COGNITIVE IMPAIRMENT: THERAPY MOMENTUM IN THE CONTINUUM OF LIFE

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EDITORIAL
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Editorial: Cognitive Impairment: Therapy Momentum in the Continuum of Life

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Editorial on the Research Topic

Cognitive Impairment: Therapy Momentum in the Continuum of Life

Cognitive impairment is hastily affecting an increasing number of people all over the globe. Despite age being a well-established risk factor, contributing to an aggregate prevalence over time, cognitive impairment is not a direct consequence of old age. Other factors and conditions may lead to mild cognitive impairment (MCI) or trigger the onset of dementia, while the same and additional factors may emerge in the continuum of the disease (Alzheimer's Association, 2020). Therefore, the goal of this Research Topic is to highlight recent advances in the field of Neuropharmacology, Pharmacoepidemiology and Geriatrics, in order to efficiently prevent or delay the onset of cognitive impairment and improve dementia management in clinical practice.

The issue comprises five contributions from four different countries. In the midst of the coronavirus disease (COVID-19) pandemic, that has emphasized the needs and difficulties in comorbidities management, most of the studies are representative of the abiding problems in the field of cognitive impairment (Wang et al., 2020). The authors explore alternative pharmacological regimens in case of MCI and dementia, propose genetic and physiological markers to monitor the efficiency of the schemes, and employ stochastic models to address biological diversity. They also propose the development of comprehensive experimental models to overcome the limitations of the previous ones.

So far, numerous rodent (mice) models have been developed to study dementia continuum, especially the most common type, Alzheimer's disease (AD) (King, 2018). These models have been useful in understanding the aggregation of amyloid peptides in plaques and in elucidating partially molecular interplay. Nevertheless, they don't necessary resonate the course of AD in humans or the formation of tangles. Papaspyropoulos et al. discuss the potential of human pluripotent stem cell-derived organoids to substitute animal models of neurodegeneration and serve as drug screening platforms.

Pharmacoepidemiology studies may be further useful in monitoring the clinical efficacy of active agents in a large scale and provide us with new insights. Cholinesterase inhibitors do not perform well in clinical practice and a high number of patients seem not to respond to the

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approved therapeutic doses. Polymorphisms, such as that of CYP2D6, may mediate drug metabolism and lead to low drug circulating levels in about two thirds of the cases (Ortner et al., 2020). In this issue, Lu et al. discuss a large array of genetic polymorphisms, such as CYPs and ATP-binding cassette transporters, as well as those involved in acetyl-or butyrylcholinesterase constitutive signaling and inhibition that influence donepezil PK/PD. They also review sex and race differences and comment on the lack of longitudinal and combined evidence. In line with the latter, Zhang et al. present a meta-analysis exploring AD therapy outcomes, taking into account drug efficacy, tolerance and patient compliance. By employing Markovian chains, correcting for the aforementioned inter-individual variability, they show that each monotherapy or combined treatment in mild to moderate AD presents discrete benefits, and that both the rarity of adverse effects, as in the case of EGb761, and efficacy may guide patient compliance.

The additional management of neuropsychiatric manifestations in people living with dementia is complex. As Zhang et al. conclude, the approved dementia therapies have no significant impact on the array. Natural extracts, such as of *Ginkgo biloba* leaves (EGb761) or *Crocus sativus* L. stigmata (Tsolaki et al., 2016) are promising, but large-scale randomized controlled trials are needed. The use of benzodiazepines, although effective in short-term, has been linked to cognitive decline, and special considerations should be made accordingly in those suffering from anxiety disorders, depression, or sleep disturbances (Salzman, 2020). Federico et al. confirm a substantial impairment of verbal memory in middle-age adult benzodiazepine abusers, and the subsequent improvement of multiple memory domains at the end of one-week clonazepam-flumazenil regimