures (RMF), portrait-features memory (PFM) (28). The scaled scores from these five categories of tests, the

total score (TS), as well as the memory quotient (MQ) of each participant were recorded at baseline and 30 days after the supplementation.

Directed memory (DM) was assessed by presenting 24 words with a recorder; subjects were then required to memorize12 words from the same category as directed by the recorded guide. One set included a set of fruits and animals, and the other set included a set of vegetables and clothes. Then, there were 12 mixed words which did not need to be remembered but were close to the words that did need to be remembered. For example: The words in the fruit category that were required to be remembered were mixed with various common non-fruit words, such as duck eggs and tofu. The subjects are then asked to recall the mixed words after the second test. This is designed to assess short term memory after hearing words.

Paired-association learning (PAL) was assessed by using 6 pairs of logically connected words and 6 pairs of words without logical connection. Each pairing was tested three times in different order to assess learning, memory and logic.

Free recall of pictures (FRP) is used to assess recall memory. The test is conducted by presenting two groups, each containing 15 stimulating pictures; the pictures are images of daily necessities and other familiar objects, presented in an ordered fashion. Subjects are asked to recall the pictures.

Recognition of meaningless figures (RMF) is used to assess short term memory through vision. The target stimuli are five types of meaningless graphics, curve closed, straight line closed, curve straight line, curves not closed and straight lines not closed. Training starts with four figures from targeted stimuli group, presentation 1 s flowed by an interval of 1 s and presented in sequence; then, mixed 20 pictures of target stimuli group with 20 pictures of other groups, presented in a random order for 3 s followed by an interval of 3 s, requiring the participant to recognize the targeted stimuli group figures.

Portrait-features memory (PFM) is designed to test for more complicated memory involving several parts of the brain. Six face sketch portraits, each showing for 9 s, with an interval of 3 s, showing the "surname", "career" and "hobby" of the portrait at the same time (such as surname Wu, actor, loves swimming), presented in sequence and repeated once. Then, presenting in another order, we asked the participant to say the last name, career and hobby when presenting each portrait.

Memory quotient (MQ) is a cognition score adjusted for age and education. CMT test is the standard test used in hospitals and research institutions in China for memory and cognition abilities.

Measurements from these 5 categories can be converted into scale points. The sum of the scaled scores from these five categories is the total score (TS) of the participant. From the table of the published CMT book, based on the participant's age and educational status, memory quotient (MQ) can be found corresponding to TS of this individual.

2.3. Data Analysis

Parallel comparisons between groups were analyzed by using *t*-test of two samples' mean. Self-reference data were analyzed by using paired *t*-test. When variance was uneven, data conversion was conducted by using *t*-test and rank sum test. Effective rate and total effective rate were calculated via x^2 test. In the case that the total number of cases in the four-square table was less than 40, or the total number of cases was equal to or greater than 40, but the theoretical number was equal to or less than 1, the exact probability method was used instead.

2.4. Instruments

The following instruments were used to evaluate general health of the participants at baseline. Automatic biochemical analyzer (A25Biosysems); automatic hematology analyzer (BC-3000 Plus); automatic urine analyzer (Uritest-300); B-ultrasound set (RH-3200); X-ray machine (Imax-1500z); ECG machine (AIKD-B-12).

2.5. Safety Parameters

The following tests were measured for all participants as safety parameters: General health indicators: mental condition, sleep, diet, excretion, heart rate, blood pressure; Blood/urine test: blood test includes white blood cells, red blood cells, hemoglobin and platelets; Blood biochemical test: checking list includes serum total protein, albumin, alanine, aminotransferase, aspartate aminotransferase, urea, creatinine, cholesterol, triglycerides, blood glucose; Chest X-rays, ECG, B-ultrasound tests: only performed once at the beginning of the study.

3. Results

There were a total 109 participants enrolled in the study, including 54 subjects for the experimental group and 55 subjects for the control group. A total of 102 subjects finished this trial, 51 subjects for the experimental group and 51 subjects for the control group. Three subjects in experimental group and four subjects in control group failed to follow up due to loss of contact; they did not provide any reason for their lack of participation. The total drop-out rate was 6.42%.

3.1. Subject Characteristics

Data from 102 subjects were collected, which includes the experimental group consisting of 51 subjects, 24 male and 27 female, average age 41.04 years, and the control group consisting of 51 subjects, 19 male and 32 female, average age 42.47 years. As seen in Table 1, the differences of memory quotient (MQ), age, gender, education level between experimental group and control group was not statistically significant ($p > 0.05$).

Table 1. Baseline distribution comparison of two groups before clinical trial.

Index	Experimental Group	Control Group	p *
MQ	60.31 ± 11.35	60.75 ± 12.31	0.854
Age (years)	41.04 ± 9.41	42.47 ± 9.40	0.444
Male/Female	24/27	19/32	0.423

* p represents the comparison between experimental group and control group.

It indicates that the two groups are comparable in MQ, age, gender and education level at the beginning of the study. No abnormality was observed from chest X-rays, ECG or abdominal B-ultrasound tests from subjects in either group.

3.2. Safety Parameters between Experimental Group and Control Group

To assess the impact of Magtein®PS on recognized safety endpoints, mental condition, sleep, diet and bowel habits were monitored throughout the study and categorized as good, normal. Blood pressure and heart rate were also tested and are shown in Table 2. There were no significant differences in these parameters between those in the experimental group compared to those in the placebo group, indicating that Magtein®PS had no negative effect on the parameters evaluated.

Table 2. General comparison between two groups before and after clinical trial.

	Experimental Group							Control Group						
	Before Clinical Trial			After Clinical Trial				Before Clinical Trial			After Clinical Trial			
	Good	Normal	Bad	Good	Normal	Bad	p [1]	Good	Normal	Bad	Good	Normal	Bad	p [1]
Mental condition	50	1	0	51	0	0	1.000	51	0	0	51	0	0	1.000
Sleep condition	50	1	0	51	0	0	1.000	51	0	0	51	0	0	1.000
Appetite	50	1	0	51	0	0	1.000	51	0	0	51	0	0	1.000
Exercise	50	1	0	51	0	0	1.000	51	0	0	51	0	0	1.000

Table 2. *Cont.*

	Experimental Group							Control Group						
	Before Clinical Trial			After Clinical Trial				Before Clinical Trial			After Clinical Trial			
	Good	Normal	Bad	Good	Normal	Bad	p [1]	Good	Normal	Bad	Good	Normal	Bad	p [1]
Systolic blood pressure (mmHg)	120.00 ± 8.60			119.31 ± 7.36			0.184	119.65 ± 7.40			119.65 ± 7.40			0.124
Diastolic blood pressure (mmHg)	76.24 ± 3.94			76.25 ± 3.82			0.957	76.55 ± 3.60			76.55 ± 3.60			0.203
Heart rate	69.39 ± 9.69			70.37 ± 7.56			0.070	71.20 ± 9.87			70.82 ± 9.87			0.583

p [1] represents the self-comparison before and after clinical trial.

As shown in Table 3, the blood biochemical levels of participants in both groups were in the normal range before and after the study. Urine tests from both groups were also normal before and after the study. Therefore, Magtein®PS had no negative effects on blood or urine biochemical parameters.

Table 3. The effect of Magtein®PS on levels of human blood and urine biochemical parameters.

Index	Before Clinical Trial			After Clinical Trial		
	Experimental Group	Control Group	p	Experimental Group	Control Group	p
Leukocyte (10^9/L)	6.15 ± 1.73	5.90 ± 1.17	0.389	6.25 ± 1.42	5.87 ± 1.46	0.180
RBC (10^{12}/L)	4.56 ± 0.6	4.38 ± 0.44	0.084	4.62 ± 0.53	4.46 ± 0.46	0.110
Platelet (10^9/L)	176.2 ± 48.65	176.2 ± 48.21	1.000	188.61 ± 50.6	200.22 ± 46.34	0.230
Hemoglobin (g/L)	136.27 ± 17.79	130.9 ± 17.93	0.132	135.94 ± 16.57	130.59 ± 19.36	0.137
Total protein (g/L)	71.70 ± 3.82	71.05 ± 3.09	0.352	74.54 ± 5.38	75.35 ± 3.78	0.384
Albumin (U/L)	46.38 ± 2.46	45.39 ± 2.29	0.067	48.15 ± 4.67	48.51 ± 2.53	0.627
Alanine Aminotransferase (U/L)	20.68 ± 15.00	23.7 ± 25.11	0.464	20.1 ± 12.92	24.00 ± 27.38	0.359
Aspartate transaminase (U/L)	19.39 ± 5.61	20.2 ± 12.23	0.671	19.04 ± 4.97	20.88 ± 12.66	0.335
Urea (mmol/L)	5.6 ± 1.6	5.31 ± 1.32	0.325	5.03 ± 1.36	4.90 ± 1.38	0.624
Creatinine (umol/L)	62.73 ± 11.16	59.61 ± 14.62	0.229	72.78 ± 13.80	68.8 ± 15.9	0.180
Blood sugar (mmol/L)	5.68 ± 0.5	6.08 ± 1.68	0.101	4.72 ± 0.57	5.17 ± 1.23	0.071
Cholesterol (mmol/L)	5.37 ± 0.69	5.18 ± 0.88	0.213	4.67 ± 0.85	4.53 ± 0.88	0.408
Triglyceride (mmol/L)	1.55 ± 1.83	1.31 ± 0.71	0.385	1.61 ± 1.45	1.63 ± 1.85	0.953
Urine test	Normal	Normal		Normal	Normal	

p represents the self-comparison before and after clinical trial.

Thus, within the parameters assessed in this study it can be concluded that Magtein®PS is safe for humans, at least in this study.

3.3. Cognitive Parameter between Experimental Group and Control Group

3.3.1. Between-Group Analysis

As seen in Table 4, after 30 days, the average score for directed memory (DM), paired-association learning (PAL), free recall of pictures (FRP), recognition of meaningless figures (RMF), portrait-features memory (PFM) and memory quotient (MQ) in the Magtein®PS group was significantly higher compared to the placebo group ($p < 0.001$).

Table 4. Effects of Magtein PS and placebo on CMT Scores and MQ score by treatment.

CMT Item	Period	Magtein® PS		Placebo		*p*-Value [b]
		Mcan ± SD	*p*-Value [a]	Mcan ± SD	*p*-Value [a]	
DM	Day 0 Day 30	10.69 ± 4.98 17.20 ± 4.26	<0.001	10.47 ± 5.00 10.98 ± 3.94	0.222	0.828 <0.001
PAL	Day 0 Day 30	8.37 ± 3.26 12.37 ± 2.61	<0.001	8.27 ± 3.56 7.7 ± 2.95	0.263	0.885 <0.001
FRP	Day 0 Day 30	11.47 ± 5.04 16.65 ± 3.07	<0.001	11.39 ± 5.11 10.75 ± 4.46	0.073	0.938 <0.001
RMF	Day 0 Day 30	11.73 ± 4.61 17.88 ± 2.73	<0.001	12.16 ± 3.54 12.24 ± 1.54	0.840	0.597 <0.001
PFM	Day 0 Day 30	9.82 ± 5.08 13.75 ± 2.42	<0.001	9.76 ± 5.11 9.20 ± 4.97	0.017	0.954 <0.001
MQ	Day 0 Day 30	60.31 ± 11.35 81.84 ± 7.18	<0.001	60.75 ± 12.31 61.73 ± 10.27	0.206	0.854 <0.001

Notes: [a] *p*-value comparison within the two study groups, [b] *p*-value comparison of changes from baseline between the two study groups. Abbreviations: CMT: Clinical Memory Test; DM: directed memory; PAL: paired-association learning; FRP: free recall of pictures; RMF: recognition of meaningless figures; PFM: portrait features memory; MQ: memory quotient.

3.3.2. Within Group Analysis

Within study comparison revealed that the average score of the Magtein®PS group after the trial was significantly higher than before the trial for all clinical memory tests ($p < 0.001$). There was no significant improvement of the average score for any clinical memory test in the placebo group before and after the trial ($p > 0.05$). Moreover, for PFM, the score was significantly worse after the trial when compared to before the trial in the placebo group ($p = 0.017$). Figure 1 summarizes the overall results on all clinical memory tests, including the 5 subcategories as well as the overall MQ scores.

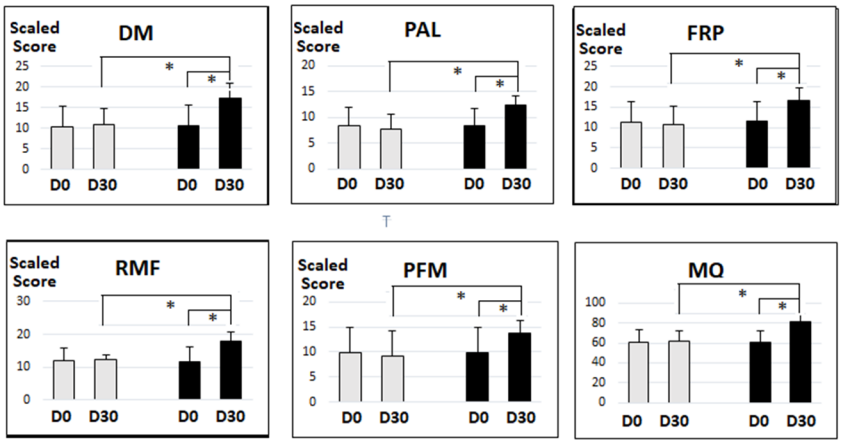

Figure 1. The significant impact of Magtein®PS on cognition. *: *p* value ($p < 0.001$), Grey box: placebo; black box: Magtein®PS. D0 = Day 0, D30 = Day 30.

3.4. Benefit of Magtein®PS on the Cognitive Function Increases with Age

A further analysis was conducted to assess any potential age-related differences in this study. As seen in Figure 2, Magtein®PS supplementation led to improved MQ in all age groups, and the impact is positively associated with age ($p < 0.001$): the older the participants, the higher the improvement from Magtein®PS intake.

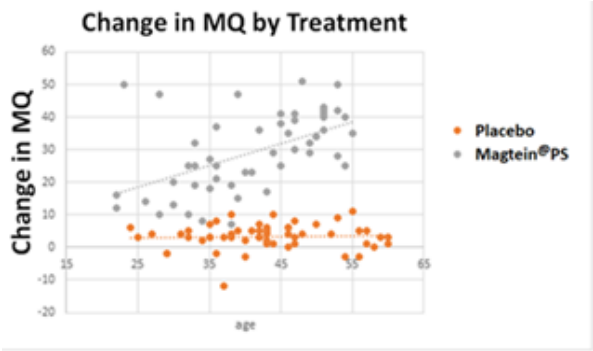

Figure 2. The effect of Magtein®PS on MQ increases with age *p* value ($p < 0.001$).

4. Discussion

The benefits associated with the individual ingredients of Magtein®PS, a magnesium L-threonate (Magtein®) and phosphatidylserine-based formulation additionally containing vitamins C and D suggest a plausible synergistic benefit. Therefore, in this study, we tested a formula containing the above listed ingredients. We found that the group receiving Magtein®PS demonstrated significant improvements ($p < 0.001$) in all categories of the cognition tests measured (Table 4, Figures 1 and 2), consistent with our synergistic hypothesis. Interestingly, the older participants experienced the highest improvement in cognition (Figure 2).

A previous publication of a double-blind, placebo-controlled human clinical examining the effects of Magtein® in older participants demonstrated significant elevation of brain Mg levels as well as cognitive abilities in the supplemented group as compared to the placebo [20]. It is interesting to note that the effective elemental magnesium levels used for the observed benefits of magnesium L-threonate (Magtein®) were 108–144 mg/day which is below the RDA of 350–420 mg/day. Thus, supplementation even when combined with dietary intake would likely be within safe limits.

Even though, in this study, we only assessed cognitive function, it is reasonable that the benefits of Magtein® may provide benefits for multiple applications. For example, it has been suggested that magnesium supplementation may be ideal for those with treatment-resistant depression [31]. Results as to the connection of magnesium intake, magnesium levels and depression are mixed. This is thought to be due to methodology in that most studies do not measure brain magnesium levels. Brain levels of magnesium are found to be lower in in patients with depression as well as other forms of mood disturbances [31], suggesting that a magnesium supplement that could readily cross the blood–brain barrier would be beneficial over those that do not readily cross the blood–brain barrier. Increasing brain levels of magnesium would provide potential for many psychological and neurological conditions such as migraine headaches, Alzheimer's disease, stroke and subjective anxiety in subjects prone to mental stress. Perhaps this explains the benefits experienced with magnesium supplementation in subjects with these conditions [4,5].

The potential for a benefit is supported by evidence related to a mechanism of action indicating that magnesium plays a key role in the regulation of N-methyl-D-aspartate (NMDA) receptor excitability in the brain. NMDA causes degeneration of neurons and magnesium intake supports healthy neurons by blocking the activity of NMDA. Blocking NMDA is thought to be a potential prevention for cognitive impairment and Alzheimer's disease [32]. Additionally, magnesium deficiency has been shown to increase inflammatory mediators leading to neuroinflammation which is said to enhance progression of cognitive impairment and dementia [33]. A recent meta-analysis of 17 randomized controlled trials showed that Mg supplementation significantly decreased serum C-reactive protein (CRP) and increased nitric oxide (NO) levels, an important vasodilator [34]. In the brain, NO enhances blood flow and has a key role in intracellular signaling in neurons [35] The proposed mechanisms of action combined with epidemiological data showing a relationship

between total magnesium and significantly higher global cognitive scores [8] support the findings of our study. The finding that the older subjects demonstrated the greatest improvement could be explained by lower brain levels of magnesium or perhaps a great degeneration of the neurons responding more notably to the increased magnesium.

The limitations of this study include a small sample size, just 51 people in the treatment group contributing to a small number of people in each age group when sub-analyses were conducted. Additionally, brain levels of magnesium were not assessed in this study, which may have been interesting to determine. Serum magnesium levels were not measured in this study as the value was not central to the purpose of this study. Further, it has been suggested that serum levels may not be the best indicator of brain levels; it may be interesting to measure this in future studies. This was not a diverse population as all study subjects were Chinese. Though subjects were instructed not to change their normal diet, we cannot entirely control for potential changes in dietary intake of magnesium during the study time period. Future studies could consider a larger, more diverse subject population. Sub-analysis for age, gender and other factors known to correlate with magnesium levels may also be interesting to evaluate. Despite a few limitations, our study was well powered and cognitive function was evaluated using a well-known and validated assessment tool.

5. Conclusions

This double-blind, placebo-controlled study demonstrates that Magtein®PS is well tolerated and safe within the parameters of this study. Our data supported the benefits of Magtein®PS on improving learning, recall, memory and cognitive abilities in this group of healthy Chinese adults. Furthermore, the benefits of Magtein®PS were observed among all ages, with older people demonstrating the most improvement. These findings may support cognitive function as well as other benefits in all age groups, especially older adults.

Author Contributions: Q.H.: Data collection and analysis, manuscript writing. C.Z.: Test preparation, data collection, statistics and analysis, manuscript writing. S.L.: Data collection, data entry, processing and verification. F.D.: Data collection, data entry and processing. W.Q.: Data collection, data entry and processing. T.Y.: Data collection, data entry and processing. Y.W.: Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team. S.H. manuscript writing and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Wu-xi Magtein Bioscience, life science and Technology Industrial Park, Huishan District, Wuxi City, P.R China.

Institutional Review Board Statement: This study was approved by the Ethics Committee of Safety Assessment and Research Center for Drug, Pesticide and Veterinary Drug of Jiangsu Province (NO. GZ01020150029-5).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Medeiros, D.M.; Wildman, R.E. *Advanced Human Nutrition*; Jones & Bartlett Learning: Burlington, MA, USA, 2018.
2. Fardet, A.; Aubrun, K.; Rock, E. Nutrition transition and chronic diseases in China (1990–2019): Industrially processed and animal calories rather than nutrients and total calories as potential determinants of the health impact. *Public Health Nutr.* **2021**, *24*, 5561–5575. [CrossRef]
3. Xu, M.; Cai, J.; Mo, X.; Liu, Q.; Zhang, J.; Wei, Y.; Liu, S.; Lin, Y.; Huang, S.; Mo, C.; et al. Association of Dietary and Plasma Magnesium with Glycaemic Markers in a Chinese Population. *Biol. Trace Elem. Res.* **2022**. [CrossRef]
4. Volpe, S.L. Magnesium in disease prevention and overall health. *Adv. Nutr.* **2013**, *4*, 378s–383s. [CrossRef]
5. Boyle, N.B.; Lawton, C.L.; Dye, L. The effects of magnesium supplementation on subjective anxiety. *Magnes. Res.* **2016**, *29*, 120–125. [CrossRef]

6. Derom, M.L.; Sayón-Orea, C.; Martínez-Ortega, J.M.; Martínez-González, M.A. Magnesium and depression: A systematic review. *Nutr. Neurosci.* **2013**, *16*, 191–206. [CrossRef]
7. Grases, G.; Pérez-Castelló, J.A.; Sanchis, P.; Casero, A.; Perelló, J.; Isern, B.; Rigo, E.; Grases, F. Anxiety and stress among science students. Study of calcium and magnesium alterations. *Magnes. Res.* **2006**, *19*, 102–106.
8. Tao, M.H.; Liu, J.; Cervantes, D. Association between magnesium intake and cognition in US older adults: National Health and Nutrition Examination Survey (NHANES) 2011 to 2014. *Alzheimer's Dement.* **2022**, *8*, e12250. [CrossRef]
9. McKee, J.A.; Brewer, R.P.; Macy, G.E.; Phillips-Bute, B.; Campbell, K.A.; Borel, C.O.; Reynolds, J.D.; Warner, D.S. Analysis of the brain bioavailability of peripherally administered magnesium sulfate: A study in humans with acute brain injury undergoing prolonged induced hypermagnesemia. *Crit. Care Med.* **2005**, *33*, 661–666. [CrossRef]
10. Fuchs-Buder, T.; Tramèr, M.R.; Tassonyi, E. Cerebrospinal fluid passage of intravenous magnesium sulfate in neurosurgical patients. *J. Neurosurg. Anesthesiol.* **1997**, *9*, 324–328. [CrossRef]
11. Slutsky, I.; Abumaria, N.; Wu, L.J.; Huang, C.; Zhang, L.; Li, B.; Zhao, X.; Govindarajan, A.; Zhao, M.G.; Zhuo, M.; et al. Enhancement of learning and memory by elevating brain magnesium. *Neuron* **2010**, *65*, 165–177. [CrossRef]
12. Sadir, S.; Tabassum, S.; Emad, S.; Liaquat, L.; Batool, Z.; Madiha, S.; Shehzad, S.; Sajid, I.; Haider, S. Neurobehavioral and biochemical effects of magnesium chloride ($MgCl_2$), magnesium sulphate ($MgSO_4$) and magnesium-L-threonate (MgT) supplementation in rats: A dose dependent comparative study. *Pak. J. Pharm. Sci.* **2019**, *32*, 277–283. [PubMed]
13. Sun, Q.; Weinger, J.G.; Mao, F.; Liu, G. Regulation of structural and functional synapse density by L-threonate through modulation of intraneuronal magnesium concentration. *Neuropharmacology* **2016**, *108*, 426–439. [CrossRef] [PubMed]
14. Fay, M.J.; Verlangieri, A.J. Stimulatory action of calcium L-threonate on ascorbic acid uptake by a human T-lymphoma cell line. *Life Sci.* **1991**, *49*, 1377–1381. [CrossRef] [PubMed]
15. Deutsch, J.C.; Butler, J.A.; Marsh, A.M.; Ross, C.A.; Norris, J.M. Rapid mass spectrometric analysis for ascorbate and related organic acids in small volumes of plasma for use in pediatric subjects. *J. Chromatogr. B Biomed. Sci. Appl.* **1999**, *726*, 79–84. [CrossRef]
16. Harding, J.J.; Hassett, P.C.; Rixon, K.C.; Bron, A.J.; Harvey, D.J. Sugars including erythronic and threonic acids in human aqueous humour. *Curr. Eye Res.* **1999**, *19*, 131–136. [CrossRef]
17. Thompson, J.A.; Markey, S.P.; Fennessey, P.V. Gas-chromatographic/mass-spectrometric identification and quantitation of tetronic and deoxytetronic acids in urine from normal adults and neonates. *Clin. Chem.* **1975**, *21*, 1892–1898. [CrossRef]
18. Lawson, A.M.; Chalmers, R.A.; Watts, R.W. Urinary organic acids in man. I. Normal patterns. *Clin. Chem.* **1976**, *22*, 1283–1287. [CrossRef]
19. Azzam, M.M.; Zou, X.T.; Dong, X.Y.; Xie, P. Effect of supplemental L-threonine on mucin 2 gene expression and intestine mucosal immune and digestive enzymes activities of laying hens in environments with high temperature and humidity. *Poult. Sci.* **2011**, *90*, 2251–2256. [CrossRef]
20. Liu, G.; Weinger, J.G.; Lu, Z.L.; Xue, F.; Sadeghpour, S. Efficacy and Safety of MMFS-01, a Synapse Density Enhancer, for Treating Cognitive Impairment in Older Adults: A Randomized, Double-Blind, Placebo-Controlled Trial. *J. Alzheimer's Dis. JAD* **2016**, *49*, 971–990. [CrossRef]
21. ter Borg, S.; Verlaan, S.; Hemsworth, J.; Mijnarends, D.M.; Schols, J.M.; Luiking, Y.C.; de Groot, L.C. Micronutrient intakes and potential inadequacies of community-dwelling older adults: A systematic review. *Br. J. Nutr.* **2015**, *113*, 1195–1206. [CrossRef]
22. Glade, M.J.; Smith, K. Phosphatidylserine and the human brain. *Nutrition* **2015**, *31*, 781–786. [CrossRef] [PubMed]
23. Turnlund, J.R.; Betschart, A.A.; Liebman, M.; Kretsch, M.J.; Sauberlich, H.E. Vitamin B-6 depletion followed by repletion with animal- or plant-source diets and calcium and magnesium metabolism in young women. *Am. J. Clin. Nutr.* **1992**, *56*, 905–910. [CrossRef] [PubMed]
24. Guillard, O.; Piriou, A.; Fauconneau, B.; Mauco, G.; Mettey, R. Unexpected toxicity induced by magnesium orotate treatment in congenital hypomagnesemia. *J. Intern. Med.* **2002**, *252*, 88–90. [CrossRef] [PubMed]
25. Spasov, A.A.; Petrov, V.I.; Iezhitsa, I.N.; Kravchenko, M.S.; Kharitonova, M.V.; Ozerov, A.A. Comparative study of magnesium salts bioavailability in rats fed a magnesium-deficient diet. *Vestn. Ross. Akad. Meditsinskikh Nauk* **2010**, 29–37.
26. Wroolie, T.E.; Chen, K.; Watson, K.T.; Iagaru, A.; Sonni, I.; Snyder, N.; Lee, W.; Reiman, E.M.; Rasgon, N.L. An 8-week open label trial of l-Threonic Acid Magnesium Salt in patients with mild to moderate dementia. *Pers. Med. Psychiatry* **2017**, *4*, 7–12. [CrossRef]
27. Ritchie, G.; Kerstan, D.; Dai, L.-J.; Kang, H.S.; Canaff, L.; Hendy, G.N.; Quamme, G.A. $1,25(OH)_2D_3$ stimulates Mg^{2+} uptake into MDCT cells: Modulation by extracellular Ca^{2+} and Mg^{2+}. *Am. J. Physiol.-Ren. Physiol.* **2001**, *280*, F868–F878. [CrossRef]
28. Cascella, M.; Vaqar, S. Hypermagnesemia. In *StatPearls*; StatPearls Publishing LLC.: Treasure Island, FL, USA, 2022.
29. Chai, B.; Gao, F.; Wu, R.; Dong, T.; Gu, C.; Lin, Q.; Zhang, Y. Vitamin D deficiency as a risk factor for dementia and Alzheimer's disease: An updated meta-analysis. *BMC Neurol.* **2019**, *19*, 284. [CrossRef]
30. Jayedi, A.; Rashidy-Pour, A.; Shab-Bidar, S. Vitamin D status and risk of dementia and Alzheimer's disease: A meta-analysis of dose-response†. *Nutr. Neurosci.* **2019**, *22*, 750–759. [CrossRef]
31. Eby, G.A., 3rd; Eby, K.L. Magnesium for treatment-resistant depression: A review and hypothesis. *Med. Hypotheses* **2010**, *74*, 649–660. [CrossRef]
32. Hynd, M.R.; Scott, H.L.; Dodd, P.R. Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. *Neurochem. Int.* **2004**, *45*, 583–595. [CrossRef]

33. Yu, X.; Guan, P.P.; Zhu, D.; Liang, Y.Y.; Wang, T.; Wang, Z.Y.; Wang, P. Magnesium Ions Inhibit the Expression of Tumor Necrosis Factor α and the Activity of γ-Secretase in a β-Amyloid Protein-Dependent Mechanism in APP/PS1 Transgenic Mice. *Front. Mol. Neurosci.* **2018**, *11*, 172. [CrossRef] [PubMed]
34. Veronese, N.; Pizzol, D.; Smith, L.; Dominguez, L.J.; Barbagallo, M. Effect of Magnesium Supplementation on Inflammatory Parameters: A Meta-Analysis of Randomized Controlled Trials. *Nutrients* **2022**, *14*, 679. [CrossRef] [PubMed]
35. Picón-Pagès, P.; Garcia-Buendia, J.; Muñoz, F.J. Functions and dysfunctions of nitric oxide in brain. *Biochim. Biophys. Acta. Mol. Basis Dis.* **2019**, *1865*, 1949–1967. [CrossRef] [PubMed]

Journal of Alzheimer's Disease 49 (2016) 971–990
DOI 10.3233/JAD-150538
IOS Press

Efficacy and Safety of MMFS-01, a Synapse Density Enhancer, for Treating Cognitive Impairment in Older Adults: A Randomized, Double-Blind, Placebo-Controlled Trial

Guosong Liu[a,d,*], Jason G. Weinger[a], Zhong-Lin Lu[b], Feng Xue[c] and Safa Sadeghpour[a]
[a]*Neurocentria, Inc., Fremont, CA, USA*
[b]*Department of Psychology, The Ohio State University, Columbus, OH, USA*
[c]*Department of Psychology, University of Southern California, Los Angeles, CA, USA*
[d]*School of Medicine, Tsinghua University, Beijing, China*

Accepted 20 August 2015

Abstract.
Background: Cognitive impairment is a major problem in elderly, affecting quality of life. Pre-clinical studies show that MMFS-01, a synapse density enhancer, is effective at reversing cognitive decline in aging rodents.
Objective: Since brain atrophy during aging is strongly associated with both cognitive decline and sleep disorder, we evaluated the efficacy of MMFS-01 in its ability to reverse cognitive impairment and improve sleep.
Methods: We conducted a randomized, double-blind, placebo-controlled, parallel-designed trial in older adult subjects (age 50–70) with cognitive impairment. Subjects were treated with MMFS-01 ($n = 23$) or placebo ($n = 21$) for 12 weeks and cognitive ability, sleep quality, and emotion were evaluated. Overall cognitive ability was determined by a composite score of tests in four major cognitive domains.
Results: With MMFS-01 treatment, overall cognitive ability improved significantly relative to placebo ($p = 0.003$; Cohen's $d = 0.91$). Cognitive fluctuation was also reduced. The study population had more severe executive function deficits than age-matched controls from normative data and MMFS-01 treatment nearly restored their impaired executive function, demonstrating that MMFS-01 may be clinically significant. Due to the strong placebo effects on sleep and anxiety, the effects of MMFS-01 on sleep and anxiety could not be determined.
Conclusions: The current study demonstrates the potential of MMFS-01 for treating cognitive impairment in older adults.

Keywords: Alzheimer's disease, cognitive impairment, cognition, composite score, L-threonate, magnesium, mild cognitive impairment, randomized clinical trial, sleep disorder, synaptic density

INTRODUCTION

Cognitive function declines with aging [1]. Cognitive impairment in elderly is a major problem that can affect activities of daily living (ADL) and quality of life [2, 3]. Although the neuropathological process underlying cognitive impairment remains elusive, the best correlate to cognitive impairment is brain atrophy [1, 4]. Brain atrophy is associated with neuronal, axonal, and synaptic loss. So far, the best structural predictor of cognitive decline is the degree of synaptic loss [5]. Since synapses are the elemental units of neural communication, synapse loss and reduction of synaptic plasticity should have a major impact on neural

*Correspondence to: Guosong Liu, Neurocentria, Inc., Fremont, CA 94538, USA. Tel.: +1 510 606 0084; E-mail: liu.guosong@gmail.com.

signaling, resulting in impaired cognition. Therapeutic strategies that prevent net synapse loss and increase synapse density may have great potential for cognitive impairment.

In our pre-clinical studies, we found that the level of brain magnesium is a critical factor controlling synapse density and plasticity. Elevating neuronal intracellular magnesium can increase functional synapse density and plasticity in cultured hippocampal neurons [6, 7]. Mechanistically, we found that intracellular magnesium in neurons serves as a critical second messenger controlling neuronal energy supply and functional synapse density [7]. In an intact rodent, treatment with conventional magnesium salts is ineffective at elevating brain magnesium and improving memory function [8]. This is because active transport systems tightly control the amount of magnesium that crosses first from digestive tract into blood, and then from blood to cerebrospinal fluid (CSF) [9]. In human, increasing blood magnesium by up to 300% only changes CSF magnesium by less than 19% [10]. To overcome this problem, we developed L-Threonic acid Magnesium salt (L-TAMS, formerly MgT), a compound that can effectively enhance CSF magnesium concentration via oral intake [8]. L-TAMS treatment increases synapse density in brain regions critical for executive function and memory, such as the prefrontal cortex and hippocampus [8, 11–13]. Furthermore, L-TAMS treatment increases the number of NR2B containing NMDA receptors, resulting in an enhancement of synaptic plasticity in aging rats and Alzheimer's disease (AD) model mice [8, 14]. At a functional level, L-TAMS treatment reverses cognitive impairment in aging rats and AD model mice [8, 14]. The increase of synapse density in aging rats is linearly correlated with memory improvement [8]. L-TAMS treatment also enhances fear memory extinction and prevents fear memory over-generalization, leading to a reduction of anxiety in rats [13, 15].

Here, we conducted a randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of a treatment regimen consisting of 12 weeks of oral intake of MMFS-01, a compound containing L-TAMS, in older adults with cognitive impairment. We used three inclusion criteria, including subjective memory complaints (SMC), sleep disorder, and anxiety, to select subjects who had cognitive impairment. Their cognitive impairment was later confirmed by an object cognitive test (Trail Making Test - Part B). Sleep and anxiety disorder were used as inclusion criteria to increase the chance of recruiting subjects who had cognitive impairment with an underlying neurodegenerative condition. This was necessary because previous studies show that SMC is not a good sole indicator of mild cognitive impairment (MCI). Many people who think they have memory issues actually have a normal cognitive profile when tested with objective memory tests. There is only a 30% chance that someone with SMC has MCI [16]. It is common that patients with brain atrophy not only have MCI but also have neuropsychiatric symptoms [17]. The common core non-cognitive symptoms in MCI patients are anxiety [18, 19] and sleep disorder [20, 21]. 47% of MCI patients have anxiety symptoms [22], and 83% of those with MCI and anxiety develop AD compared to only 41% of those with MCI without anxiety [23]. Recent studies show sleep disorder is strongly correlated with cognitive impairment [20], and even the chance of getting AD [24].

Our efficacy evaluation included determination of body magnesium status, tests of cognition in four domains (executive function, working memory, attention, and episodic memory), and measurements of sleep quality and emotional state [25].

MATERIALS AND METHODS

Study design

This was a 12-week parallel-designed, randomized, single-site, double-blind, placebo-controlled clinical trial that compared MMFS-01 and placebo. MMFS-01 is a compound containing L-TAMS, trademarked under the name ClariMem®.

Participants

Subjects were men or women between 50 and 70 years of age with self-reported complaints of cognition (memory and concentration), and with anxiety and sleep disorder. Subjects had a Mini-Mental State Examination score (MMSE) equal to or greater than 24. Sleep difficulties defined by a score of greater than 5 on the Pittsburgh Sleep Quality Index (PSQI), and the presence of mild-to-moderate anxiety, with scores $\geq$12 and $\leq$28 on the Hamilton Anxiety Questionnaire sub-score A (HAM-A), were required for inclusion in the study [26].

Exclusion criteria included active heart disease; uncontrolled high blood pressure ($\geq$140/90 mmHg); renal or hepatic impairment/disease; Type I or II diabetes; bipolar disorder; Parkinson's disease; AD; dementia; unstable thyroid disease; diagnosed major affective disorder; psychiatric disorder (hospitalized in

the past year); immune disorder (such as HIV/AIDS); a history of cancer (except localized skin cancer without metastases or in situ cervical cancer) within 5 years prior to screening; current use of calcium channel blockers, SSRIs or anxiolytics other than benzodiazepines as needed, with "as needed" defined as less than 5 times per month; current use of any medications that are known to interact with magnesium including loop, thiazide, or potassium-sparing diuretics, muscle relaxants, penicillamine, corticosteroids, magnesium-containing antacids or other magnesium containing products; use less than 7 days before the randomization visit of calcium channel blockers, any anxiolytics or SSRIs; current use of antibiotics (a washout period of 2 weeks was allowed); presence of an unstable dose of medication (defined as fewer than 90 days at the same dose); presence of an allergy or sensitivity to any ingredient in the test product; hepatic or renal dysfunction as evidenced by ALT, AST, AP being $\geq$2 times the upper limit of normal or serum creatinine value $\geq$2.0 mg/; history of drug or alcohol abuse in the past 12 months or had begun/stopped smoking $\leq$6 months ago or had plans to begin/quit smoking; possibility that the subject may become pregnant as shown by lack of birth control use, pre-menopausal status or absence of hysterectomy; status of pregnancy, lactation or plans to become pregnant during the study period; participation in another research study either presently or within 30 days prior to the screening visit; any condition, abnormality, medication usage or clinically significant clinical laboratory findings that, in the opinion of the investigator, would compromise the safety of the subject or the quality of the study data. Subjects were allowed to take medications if the medication was not part of the exclusion criteria and the dose was unchanged at least 90 days before screening and throughout the study.

Subjects stopped taking any dietary supplements at least 7 days prior to randomization, and maintained cessation during the study. They refrained from alcohol consumption or exercise for at least 24 hours prior to each test visit. No changes to the methods, including eligibility requirements and dosing, were made after commencement of the trial.

Recruitment and randomization

Subject randomization began in November 2012, and recruitment was completed in June 2013. A total of 51 subjects (age 50–70) were recruited by Miami Research Associates (MRA) and enrolled in a randomized double-blind, placebo-controlled trial conducted at MRA (Miami). Data for all subjects at every time point were collected by MRA at their Miami clinical laboratory.

Before the study began, the protocol, informed-consent form, and other information provided to subjects and caregivers were reviewed and approved by the Aspire IRB (September 20, 2012). Subjects were randomly assigned to the MMFS-01 or placebo group in a ratio of 1:1, using a block-2 randomization schedule. Subjects received a sequential number corresponding to the order in which they entered the study. Study sponsors, investigators, research coordinators, attending care teams, and subjects were blinded to treatment group. The consulting statistician locked the database of data elements and unblinded it by accessing the table of randomized assignments and merging them into the data tables.

Dosage

Dosage was set to correspond to approximately 25 mg/kg/day. To accomplish this, subjects between 50 and 70 kg took 1.5 g/day, and subjects between 70 and 100 kg took 2 g/day of MMFS-01. At conclusion of the study, 8 subjects (35 percent) were taking 1.5 g of MMFS-01 per day, and 15 subjects (65 percent) were taking 2 g of MMFS-01 per day.

Power analysis

Enrollment for this study was targeted at 50 subjects (25 per group). Prior to this study, this compound was given open-label to a small number of subjects (L-TAMS has self-affirmed/FDA-affirmed GRAS status). These subjects' subjective feeling was a significant improvement in anxiety, sleep, and mental clarity. They had significant improvement in anxiety based on the HAM-A questionnaire. Therefore, we powered the analysis in this trial by reduction of HAM-A score. We predicted the treatment would lead to a 50% reduction in HAM-A score, with a SD of HAM-A scores of approximately $\pm$ 10 score points [27]. Assuming a serial coefficient correlation of about 0.5 for HAM-A scores at baseline and 12 weeks, the within-group SD of the 12-week changes would also be $\pm$ 10 score points. With the use of an unpaired Student *t*-test with a significance level of 0.05, a total enrollment of 50 subjects (40 completers if 20% attrition) was required for the study to be able to detect differences of about a 45% reduction in HAM-A score. We assumed an attrition rate of 20% in line with previous experience by the contract research organization that ran

the study, MRA. Even if the attrition rate had been as high as 32%, there would have been enough analyzable subjects (34 subjects) to provide 87% power in detecting a clinically meaningful 50% HAM-A score reduction.

Efficacy endpoints

Efficacy assessments were made at Baseline Visit, Week 6 Visit, and Week 12 Visit. The change in the body's magnesium status was quantified by assessing blood magnesium concentration (plasma Mg^{2+}), urine magnesium concentration normalized by the estimated glomerular filtration rate (uMg^{2+}/GFR), and intracellular magnesium concentration (Red Blood Cell; RBC Mg^{2+}). The key functional efficacy outcome measures included measurements of cognitive abilities, sleep quality, and affect.

TMT-B test

The Trail Making Test – Part B (TMT-B) assesses executive function as well as impulsivity, visual search, visual attention, and motor speed [28]. In the test, subjects were required to connect a series of label circles that constituted a trail. Scores were calculated as the inverse of the time (in milliseconds) it took the subject to complete the task (all 25 circle connections), representing speed. Scores from subjects unable to complete the task in the maximum allotted time (360 seconds), or from those who quit prior to the maximum allotted time, were scaled to the time to complete 25 circle connections before converting to speed. Six out of 44 (13%) subjects did not complete the task at least once with a total of 9 occurrences, 5 at baseline, 3 at Week 6, and 1 at Week 12. Higher speeds reflected better performance.

DigitSpan test

The DigitSpan test assesses working memory performance. Scores were based on the length of the longest sequence of digits (consecutive numbers) subjects could remember and thus ranged from 0 without an upper bound, with higher scores reflecting better performance.

Eriksen Flanker Congruent/Incongruent test

The Eriksen Flanker Congruent/Incongruent test assesses attention, that is, cognitive processes involved in detection and recognition of targets in the presence of distracting information [29]. A target directional arrow was flanked by either arrows in the same (congruent) or opposite direction (incongruent). The average time to correctly select the target arrow's direction was recorded. The incongruent task was more difficult than the congruent task because the congruent task did not require response inhibition and was less confounded by training effects. Therefore, the response times in the congruent condition were subtracted from those in the incongruent condition to remove training effects and discern effects on attention. The opposite of this difference was reported so higher scores reflected better performance.

Face-Name association test

Finally, the Face-Name Association test assesses hippocampal-dependent episodic memory [30]. Twenty faces with twenty fictional popular first names were shown on screen. Subjects were then asked to remember and later recognize each face and name pair when presented with the same or novel face and name pairs. Using signal detection theory, the hit rate, false alarm rate, and sensitivity index (d') were calculated, where $d' = z_{(\text{hit rate})} - z_{(\text{false alarm rate})}$. d' showed how well the subject distinguished old from new. Hit rate was defined as a correct identification of an old face and name pair and false alarm as an incorrect identification of a new face and name pair. Higher scores reflected improved performance with scores above three indicative of a near perfect score.

Composite score

Scores from several cognitive tests, evaluating four domains of cognition—executive function, working memory, attention, and episodic memory—were combined to produce a composite score to assess overall cognitive ability [25]. The cognitive tests included TMT-B for executive function [31], DigitSpan for working memory capacity [32], Face-Name Association for episodic memory [33], and Eriksen Congruent/Incongruent Flanker [29] for attention.

The composite score was calculated as the average of the four individual z scores ($\bar{z}$). z scores were calculated for each subject on each test using the formula $z = \frac{x-\mu_b}{\sigma_b}$, where μ_b is the mean of all subjects (MMFS-01 and placebo combined) at baseline and σ_b is the standard deviation (SD) of all subjects at baseline. Baseline means and SDs were used to convert the raw scores of Week 6 and Week 12 to z scores in order to determine the treatment effects (change from

baseline) of MMFS-01 versus placebo for each subject for each test.

Effect size (Cohen's d) was determined for each of the cognitive endpoints at Week 6 and Week 12 using the formula

Cohen's $d = \frac{(\bar{X}_{n,\Delta MMFS-01})-(\bar{X}_{n,\Delta Placebo})}{\sigma_{pooled}}$, where $\bar{X}_n$ was the mean of the change from baseline values in the MMFS-01 or placebo group at either Week 6 or Week 12 and σ_{pooled} was the pooled SD of the change from baseline of the MMFS-01 and placebo groups at either Week 6 or Week 12. Pooled SD was calculated using the formula

$$\sigma_{pooled} = \sqrt{\frac{\left[(n_{MMFS-01}-1)(\sigma_{\Delta MMFS-01})^2+(n_{Placebo}-1)(\sigma_{\Delta Placebo})^2\right]}{\left[(n_{MMFS-01}+n_{Placebo})-2\right]}}.$$

Sleep

Sleep quality was measured with PSQI [26]. PSQI is a self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval. Higher scores indicated worse sleep quality. Based on previous research, a global PSQI score greater than 5 yields a diagnostic sensitivity of 89.6% and specificity of 86.5% (kappa = 0.75, p less than 0.001) in distinguishing good and poor sleepers [26].

Emotion

Affective personality was assessed with the HAM-A and the Positive and Negative Affect Schedule (PANAS). The HAM-A is a rating scale used in both clinical and research settings to measure the severity of psychic and somatic anxiety symptoms [34]. It did not provide any standardized probe questions and was administered by a clinician (subject did not complete the questionnaire by his/herself). Scores ranged from 0 to 56 where $\leq$17 indicated mild severity, 18 to 24 mild to moderate severity, 25 to 30 moderate to severe severity, and >30 severe severity. The PANAS is a self-rated tool used to measure positive and negative affect over a 1-week time interval, and consists of two 10-item scales, one for Positive Affect and the other for Negative Affect [35]. Subjects were asked to rate different feelings and emotions using the following Likert scale: 1 = very slightly or not at all, 2 = a little, 3 = moderately, 4 = quite a bit and 5 = extremely. Scores for each scale ranged from 10 to 50. Higher positive affect scores represented more positive affect, and thus, better outcomes. Higher negative affect scores represented more negative affect, and thus, worse outcomes.

Cognitive ability fluctuation analysis

The fluctuation of cognitive ability over time was evaluated by calculating variance of the change in composite score from Week 6 to Week 12 of individual subjects, with the formula $\sigma^2 = \frac{\sum(X_{Week\ 12}-X_{Week\ 6})^2}{n-1}$. The fluctuations of cognition of the placebo group and the MMFS-01 group were calculated separately.

Tolerability and safety

Safety evaluations included recording all adverse events, results of laboratory tests (comprehensive metabolic panel, uric acid, and complete blood count with differential), vital signs, body weight, and subjective remarks.

Adverse events were listed, MedDRA encoded, grouped by general type of event (gastrointestinal, neurologic, cardiac, etc.), and cross-tabulated by event type and product group. The principal investigator catalogued adverse events as mild, moderate, or severe according to the following definitions: Mild (causing no limitation in normal activities), Moderate (causing some limitation in normal activities), and Severe (causing significant limitation in or the inability to perform normal activities). A central laboratory conducted all laboratory evaluations. Of the 47 adverse events, 13 events, occurring in 10 subjects, were judged by the principal investigator to be probably or possibly related to the study product. Probably and possibly-related adverse events were considerably more prevalent in the placebo group than in the MMFS-01 group (9 and 4 events, in 6 and 4 subjects, respectively). The predominant adverse events were related to gastrointestinal function (affecting 5 of 25 subjects (20.0%) in the MMFS-01 group and 4 of 26 subjects (15.4%) in placebo group, $p = 0.726$) or infections/infestations (affecting 4 of 25 subjects (16%) in the MMFS-01 group and 6 of 26 subjects (23%) in placebo group, $p = 0.726$).

Statistical analysis

The safety population consisted of subjects who received at least one dose of any study product, and who had any subsequent encounter with the study site. The efficacy population included all subjects who completed all scheduled visits, had no protocol deviations that in the judgment of the principal investigator would have invalidated their efficacy data (see product compliance section below). Only data from subjects that completed all visits were included in the

statistical analysis; therefore, there were no missing data values in the dataset, and imputation was not required.

Statistical analyses for cognitive tests and body magnesium status variables were performed with SPSS and R. For categorical variables, difference in the distribution of categories between the different treatment groups was tested for nominal significance by the Chi-Square test, in SPSS or GraphPad Prism. Formal statistical tests were performed for cognitive endpoints and magnesium status using a univariate analysis of covariance (ANCOVA) model at Week 6 and Week 12 with baseline values as a covariate. For safety endpoints, changes were tested for significance by the paired Student *t*-test, or the non-parametric Wilcoxon signed-ranks test if necessary. Differences in adverse event patterns between product groups were tested by the Fisher's Exact test.

Longitudinal repeated measures ANCOVA analyses using observed data without any data imputation were used to determine the overall effect from baseline of MMFS-01 compared to placebo. The model included the categorical fixed effects of treatment (MMFS-01 versus placebo), week (6 and 12), and treatment-by-week interaction, as well as the continuous fixed covariate of baseline measurement. Normality of distribution and equality of variance were determined using the Shapiro-Wilk's test and Levene's test, respectively. For endpoint values that violated either test, additional bootstrapping was employed, using resampling methods. In the ANCOVA analyses, in order to simulate the F-distribution under the null hypothesis, resampling techniques were used to permute the treatment labels, time point labels, and baseline values. For each of the 10,000 random permutations, F-statistics for the ANCOVA model were computed, and used to compute a percentile *p*-value for the dataset. Bootstrapping was used for TMT-B, Mg^{2+} Urine, and Mg^{2+} Plasma. In one exception, to determine treatment differences at Week 6 and Week 12 between the MMFS-01 and placebo groups for percent change in RBC magnesium concentration, an analysis of variance (ANOVA) model was used instead of ANCOVA.

As this was not a pivotal Phase-III clinical trial, it was not required to control the *study-wise* Type-1 error rate to a specified alpha level. Each efficacy endpoint was considered an independent question of interest, with a hypothesized difference, and was tested independently using a two-tailed 0.05 alpha level ($p \leq 0.05$ required for a conclusion of statistical significance). No interim analysis was performed for this study.

To determine outliers, individual data for each test was analyzed. If a baseline score was greater than 2 SDs away from the mean then that data point was considered to be an outlier, and therefore excluded. Of the four cognitive tests, outliers were only found on the Flanker test. Out of 44 baseline data points, 3 subjects were removed (1 MMFS-01, 2 placebo) from the analysis of the Flanker test. Additionally, we found some ceiling effects in the Face-Name test, in which some subjects had a near perfect baseline score (>3). Therefore, we set 3 as the threshold for the ceiling baseline Face-Name score. Out of 44 data points, 3 subjects were removed (2 MMFS-01, 1 Placebo) from the analysis of the Face-Name test. We removed the contribution of any excluded subject to the composite score so the excluded data points did not erroneously skew the composite score. Except for outliers and scores at the ceiling, all data were included for all subjects for all outcome measurement analyses.

Product compliance

Compliance was measured via the pill counting method, by documenting the number of calendar days between visits and the number of pills that should have been taken. Subject compliance was recorded as a percent of the prescribed amount for each visit and then averaged to produce an overall compliance figure. Per the original protocol, 80–120% compliance was considered acceptable. Of 44 subjects in the per protocol population, 41 returned their unused pills and were in the acceptable range. The remaining 3 did not return their pills, but were determined to be within the acceptable range of compliance based on the estimation of the PI, using MRA staff's familiarity with the subject and/or subject's compliance during other testing phase(s) of the study to make this decision. Therefore, all 44 subjects were considered compliant.

Funding and sponsor involvement

The study was funded by Neurocentria Inc., CA, USA, and designed jointly by Neurocentria and MRA. The study was executed and data was collected by MRA who vouched for its integrity, with Dr. Diane Krieger (MRA) serving as the Principal Investigator. Statistical analysis of several efficacy variables including affective, sleep quality and clinical impression tests, and all safety variables including adverse events was carried out by MRA. Neurocentria conducted statistical analysis for cognitive tests and body magnesium status variables. Neurocentria wrote the paper

through an iterative review process. ClinicalTrials.gov number, NCT02363634.

RESULTS

Study population

The mean subject age was 57.3 ± 5.2 years, with 71% female. Baseline demographic and background characteristics are summarized in Table 1; there were no significant differences in these characteristics between the treatment and control groups. 66.7% of the subjects (34 of 51) had coexisting medical conditions at baseline. The most common conditions were gastrointestinal (10 subjects; 19.6%). None of the subjects were taking CNS medications and there were no significant differences between groups in the presence of coexisting diseases or medication use.

25 subjects received MMFS-01 (Neurocentria, Inc., Fremont, California, USA), and 26 received placebo. 7 subjects (14%) discontinued the study prematurely: 2 (7.7%) in the MMFS-01 group and 5 (19%) in the placebo group (Fig. 1). Withdrawn consent was the primary reason for discontinuation. The remaining 44 subjects completed the study and were included in the efficacy analysis.

Table 1
Baseline characteristics according to treatment group

Characteristic	Placebo ($n = 26$)	MMFS-01 ($n = 25$)
Age - y ± SD	57.6 ± 4.4	57.1 ± 6.0
Gender - no. (%)		
Male	8 (31%)	7 (28%)
Female	18 (69%)	18 (72%)
Ethnicity - no. (%)		
Hispanic	25 (96%)	22 (88%)
Non-Hispanic	1 (4%)	3 (12%)
Race - no (%)		
African-American	3 (12%)	2 (8%)
Caucasian	23 (88%)	23 (92%)
Medical History - no. (%)		
Cardiovascular	10 (38%)	9 (36%)
Dermatological	0 (0%)	3 (12%)
Ears/Nose/Throat/Mouth/Eyes	6 (23%)	6 (24%)
Endocrine/Metabolic	7 (27%)	5 (20%)
Gastrointestinal	14 (54%)	11 (44%)
Musculoskeletal	8 (31%)	12 (48%)
Neurological	10 (38%)	11 (44%)
Renal/Genitourinary	1 (4%)	5 (20%)
CNS Medication - no. (%)	0 (0%)	0 (0%)
Height - cm ± SD	159.7 ± 9.7	161.0 ± 9.1
Weight - kg ± SD	73.2 ± 12.9	73.1 ± 10.4
MMSE Score ± SD	28.2 ± 1.3	27.8 ± 1.6

Efficacy

The effects of MMFS-01 on body magnesium levels

We determined the change in body magnesium status by quantifying magnesium in urine (excretion), plasma (extracellular), and RBC (intracellular). Excreted magnesium was measured to estimate the relative amount of absorbed magnesium, because magnesium excreted in urine is proportional to absorbed magnesium, provided that the subject has normal kidney function for mineral reabsorption (i.e., the higher the absorption of magnesium, the higher the excretion) [36]. Treatment with MMFS-01 for 12 weeks resulted in a significant increase in the excretion rate of magnesium relative to placebo ($p = 0.027$). Plasma magnesium concentration is tightly controlled by homeostatic mechanisms, and plasma magnesium concentration is hardly changed by conventional oral magnesium supplementation [37]. While magnesium was initially higher in the plasma of subjects taking MMFS-01 (Week 6) versus placebo, there was no difference between the two groups at Week 12, due to a change in plasma magnesium concentration in the placebo group from Week 6 to Week 12. This difference is indicated by a significant treatment × time interaction between MMFS-01 and placebo ($p < 0.05$). Finally, RBC magnesium concentration increased in MMFS-01 treated subjects from baseline to Week 12 (3.3 ± 1.9%) and from Week 6 to Week 12 (3.0 ± 2.0%) compared with a reduction in placebo treated subjects at Week 12 (–0.6% ± 1.8%) and from Week 6 to Week 12 (–3.6 ± 2.1%, $p = 0.019$). The body magnesium results are summarized in Table 2. These results suggest that the dosage of MMFS-01 was effective at loading magnesium into the body.

The effects of MMFS-01 on cognitive abilities

The effect of MMFS-01 on cognitive ability was evaluated in four cognitive domains: executive function, working memory, attention, and episodic memory by administration of the Trail Making, DigitSpan, Flanker, and Face-Name tests, respectively, at Baseline, Week 6, and Week 12 (Table 3). These cognitive tests were chosen based on the current consensus that multiple domains of cognition should be evaluated to determine cognitive impairment [38]. The cognitive domains we selected were similar to those included in the Alzheimer's Disease Cooperative Study - Preclinical Alzheimer Cognitive Composite (ADCS-PACC), are in line with recent recommendations by the U.S. Food and Drug Administration, and are reliable

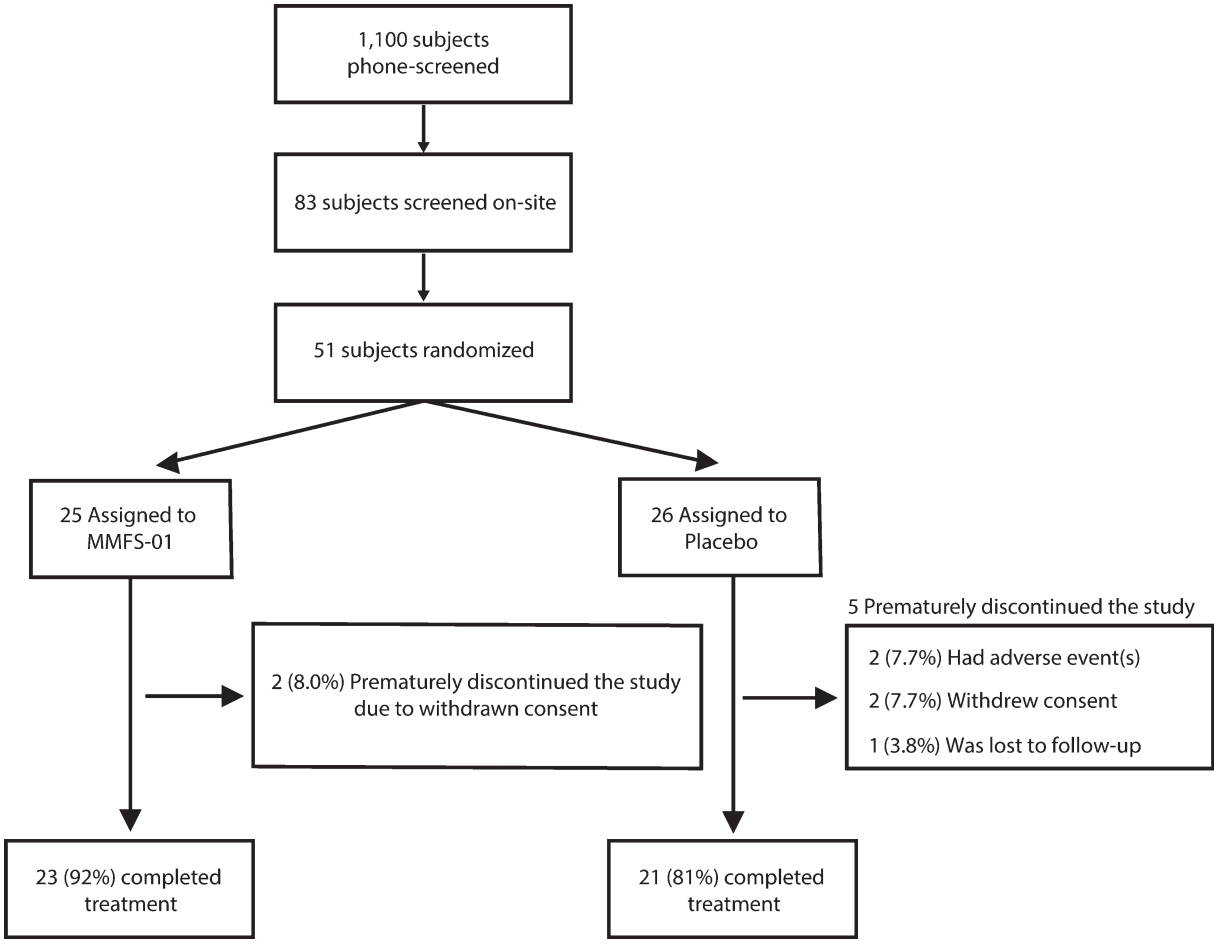

Fig. 1. Study assignment and outcomes. All subjects who withdrew were evaluated for the presence of an adverse event. If an adverse event was determined as the reason for withdrawn consent then "had adverse event(s)" was listed as the reason for premature discontinuation.

Table 2
Change from baseline in physiological measures

Endpoint	Baseline Score	Week 6 change from baseline	Week 6 p value (between groups)	Week 12 change from baseline	Week 12 p value (between groups)	Total Treatment p value
Physiological						
Mg^{2+} Urine (mg/ml)						
MMFS-01	0.061 ± 0.003	0.026 ± 0.007	0.140	0.025 ± 0.007	0.048*	0.027*
Placebo	0.062 ± 0.006	0.012 ± 0.006		0.005 ± 0.007		
$[Mg^{2+}]$ Plasma (mg/dL)						
MMFS-01	2.04 ± 0.033	0.100 ± 0.032	0.026*	0.065 ± 0.030	0.808	0.119
Placebo	2.06 ± 0.041	–0.002 ± 0.043		0.052 ± 0.035		
$[Mg^{2+}]_i$ RBC (mg/10^{11} cells)						
MMFS-01	1.15 ± 0.038	0.003 ± 0.026	0.217	0.032 ± 0.023	0.262	0.829
Placebo	1.19 ± 0.035	0.049 ± 0.026		–0.009 ± 0.021		

Mean ± SEM. *significant $p < 0.05$.

for testing cognitive deficits and improvements [39, 40].

MMFS-01 treatment resulted in a significant overall treatment effect in TMT-B ($p = 0.047$; Table 3 and Fig. 2A). Performance speed in TMT-B (Fig. 2A), reflecting executive function and cognitive processing, improved from baseline at Week 6. The mean improvement (MI) was 2.0 ± 0.8 ms^{-1} at Week 6 and 2.1 ± 0.8 ms^{-1} at Week 12 in the MMFS-01 group, corresponding to improvements of 19.1% (Week 6) and 19.9% (Week 12). There was little improvement from baseline in the placebo group at Week 6 (MI = 0.1 ± 0.5 ms^{-1}) and Week 12 (MI = 0.2 ± 0.8 ms^{-1}). These results correspond to an effect size (Cohen's d) of 0.58 at Week 6 and 0.51 at Week 12 (Table 3).

The DigitSpan test assesses working memory capacity. Subjects receiving MMFS-01 improved their DigitSpan scores (Table 3 and Fig. 2B) at Week 6 (MI = 1.61 ± 0.48 consecutive numbers) compared to those receiving placebo (MI = 0.10 ± 0.59 consecutive numbers). This difference was significant ($p = 0.023$, Cohen's $d = 0.61$), representing a 13.1% net improvement. At Week 12, the improvement persisted in the MMFS-01 group (MI = 1.43 ± 0.55 consecutive numbers), but there was an increase of the test scores in the placebo group (MI = 0.67 ± 0.54 consecutive numbers). Therefore, the difference between the MMFS-01 and placebo groups was not significantly different at Week 12 ($p = 0.225$).

We used the Flanker test (Table 3 and Fig. 2C) to evaluate attention capability. The opposite of the difference between incongruent and congruent test times was used to represent the test score (see Materials and Methods for explanation). We observed improved test scores relative to baseline in the MMFS-01 group, but the improvements were not statistically different from that of the placebo group at either Week 6 or Week 12, and there was not an overall treatment effect. Flanker test time improved by 34.9% (Week 6) and 38.2% (Week 12) in subjects receiving MMFS-01; however, times of subjects receiving placebo also improved at Week 6 (14.3%) and Week 12 (32.3%). These results suggest that there were significant training effects in this test, which reduced the test's utility for evaluating efficacy of treatment.

We used the Face-Name association test to evaluate episodic memory (Table 3 and Fig. 2D). In subjects receiving MMFS-01, test scores did not significantly change from baseline at Week 6 (7.1%, $p = 0.460$), but improved significantly from baseline at Week 12 (37.6%, $p = 0.003$). However, similarly, the test scores in the placebo group did not improve at Week 6, but increased from baseline by 16.2% at Week 12, although not significantly ($p = 0.207$). Despite a 21.4% net improvement at Week 12 with MMFS-01 treatment, improvement in the MMFS-01 group was not significantly better than improvement in the placebo group ($p = 0.089$, Cohen's $d = 0.44$).

Finally, to evaluate the overall cognitive ability of each subject, we calculated the composite score of all subjects at baseline, Week 6, and Week 12. Each individual score from each cognitive test was converted to a z score and the z scores from the four tests were averaged ($\bar{z}$) to obtain the composite score for each subject. The cognitive tests we selected evaluated major domains of overall cognitive ability (Table 3 and Fig. 2E). The composite score $\bar{z}$ of subjects treated with MMFS-01 improved significantly compared to placebo at Week 6 ($p = 0.017$) and Week 12 ($p = 0.003$), and had a significant overall treatment effect ($p = 0.001$). Subjects treated with MMFS-01 had a MI of 0.41 ± 0.12 $\bar{z}$ at Week 6 and 0.60 ± 0.13 $\bar{z}$ at Week 12 compared to 0.06 ± 0.08 $\bar{z}$ at Week 6 and 0.03 ± 0.14 $\bar{z}$ at Week 12 for subjects treated with placebo. The effect size was 0.74 at Week 6 and 0.91 at Week 12. Based on the typical scale for effect size where 0.2–0.5 is small, 0.5–0.8 is medium, and $\geq$0.8 is large [41], the improvement of overall cognitive ability induced by MMFS-01 treatment was robust [41].

To determine if improvement in overall cognitive ability persisted from Week 6 to Week 12 in individual subjects we plotted the composite score change from baseline at Week 6 versus the change from baseline at Week 12. The degree of improvement at Week 6 was significantly correlated with the degree of improvement from baseline at Week 12 ($R = 0.72$, $p < 0.001$; Supplemental Figure 1B). Conversely, in the placebo group, there was no correlation between change in composite score at Week 6 and change in composite score from baseline at Week 12 ($R = 0.07$, $p = 0.753$; Supplementary Figure 1A). This analysis suggests that the treatment effects of MMFS-01 persisted in individual subjects.

MMFS-01 treatment reduces fluctuation in overall cognitive ability

Fluctuation of cognitive ability is an early sign of cognitive impairment [42]. It is reported that 85% of MCI patients have fluctuations over time in their cognitive ability [43]. To evaluate if the subjects' cognitive ability fluctuated, for each subject we plotted the composite score change from baseline at Week 6 and

Table 3
Change from baseline in cognitive measures

Endpoint	Baseline Score (Mean ± SEM)	Week 6 change from baseline	Week 6 p value (between groups)	Week 6 Effect Size Cohen's d (95% C.I.)	Week 12 change from baseline	Week 12 p value (between groups)	Week 12 Effect Size Cohen's d (95% C.I.)	Total Treatment p value
Cognitive TMT-B (ms^{-1})								
MMFS-01	10.6 ± 1.0	2.0 ± 0.8	0.066	0.58 (–0.03–1.17)	2.1 ± 0.8	0.116	0.51 (–0.10–1.10)	0.047*
Placebo	11.2 ± 0.9	0.1 ± 0.5			0.2 ± 0.8			
DigitSpan (consecutive #s)								
MMFS-01	11.52 ± 0.59	1.61 ± 0.48	0.023*	0.61 (–0.01–1.20)	1.43 ± 0.55	0.225	0.30 (–0.3–0.89)	0.064
Placebo	11.05 ± 0.50	0.10 ± 0.59			0.67 ± 0.54			
Flanker: –1(Incongruent - Congruent) (s)								
MMFS-01	0.13 ± 0.03	0.04 ± 0.03	0.964	0.27 (–0.89–0.35)	0.05 ± 0.03	0.440	0.15 (–0.76–0.47)	0.660
Placebo	0.09 ± 0.02	0.01 ± 0.02			0.03 ± 0.02			
Face-Name (d')								
MMFS-01	1.70 ± 0.14	0.12 ± 0.16	0.484	0.10 (–0.51–0.72)	0.64 ± 0.19	0.089	0.44 (–0.18–1.05)	0.103
Placebo	1.57 ± 0.14	0.04 ± 0.18			0.25 ± 0.19			
Overall Cognitive Ability ($\bar{z}$)								
MMFS-01	–0.025 ± 0.12	0.41 ± 0.12	0.017*	0.74 (0.12–1.34)	0.60 ± 0.13	0.003**	0.91 (0.27–1.51)	0.001**
Placebo	–0.002 ± 0.11	0.06 ± 0.08			0.03 ± 0.14			

Mean ± SEM. *significant $p < 0.05$. **significant $p < 0.01$.

Week 12 (Fig. 3A-D). In the placebo group, subjects' composite scores changed dramatically both positively and negatively from baseline (Fig. 3A, C), confirming the existence of cognitive variance in subjects in the current study. Interestingly, in the MMFS-01 treated group, changes from baseline at both Week 6 and Week 12 were mostly positive (Fig. 3B, D). Thus, MMFS-01 treatment appeared to reduce negative fluctuations in overall cognitive ability.

To quantify the effect of MMFS-01 on the fluctuation of cognitive ability, we compared the variance of composite scores between MMFS-01 and placebo groups (Fig. 3E). We calculated variance of individual subjects' composite score between Week 6 and Week 12 (see Methods for equation). We did not use change from Baseline to Week 6 to avoid the pre-existing cognitive fluctuation prior to treatment. Variance of the composite scores in the placebo group was $\sigma^2 = 0.53$ whereas variance in MMFS-01 treated group was $\sigma^2 = 0.22$, a reduction of 57.6%. This analysis included all subjects, even those whose composite score did not improve at Week 6 ($n = 7$ of 23), so any delayed improvement that occurred from Week 6 to Week 12 contributed to this variance. When we only considered subjects whose composite score improved at Week 6 ($n = 16$ of 23), variance was even smaller ($\sigma^2 = 0.14$), representing a 72.8% reduction in variance (Fig. 3E). Therefore, MMFS-01 treatment might also help reduce cognitive fluctuation.

Change in intracellular magnesium predicted the improvement of cognitive abilities

We noticed that the composite scores of subjects in the treatment group did not improve uniformly and in particular, four subjects had little or no improvement after 12 weeks of treatment. Our pre-clinical studies indicate that the increase in intracellular magnesium concentration in neurons is essential for the increase in synapse density (unpublished data) and elevation of CSF magnesium is an important intermediary molecule in the mechanism of action through which our compound leads to an improvement in cognitive abilities [8]. In principle, the increase in intracellular magnesium in neurons should be a predictor of the improvement of cognitive abilities. Unfortunately, current technology does not permit safe quantification of intracellular magnesium of neurons in human. Therefore, we decided to use intracellular magnesium of RBCs as a surrogate marker. Although intracellular magnesium in peripheral cells may not be a true indicator of brain magnesium, it provided a reference for the loading effectiveness of magnesium into cells.

Remarkably, the percent change of RBC intracellular magnesium concentration predicted, with statistical significance, the enhancement in overall cognitive ability (composite score) in the MMFS-01 group ($R = 0.49$; $p = 0.021$; Fig. 3G), but not in the placebo group ($R = 0.22$; $p = 0.334$; Fig. 3F). Controlling for the effects of baseline composite score (see below), the correlation

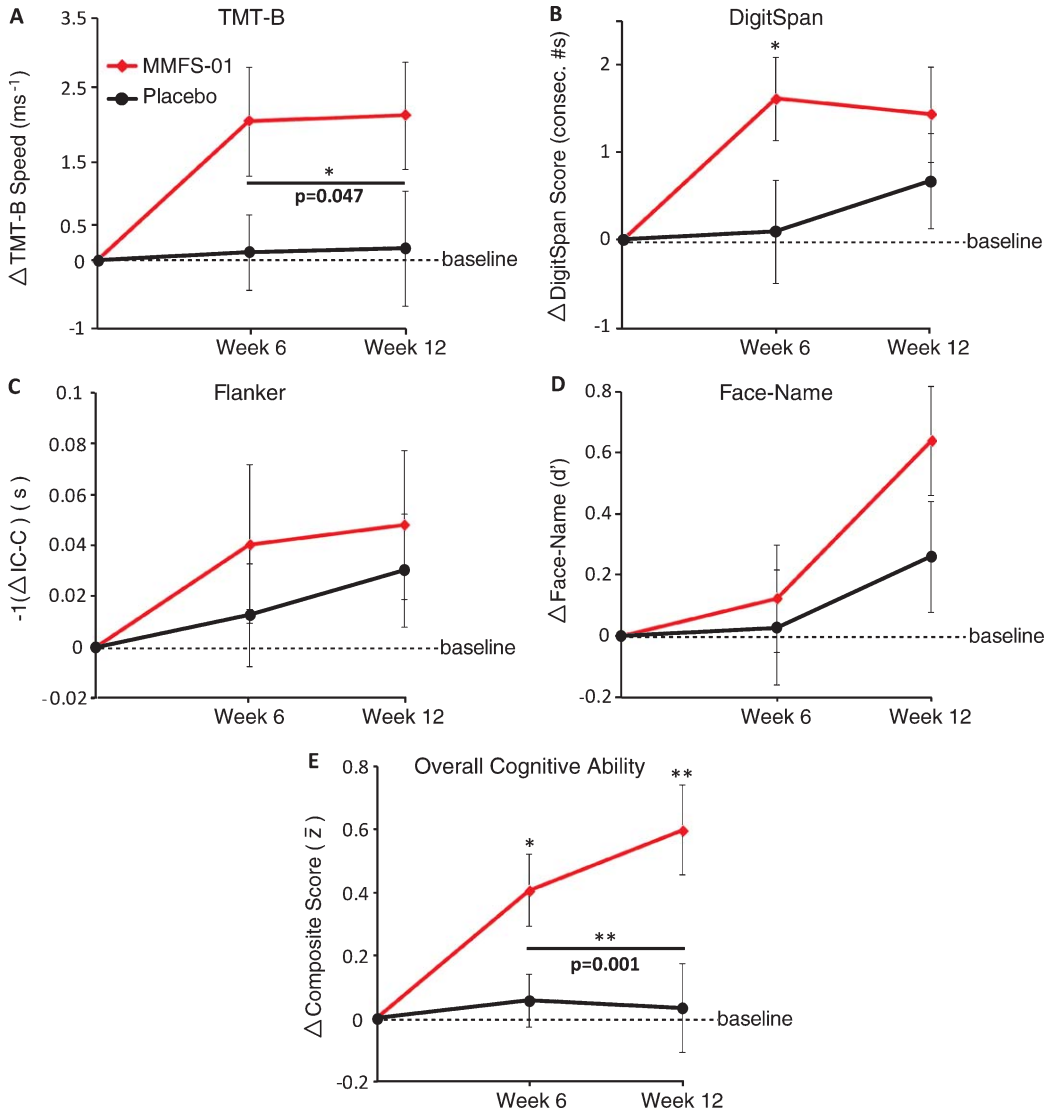

Fig. 2. Cognitive endpoints for MMFS-01 and placebo. Change from baseline (dashed line) was evaluated at Week 6 and Week 12 for MMFS-01 (red line) and placebo (black line) treated groups in four cognitive tests: TMT-B (A), DigitSpan (B), Flanker (C), and Face-Name (D). TMT-B is presented as speed (milliseconds) to complete 25 circle connections, DigitSpan as the number of consecutive numbers (consec. #s) repeated without error, Flanker as the opposite of the difference between Congruent time and Incongruent time –1 (IC-C) in seconds, and Face-Name as relative d' score. The opposite of change in IC-C is shown to illustrate positive change for improvement in the task. Overall cognitive ability (composite score) is the average of the z scores ($\bar{z}$) of the four cognitive tests, presented as the change in composite score from baseline (E). Asterisk over individual time points denotes significance between MMFS-01 and placebo only at that time point whereas asterisk over line between Week 6 and Week 12 denotes a significant overall treatment effect. $^{*}p < 0.05$, $^{**}p < 0.01$. All values are mean ± SEM.

between the percent change of RBC intracellular magnesium concentration and the change in composite score at Week 12 further improved (denoted as R' = 0.54; $p = 0.012$; Fig. 3G), with no significant change in the placebo group (R' = 0.25, $p = 0.294$; Fig. 3F).

There was also a small but non-significant inverse correlation between baseline composite score and the change in composite score at Week 12 in the MMFS-01 group (R = –0.34; $p = 0.126$; Fig. 3I), that was not present in the placebo group (R = –0.18; $p = 0.442$; Fig. 3H). Controlling for the percent change of intracellular magnesium, the correlation between baseline composite score and change in composite improved nearly to statistical significance in the MMFS-01 group

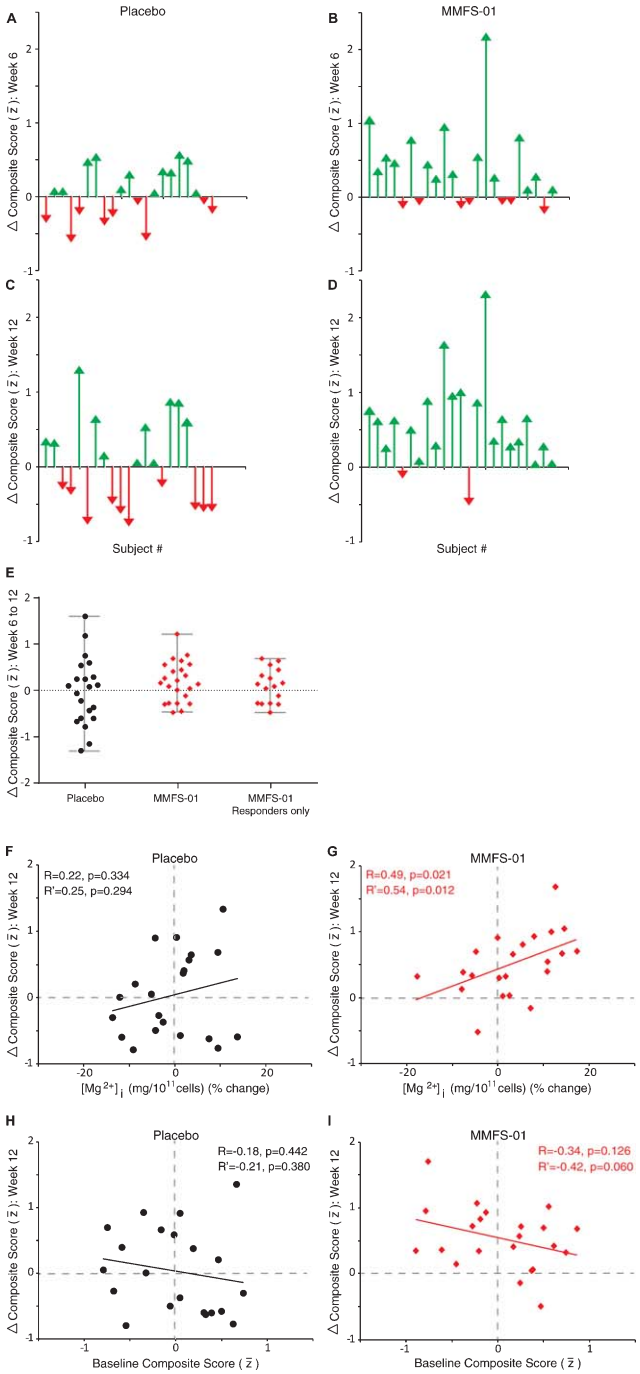

Fig. 3. Analysis of composite score fluctuation. A–D) Individual subject change from baseline composite score at Week 6 and Week 12. Each arrow represents an individual subject, ordered as subject number determined by the order in which each enrolled in the study. Green arrows indicate an increase from baseline in composite score and red arrows indicate a decrease from baseline in composite score. E) Change in composite score from Week 6 to Week 12 for each subject. Bars indicate range of data. Only subjects in the MMFS-01 group who had a positive composite score at Week 6 were included in the "Responders only" group (far right). F, G) Correlations (R) were determined between the percent change of RBC intracellular magnesium concentration and the change from baseline in composite score at Week 12 for placebo treated (F) and MMFS-01 treated (G) subjects. H, I) Correlations (R) were also determined between the baseline composite score and the change from baseline in composite score at Week 12 for placebo treated (H) and MMFS-01 treated (I) subjects. To eliminate contribution to the correlation from other factors, either percent change of RBC intracellular magnesium concentration or baseline composite score was controlled for while calculating each correlation. These correlations (not graphed) are denoted as R'.

(R' = –0.42; $p = 0.060$; Fig. 3I) but not in the placebo group (R' = –0.21; $p = 0.380$; Fig. 3H). These data suggest that MMFS-01 might be more effective at improving the overall cognitive ability of subjects with greater cognitive deficits.

Clinical significance of MMFS-01

Analysis of data from the cognitive tests demonstrated that the improvement of cognitive abilities by MMFS-01 treatment was statistically significant. We carried out further analysis to determine the clinical significance of MMFS-01 treatment. One way to quantify clinical significance is to determine how much cognitive deficit is reversed by comparing test scores with normative data of age-matched subjects. Unfortunately, normative data for our composite score is not available. However, normative data for TMT-B is available from cognitively competent subjects from age 18 to 89 years (referred to hereafter as Tombaugh study), and performance on TMT-B declines with age [44]. We compared results from our study with data from the Tombaugh study. Subjects in our study took significantly longer (125.7 ± 17.6 s) to complete the TMT-B task than age-matched (average age 50–70 years) cognitively normal subjects in the Tombaugh study (75.0 ± 1.3 s; $p < 0.0001$), confirming that subjects in our study indeed had executive function decline (Fig. 4A), and a mild cognitive impairment.

To quantify how much cognitive impairment was reversed, we plotted average speed of performance on TMT-B as a function of age. The youngest age group, age 18–24, performed the fastest, so all other age groups were normalized to the 18–24 age group. Strikingly, performance of cognitively normal subjects on the TMT-B task declined linearly with age (R = –0.99, $p = 10^{-8}$), at a rate of 1.04% per year (Fig. 4B). Average TMT-B speed for all subjects we studied was about 10% lower than age-matched controls. Following 12 weeks of MMFS-01 treatment there was an average increase of 10.3 ± 3.8% in TMT-B speed, such that their speed was close to that of their age-matched controls.

With this data, we assigned each subject a "brain age" that corresponded to that subject's speed relative to the normative TMT-B data. The difference between each subject's actual age and brain age was representative of the degree of executive function decline. For example, a 50-year-old subject who performed approximately 10% worse on the TMT-B test than a normal 50-year-old had a brain age that corresponded approximately to a cognitively normal 60-year-old, and therefore had a 10-year deficit. The average age of all subjects who completed the current study was 57.8 ± 0.8 years (Fig. 4B blue arrow), but their average brain age at baseline was 68.3 ± 3.0 years (Fig. 4B, red arrow), suggesting that the subjects in the current study had about 10 years of cognitive impairment. After 6 weeks of treatment, the average brain age of the MMFS-01 group deceased from 69.6 ± 4.2 years to 60.6 ± 5.6 years, an improvement of 9.0 ± 3.5 years (Fig. 4C, top right panel), and persisted after 12 weeks of treatment with 9.4 ± 3.5 years of improvement (Fig. 4B, green arrow; 4C, bottom right panel). In contrast, there was little change in the average brain age in the placebo group, improving 0.6 ± 2.3 years at Week 6 (Fig. 4C, top left panel) and 0.8 ± 3.5 years at Week 12 (Fig. 4C, bottom left panel). These data demonstrate that MMFS-01 treatment was effective in our subjects at reversing cognitive impairment almost back to normal ability relative to age.

Using elevation of RBC intracellular magnesium as a biomarker to screen for responders, we found that 15 of 22 subjects in the MMFS-01 group (68.2%) responded to MMFS-01 treatment. When the brain age of only the responders was calculated, the improvement at Week 12 was 14.6 ± 3.9 years, indicating an even greater reduction in cognitive impairment among magnesium responders than all subjects receiving MMFS-01. On the other hand, these data also show approximately 30% of the subjects did not respond to MMFS-01 treatment.

Safety and tolerability

The effects of MMFS-01 on sleep quality and emotion

We also evaluated the effects of MMFS-01 treatment on neuropsychiatric symptoms (Table 4). Subjects in the placebo group had significant changes in affect, anxiety, and sleep at Week 6 and Week 12, as reflected by the subjective tests HAM-A, PANAS: Positive Affect, PANAS: Negative Affect, and PSQI, suggesting that there were significant placebo effects on all three. MMFS-01 treatment had similar effects on affect, anxiety, and sleep, but was not significantly better or worse than placebo. Therefore, in this trial, using these subjective measures, MMFS-01 treatment did not have an effect on sleep or anxiety. Importantly though, MMFS-01 treatment did not make anxiety, sleep disorder, or affect worse.

Adverse events

The safety population was composed of 25 subjects in the MMFS-01 group and 26 subjects in the placebo group. A total of 47 adverse events were observed

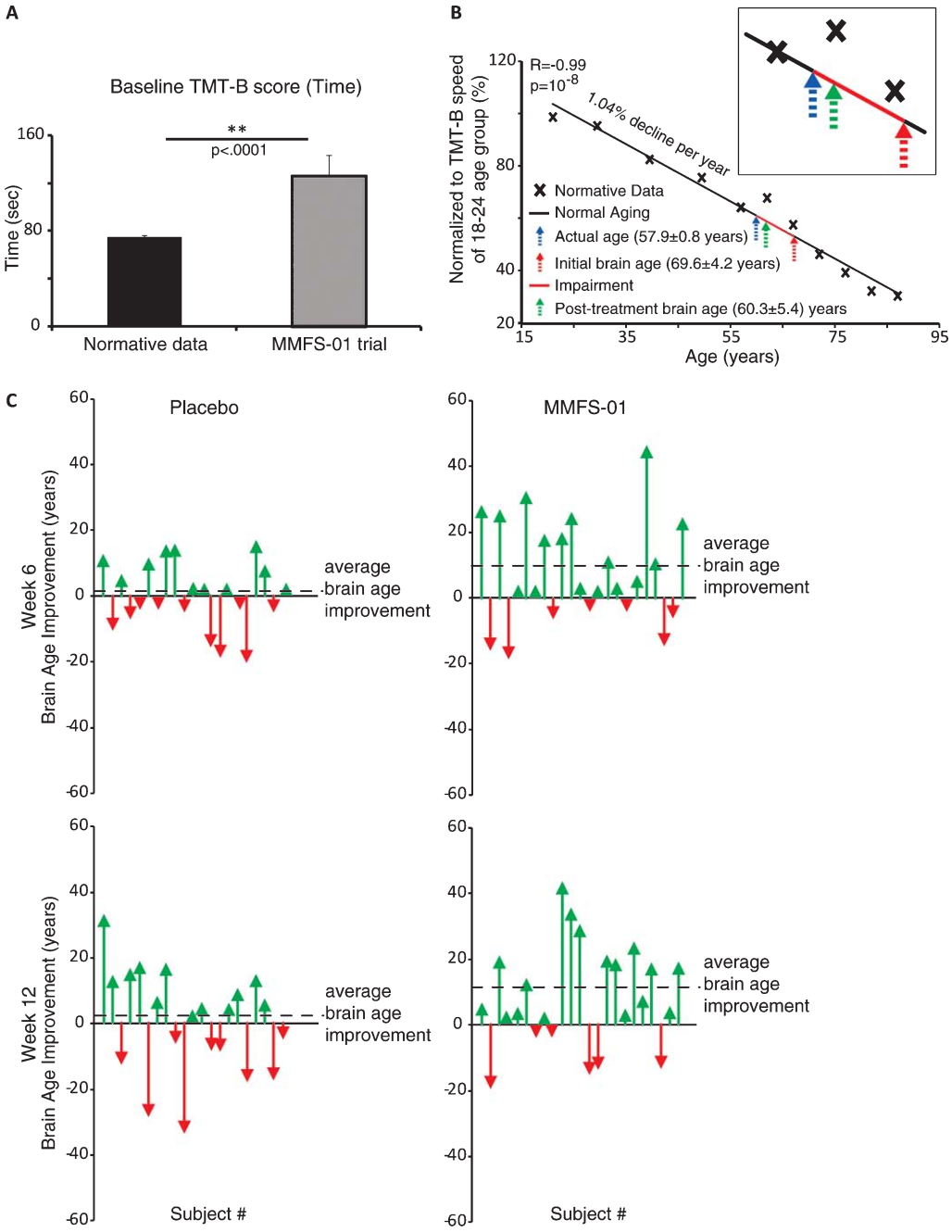

Fig. 4. Reversal of executive function deficits in MMFS-01 treated subjects. A) Average TMT-B time was compared to age-matched normative data [44]. B) Relationship between age and normalized TMT-B speed (percent normalized to peak speed; 100% = 18–24 age group) was graphed from normative data (ages 18–89 separated in 11 different age groups). TMT-B speed declines linearly ($R = -0.99$, $p = 10^{-8}$) at a rate of 1.04% per year (black line). Shown on the graph are the location where TMT-B speed corresponds to the average actual age of all subjects in the study (blue arrow), the initial brain age of subjects in the MMFS-01 group (red arrow), and the brain age of subjects following 12 weeks of MMFS-01 treatment (green arrow). The average impairment in brain age of the subjects at the beginning of the trial, relative to age-matched controls from the normative data set, is depicted along the linear trendline (red line). The area of the graph corresponding to the age range of subjects in the study (50–70 years) is enlarged in the inset. C) Change in brain age from baseline for each subject in the MMFS-01 group at Week 6 (top right panel) and Week 12 (bottom right panel) and placebo group at Week 6 (top left panel) and Week 12 (bottom left panel). Each arrow indicates an individual subject, ordered as subject number determined by the order in which each enrolled in the study. Green arrows indicate brain age improvement and red arrows indicate brain age decline relative to baseline. The average brain age improvement is indicated by a dashed line.

Table 4
Change from baseline in emotional and sleep measures

Endpoint	Baseline Score	Week 6			Week 12		
		change from baseline	*p* value (change from baseline)	*p* value (between group difference)	change from baseline	*p* value (change from baseline)	*p* value (between group difference)
Emotional							
HAM-A							
MMFS-01	17.1 ± 3.0	–6.3 ± 3.6	<0.001***	0.876	–7.3 ± 5.8	<0.001***	0.396
Placebo	17.2 ± 2.5	–6.1 ± 3.4	<0.001***		–8.6 ± 4.1	<0.001***	
PANAS: Positive Affect							
MMFS-01	28.3 ± 6.6	4.7 ± 5.2	<0.001***	0.648	3.9 ± 7.0	0.014*	0.596
Placebo	27.7 ± 8.1	3.8 ± 8.1	0.046*		5.1 ± 8.2	0.01*	
PANAS: Negative Affect							
MMFS-01	24.8 ± 6.4	–7.6 ± 7.5	<0.001***	0.556	–8.3 ± 8.9	0.001**	0.371
Placebo	23.9 ± 6.0	–6.3 ± 5.9	<0.001***		–6.4 ± 9.0	0.008**	
Sleep							
PSQI							
MMFS-01	13.7 ± 2.6	–4.2 ± 3.8	<0.001***	0.415	–4.8 ± 4.5	<0.001***	0.279
Placebo	12.9 ± 2.4	–3.2 ± 3.7	<0.001***		–6.1 ± 3.3	<0.001***	

Mean ± SEM. *significant $p < 0.05$. **significant $p < 0.01$. ***significant $p < 0.001$.

Table 5
All adverse events observed in the study

Adverse Event	# of Events		# of Subjects	
	MMFS-01 ($n = 25$)	Placebo ($n = 26$)	MMFS-01 ($n = 25$)	Placebo ($n = 26$)
Gastrointestinal disorders	5	6	5	4
General disorders and administration site conditions	0	2	0	2
infections and infestations	4	7	4	6
injury, poisoning and procedural complications	0	1	0	1
Musculoskeletal and connective tissue disorders	3	0	3	0
Nervous system disorders	1	7	1	5
Psychiatric disorders	1	3	1	3
Respiratory, thoracic and mediastinal disorders	2	2	2	1
Skin and subcutaneous tissue disorders	0	1	0	1
Surgical and medical procedures	0	1	0	1
Vascular disorders	1	0	1	0
All Organ Systems	17	30	13	15

among 28 of the 51 subjects in the safety population, experienced approximately equally among subjects in placebo and MMFS-01 groups (15 and 13 subjects, respectively; Table 5). Individual events were considerably more prevalent in the placebo group than in the MMFS-01 group (30 and 17 events, respectively). Most adverse events were mild, and no serious adverse events were observed during the course of the study. No significant changes in body weight, systolic blood pressure, diastolic blood pressure, or heart rate were observed. For additional tolerability and safety information, see Materials and Methods section.

DISCUSSION

This study sought to determine the effects of MMFS-01, a synapse density enhancer, on cognitive ability, sleep disorder, and anxiety in older adult subjects with cognitive impairment. The inclusion criteria we chose, including SMC, sleep disorder and anxiety, of which the latter two are strongly associated with cognitive impairment [18, 20], enriched our population for those who had an underlying cognitive impairment. Indeed, the subjects recruited in this study had a mild cognitive impairment (approximately 10 years) compared to age-matched controls from normative data (Fig. 4). Therefore, the results from this study are more appropriately interpreted as a reduction of cognitive impairment than as an enhancement of cognitive function.

MMFS-01 treatment resulted in an improvement in multiple individual cognitive domains. After 6 weeks of treatment with MMFS-01, we observed improvements in executive function (TMT-B), and working memory (DigitSpan), both associated with

the prefrontal cortex, and after 12 weeks, we observed improvement in episodic memory, associated with the hippocampus [11, 12]. These observations suggest that the mechanisms of action of MMFS-01 might work at different time scales in different brain areas.

Although there was a significant overall improvement in executive function ($p = 0.047$, Cohen's $d = 0.51$ at Week 12), it was unclear how this improvement would impact the subject's daily function. It is common practice in evaluating clinical trial data to not only evaluate effect size but to also evaluate clinical significance. For example, in Parkinson's disease clinical trials, a total score change of 8 points in the Unified Parkinson's Disease Rating Scale is considered to be clinically significant because it demonstrates a meaningful functional improvement in patients' quality of life even though statistical significance can be achieved with a lower score change [45]. Here, we show that the improvement on the TMT-B test reflected an approximate 9-year improvement in executive function, which might have a meaningful effect on the subject's quality of life.

Even though the improvement in executive function was clinically significant, each of the individual cognitive tests only provided information on one cognitive domain. To evaluate the efficacy of new therapies for reducing cognitive impairment, one needs to evaluate the change of overall cognitive ability. Conventionally, overall cognitive ability is determined by a composite score calculated from a set of cognitive tests [46, 47]. Currently, a standardized set of individual cognitive tests has not been established. Cognitive domains that decline significantly with age include attention/working memory, executive function, episodic memory, and visuo-spatial ability [46]. Several attempts have been made to establish composite tests that can be used to quantify overall cognitive abilities across these domains. The Alzheimer's Disease Cooperative Study-Preclinical Alzheimer Cognitive Composite (ADCS-PACC), the Alzheimer's Prevention Initiative composite cognitive test score, and the UCSD Performance-Based Skills Assessment (UPSA) are some of the examples [25, 48, 49]. Our study evaluated the same cognitive domains (albeit without evaluation of visuo-spatial ability). Each test we chose to evaluate specific domain function is extensively used and highly sensitive, as each has a large dynamic range with limited ceiling and floor effects [46]. Thus, the composite score in the current study should be valid to represent overall cognitive ability.

We determined that MMFS-01 improved overall cognitive ability (composite score) both in absolute terms and compared to the placebo group. The effect size for change in overall cognitive ability was robust ($d = 0.91$ at Week 12), generating enough statistical power in a sample size of only 44 subjects. The effect size was significantly larger for overall cognitive ability than for individual cognitive tests. The possible interpretation is that subjects in the MMFS-01 group who improved in one domain typically improved in the other domains, whereas, in the placebo group, subjects who improved in one domain often had no change or decreased in the other domains.

The consistency in improvement among the different cognitive domains for individual subjects in the MMFS-01 group was in line with our observation that MMFS-01 reduced fluctuations in cognitive ability (Fig. 3). Cognitive fluctuation is a known phenomenon in those with cognitive impairment and is likely due to variations in the activity of neural networks [42, 43]. These fluctuations can have a dramatic impact on subject's performance on cognitive testing over time [50]. Indeed, large fluctuations in cognitive ability over time were observed in subjects in the placebo group. The reduction in cognitive fluctuation with MMFS-01 treatment might be a more noteworthy observation than the increase in overall cognitive ability because from a clinical perspective it might be most important to reduce the number of "bad days" a patient has. This might result in higher overall functionality and quality of life. If so, the reduction of cognitive fluctuation may be a meaningful outcome measure and could be included as an efficacy endpoint in future clinical trials.

Since not all subjects responded to MMFS-01 treatment, a biomarker that predicts responders would be ideal in a clinical setting. Our pre-clinical work indicates that the mechanism of action of MMFS-01 is increased structural and functional synapse density, mediated by an elevation of neuronal intracellular magnesium concentration [7, 8]. Consistent with this mechanism, we identified a biomarker—the percent change in RBC magnesium concentration at 12 weeks of treatment—that predicted treatment response (or lack thereof). If confirmed, the biomarker can potentially be used to predict the outcome of MMFS-01 therapy and help identify the subjects who are more likely to benefit from MMFS-01 treatment.

At the onset of this trial, in addition to determining the effects of MMFS-01 treatment on cognitive ability, we also sought to determine its effects on emotion and sleep. The large placebo effects observed in this study, typical in these types of trials [51], is unfortunate because it prevented us from determining the true effects of MMFS-01 on emotion and sleep (Table 4).

With the current subjective measures it is difficult to determine the effects of MMFS-01 on emotion and sleep. One possible way to solve this problem is to use objective evaluators of emotion. For example, our animal studies show that L-TAMS treatment can enhance fear extinction [13], and such experiments can be done in humans [52]. We plan to use more objective measures to test the effects of MMFS-01 treatment on anxiety and sleep in a future trial.

This trial also evaluated the safety of MMFS-01. Importantly, the adverse event profile was similar between the MMFS-01 and placebo groups, with nearly all events classified as mild and none as serious. This is promising because any treatment for age-related cognitive decline or any other pre-AD cognitive impairment needs to have an extremely good safety and tolerance profile, since patients will likely take the medicine for many years.

There is currently no effective way to reverse age-related cognitive decline or MCI. Numerous efforts with different approaches have had minimal effect. For example, pharmacological or dietary supplemental treatments using cholinesterase inhibitors, statins, or vitamin E are ineffective at reducing cognitive deficits or delaying onset of AD in MCI patients [53–56]. The only treatment showing consistent positive results is physical exercise, but with a modest effect size (in the Cohen's $d = 0.30$ range) [57–59]. Recent studies utilizing mental exercise therapy have shown some exciting potential [60–65], albeit with small effect sizes. Interestingly, we found in our pre-clinical studies that a combination of L-TAMS treatment and environmental enrichment/physical exercise can further enhance the cognitive ability of aging rodents (unpublished observation).

Study limitations

Although this study showed strong efficacy of MMFS-01 for improving cognitive ability, one caution we have is that the population of subjects we studied not only had cognitive impairment but also had common neuropsychiatric symptoms including anxiety and sleep disorder. Therefore, we do not know whether our compound will be equally effective in people with cognitive impairment but without neuropsychiatric symptoms. Nevertheless, since approximately 50% of MCI patients have anxiety [22], even if MMFS-01 only works for this subtype of MCI patients, it still would represent a significant portion of MCI patients. There are several other limitations to our study that should be considered when interpreting the results. One of them is the relatively small sample size recruited at only one study site. Due to the inhomogeneity of human genetic background and environment, a larger trial size with more geographical locations and more ethnic diversity is needed. Another important limitation is trial length. A longer trial will help determine the long-term outcome of MMFS-01 treatment and whether MMFS-01 will delay onset to AD/dementia. Finally, it is not known if MMFS-01 can reverse cognitive impairment in those with more severe cognitive deficits, such as AD. To evaluate this, we are currently testing MMFS-01 in another trial with mild and moderate AD patients.

CONCLUSIONS

In summary, the current study demonstrated efficacy of MMFS-01, a compound designed to increase brain synapse density, on restoration of cognitive abilities. This study highlights the importance of increasing neuronal intracellular magnesium, a key intermediary of synapse density control, on improving cognitive abilities in older adults.

ACKNOWLEDGMENTS

We thank the volunteers and site staff who participated in this study, including Dr. John Pezzullo, MRA, for creating the randomization schedule and log, carrying out statistical analysis on emotion and sleep endpoints, and generating the clinical project report. We acknowledge and thank Dr. Diane Krieger, Dr. Douglas Kalman, Mr. Adam Samson, and Ms. Samantha Feldman, MRA, for study execution, data collection, and statistical analysis and interpretation of safety data (including adverse events), and subjective endpoints (including anxiety, mood, and sleep quality). We thank Dr. Alan Garfinkel and Dr. Nick Wisniewski, University of California Los Angeles, for their help with statistical bootstrapping analysis. We also thank Dr. Jack Feldman, University of California Los Angeles, for his critical review and helpful insight of the manuscript.

This work is supported by Neurocentria, Inc.

Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/15-0538r2).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-150538.

REFERENCES

[1] Jack CR Jr, Wiste HJ, Weigand SD, Knopman DS, Vemuri P, Mielke MM, Lowe V, Senjem ML, Gunter JL, Machulda MM, Gregg BE, Pankratz VS, Rocca WA, Petersen RC (2015) Age, sex, and APOE epsilon4 effects on memory, brain structure, and beta-amyloid across the adult life span. *JAMA Neurol* **72**, 511-519.

[2] Perneczky R, Pohl C, Sorg C, Hartmann J, Tosic N, Grimmer T, Heitele S, Kurz A (2006) Impairment of activities of daily living requiring memory or complex reasoning as part of the MCI syndrome. *Int J Geriatr Psychiatry* **21**, 158-162.

[3] Farias ST, Mungas D, Reed BR, Harvey D, Cahn-Weiner D, Decarli C (2006) MCI is associated with deficits in everyday functioning. *Alzheimer Dis Assoc Disord* **20**, 217-223.

[4] Fox NC, Scahill RI, Crum WR, Rossor MN (1999) Correlation between rates of brain atrophy and cognitive decline in AD. *Neurology* **52**, 1687-1689.

[5] Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R (1991) Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Ann Neurol* **30**, 572-580.

[6] Slutsky I, Sadeghpour S, Li B, Liu G (2004) Enhancement of synaptic plasticity through chronically reduced Ca2+ flux during uncorrelated activity. *Neuron* **44**, 835-849.

[7] Zhou H, Liu G (2015) Regulation of density of functional presynaptic terminals by local energy supply. *Mol Brain* **8**, 42.

[8] Slutsky I, Abumaria N, Wu LJ, Huang C, Zhang L, Li B, Zhao X, Govindarajan A, Zhao MG, Zhuo M, Tonegawa S, Liu G (2010) Enhancement of learning and memory by elevating brain magnesium. *Neuron* **65**, 165-177.

[9] Kim YJ, McFarlane C, Warner DS, Baker MT, Choi WW, Dexter F (1996) The effects of plasma and brain magnesium concentrations on lidocaine-induced seizures in the rat. *Anesth Analg* **83**, 1223-1228.

[10] McKee JA, Brewer RP, Macy GE, Phillips-Bute B, Campbell KA, Borel CO, Reynolds JD, Warner DS (2005) Analysis of the brain bioavailability of peripherally administered magnesium sulfate: A study in humans with acute brain injury undergoing prolonged induced hypermagnesemia. *Crit Care Med* **33**, 661-666.

[11] Burgess N, Maguire EA, O'Keefe J (2002) The human hippocampus and spatial and episodic memory. *Neuron* **35**, 625-641.

[12] Kane MJ, Engle RW (2002) The role of prefrontal cortex in working-memory capacity, executive attention, and general fluid intelligence: An individual-differences perspective. *Psychon Bull Rev* **9**, 637-671.

[13] Abumaria N, Yin B, Zhang L, Li XY, Chen T, Descalzi G, Zhao L, Ahn M, Luo L, Ran C, Zhuo M, Liu G (2011) Effects of elevation of brain magnesium on fear conditioning, fear extinction, and synaptic plasticity in the infralimbic prefrontal cortex and lateral amygdala. *J Neurosci* **31**, 14871-14881.

[14] Li W, Yu J, Liu Y, Huang X, Abumaria N, Zhu Y, Xiong W, Ren C, Liu XG, Chui D, Liu G (2014) Elevation of brain magnesium prevents synaptic loss and reverses cognitive deficits in Alzheimer's disease mouse model. *Mol Brain* **7**, 65.

[15] Abumaria N, Luo L, Ahn M, Liu G (2013) Magnesium supplement enhances spatial-context pattern separation and prevents fear overgeneralization. *Behav Pharmacol* **24**, 255-263.

[16] Mitchell AJ (2008) The clinical significance of subjective memory complaints in the diagnosis of mild cognitive impairment and dementia: A meta-analysis. *Int J Geriatr Psychiatry* **23**, 1191-1202.

[17] Lee GJ, Lu PH, Hua X, Lee S, Wu S, Nguyen K, Teng E, Leow AD, Jack CR Jr, Toga AW, Weiner MW, Bartzokis G, Thompson PM, Alzheimer's Disease Neuroimaging, Initiative (2012) Depressive symptoms in mild cognitive impairment predict greater atrophy in Alzheimer's disease-related regions. *Biol Psychiatry* **71**, 814-821.

[18] Bierman EJ, Comijs HC, Jonker C, Beekman AT (2007) Symptoms of anxiety and depression in the course of cognitive decline. *Dement Geriatr Cogn Disord* **24**, 213-219.

[19] Schaie KW, Willis SL, Caskie GI (2004) The Seattle longitudinal study: Relationship between personality and cognition. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn* **11**, 304-324.

[20] Mander BA, Rao V, Lu B, Saletin JM, Lindquist JR, Ancoli-Israel S, Jagust W, Walker MP (2013) Prefrontal atrophy, disrupted NREM slow waves and impaired hippocampal-dependent memory in aging. *Nat Neurosci* **16**, 357-364.

[21] Chaves da Silva RAP (2015) Sleep disturbances and mild cognitive impairment: A review. *Sleep Science* **8**, 36-41.

[22] Apostolova LG, Di LJ, Duffy EL, Brook J, Elashoff D, Tseng CH, Fairbanks L, Cummings JL (2014) Risk factors for behavioral abnormalities in mild cognitive impairment and mild Alzheimer's disease. *Dement Geriatr Cogn Disord* **37**, 315-326.

[23] Palmer K, Berger AK, Monastero R, Winblad B, Backman L, Fratiglioni L (2007) Predictors of progression from mild cognitive impairment to Alzheimer disease. *Neurology* **68**, 1596-1602.

[24] Ju YE, McLeland JS, Toedebusch CD, Xiong C, Fagan AM, Duntley SP, Morris JC, Holtzman DM (2013) Sleep quality and preclinical Alzheimer disease. *JAMA Neurol* **70**, 587-593.

[25] Langbaum JB, Hendrix SB, Ayutyanont N, Chen K, Fleisher AS, Shah RC, Barnes LL, Bennett DA, Tariot PN, Reiman EM (2014) An empirically derived composite cognitive test score with improved power to track and evaluate treatments for preclinical Alzheimer's disease. *Alzheimers Dement* **10**, 666-674.

[26] Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ (1989) The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatry Res* **28**, 193-213.

[27] Amsterdam JD, Li Y, Soeller I, Rockwell K, Mao JJ, Shults J (2009) A randomized, double-blind, placebo-controlled trial of oral Matricaria recutita (chamomile) extract therapy for generalized anxiety disorder. *J Clin Psychopharmacol* **29**, 378-382.

[28] Arbuthnott K, Frank J (2000) Trail making test, part B as a measure of executive control: Validation using a set-switching paradigm. *J Clin Exp Neuropsychol* **22**, 518-528.

[29] Eriksen B, Eriksen C (1974) Effects of noise letters upon the identification of a target letter in a nonsearch task. *Percept Psychophys* **16**, 143-149.

[30] Zeineh MM, Engel SA, Thompson PM, Bookheimer SY (2003) Dynamics of the hippocampus during encoding and retrieval of face-name pairs. *Science* **299**, 577-580.
[31] Ashendorf L, Jefferson AL, O'Connor MK, Chaisson C, Green RC, Stern RA (2008) Trail Making Test errors in normal aging, mild cognitive impairment, and dementia. *Arch Clin Neuropsychol* **23**, 129-137.
[32] Kurt P, Yener G, Oguz M (2011) Impaired digit span can predict further cognitive decline in older people with subjective memory complaint: A preliminary result. *Aging Ment Health* **15**, 364-369.
[33] James LE, Fogler KA, Tauber SK (2008) Recognition memory measures yield disproportionate effects of aging on learning face-name associations. *Psychol Aging* **23**, 657-664.
[34] Hamilton M (1959) The assessment of anxiety states by rating. *Br J Med Psychol* **32**, 50-55.
[35] Watson D, Clark LA, Tellegen A (1988) Development and validation of brief measures of positive and negative affect: The PANAS scales. *J Pers Soc Psychol* **54**, 1063-1070.
[36] Walker AF, Marakis G, Christie S, Byng M (2003) Mg citrate found more bioavailable than other Mg preparations in a randomised, double-blind study. *Magnes Res* **16**, 183-191.
[37] Muhlbauer B, Schwenk M, Coram WM, Antonin KH, Etienne P, Bieck PR, Douglas FL (1991) Magnesium-L-aspartate-HCl and magnesium-oxide: Bioavailability in healthy volunteers. *Eur J Clin Pharmacol* **40**, 437-438.
[38] Dubois B, Feldman HH, Jacova C, Cummings JL, Dekosky ST, Barberger-Gateau P, Delacourte A, Frisoni G, Fox NC, Galasko D, Gauthier S, Hampel H, Jicha GA, Meguro K, O'Brien J, Pasquier F, Robert P, Rossor M, Salloway S, Sarazin M, de Souza LC, Stern Y, Visser PJ, Scheltens P (2010) Revising the definition of Alzheimer's disease: A new lexicon. *Lancet Neurol* **9**, 1118-1127.
[39] Donohue MC, Sperling RA, Salmon DP, Rentz DM, Raman R, Thomas RG, Weiner M, Aisen PS (2014) The preclinical Alzheimer cognitive composite: Measuring amyloid-related decline. *JAMA Neurol* **71**, 961-970.
[40] Food and Drug Administration (2013) FDA Guidance for Industry. Alzheimer's disease: Developing drugs for the treatment of early stage disease. Food and Drug Administration, Washington, DC.
[41] Cohen J (2013) Statistical power analysis for the behavioral sciences, Academic press.
[42] Palop JJ, Chin J, Mucke L (2006) A network dysfunction perspective on neurodegenerative diseases. *Nature* **443**, 768-773.
[43] Kaduszkiewicz H, Eisele M, Wiese B, Prokein J, Luppa M, Luck T, Jessen F, Bickel H, Mosch E, Pentzek M, Fuchs A, Eifflaender-Gorfer S, Weyerer S, Konig HH, Brettschneider C, van den Bussche H, Maier W, Scherer M, Riedel-Heller SG (2014) Prognosis of mild cognitive impairment in general practice: Results of the German AgeCoDe study. *Ann Fam Med* **12**, 158-165.
[44] Tombaugh TN (2004) Trail Making Test A and B: Normative data stratified by age and education. *Arch Clin Neuropsychol* **19**, 203-214.
[45] Schrag A, Sampaio C, Counsell N, Poewe W (2006) Minimal clinically important change on the unified Parkinson's disease rating scale. *Mov Disord* **21**, 1200-1207.
[46] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 270-279.
[47] Huang Y, Ito K, Billing CB Jr, Anziano RJ (2015) Development of a straightforward and sensitive scale for MCI and early AD clinical trials. *Alzheimers Dement* **11**, 404-414.
[48] Goldberg TE, Koppel J, Keehlisen L, Christen E, Dreses-Werringloer U, Conejero-Goldberg C, Gordon ML, Davies P (2010) Performance-based measures of everyday function in mild cognitive impairment. *Am J Psychiatry* **167**, 845-853.
[49] Ayutyanont N, Langbaum JB, Hendrix SB, Chen K, Fleisher AS, Friesenhahn M, Ward M, Aguirre C, Acosta-Baena N, Madrigal L, Munoz C, Tirado V, Moreno S, Tariot PN, Lopera F, Reiman EM (2014) The Alzheimer's prevention initiative composite cognitive test score: Sample size estimates for the evaluation of preclinical Alzheimer's disease treatments in presenilin 1 E280A mutation carriers. *J Clin Psychiatry* **75**, 652-660.
[50] Escandon A, Al-Hammadi N, Galvin JE (2010) Effect of cognitive fluctuation on neuropsychological performance in aging and dementia. *Neurology* **74**, 210-217.
[51] Huedo-Medina TB, Kirsch I, Middlemass J, Klonizakis M, Siriwardena AN (2012) Effectiveness of non-benzodiazepine hypnotics in treatment of adult insomnia: Meta-analysis of data submitted to the Food and Drug Administration. *BMJ* **345**, e8343.
[52] Schiller D, Delgado MR (2010) Overlapping neural systems mediating extinction, reversal and regulation of fear. *Trends Cogn Sci* **14**, 268-276.
[53] Russ TC, Morling JR (2012) Cholinesterase inhibitors for mild cognitive impairment. *Cochrane Database Syst Rev* **9**, CD009132.
[54] Bettermann K, Arnold AM, Williamson J, Rapp S, Sink K, Toole JF, Carlson MC, Yasar S, Dekosky S, Burke GL (2012) Statins, risk of dementia, and cognitive function: Secondary analysis of the ginkgo evaluation of memory study. *J Stroke Cerebrovasc Dis* **21**, 436-444.
[55] Farina N, Isaac MG, Clark AR, Rusted J, Tabet N (2012) Vitamin E for Alzheimer's dementia and mild cognitive impairment. *Cochrane Database Syst Rev* **11**, CD002854.
[56] Dysken MW, Sano M, Asthana S, Vertrees JE, Pallaki M, Llorente M, Love S, Schellenberg GD, McCarten JR, Malphurs J, Prieto S, Chen P, Loreck DJ, Trapp G, Bakshi RS, Mintzer JE, Heidebrink JL, Vidal-Cardona A, Arroyo LM, Cruz AR, Zachariah S, Kowall NW, Chopra MP, Craft S, Thielke S, Turvey CL, Woodman C, Monnell KA, Gordon K, Tomaska J, Segal Y, Peduzzi PN, Guarino PD (2014) Effect of vitamin E and memantine on functional decline in Alzheimer disease: The TEAM-AD VA cooperative randomized trial. *JAMA* **311**, 33-44.
[57] Chapman SB, Aslan S, Spence JS, Defina LF, Keebler MW, Didehbani N, Lu H (2013) Shorter term aerobic exercise improves brain, cognition, and cardiovascular fitness in aging. *Front Aging Neurosci* **5**, 75.
[58] Colcombe S, Kramer AF (2003) Fitness effects on the cognitive function of older adults: A meta-analytic study. *Psychol Sci* **14**, 125-130.
[59] McDonnell MN, Smith AE, Mackintosh SF (2011) Aerobic exercise to improve cognitive function in adults with neurological disorders: A systematic review. *Arch Phys Med Rehabil* **92**, 1044-1052.
[60] Anguera JA, Boccanfuso J, Rintoul JL, Al-Hashimi O, Faraji F, Janowich J, Kong E, Larraburo Y, Rolle C, Johnston E, Gazzaley A (2013) Video game training enhances cognitive control in older adults. *Nature* **501**, 97-101.

[61] Rebok GW, Ball K, Guey LT, Jones RN, Kim HY, King JW, Marsiske M, Morris JN, Tennstedt SL, Unverzagt FW, Willis SL (2014) Ten-year effects of the advanced cognitive training for independent and vital elderly cognitive training trial on cognition and everyday functioning in older adults. *J Am Geriatr Soc* **62**, 16-24.

[62] Smith GE, Housen P, Yaffe K, Ruff R, Kennison RF, Mahncke HW, Zelinski EM (2009) A cognitive training program based on principles of brain plasticity: Results from the Improvement in Memory with Plasticity-based Adaptive Cognitive Training (IMPACT) study. *J Am Geriatr Soc* **57**, 594-603.

[63] Cheng ST, Chow PK, Song YQ, Yu EC, Chan AC, Lee TM, Lam JH (2014) Mental and physical activities delay cognitive decline in older persons with dementia. *Am J Geriatr Psychiatry* **22**, 63-74.

[64] Bozoki A, Radovanovic M, Winn B, Heeter C, Anthony JC (2013) Effects of a computer-based cognitive exercise program on age-related cognitive decline. *Arch Gerontol Geriatri* **57**, 1-7.

[65] Owen AM, Hampshire A, Grahn JA, Stenton R, Dajani S, Burns AS, Howard RJ, Ballard CG (2010) Putting brain training to the test. *Nature* **465**, 775-778.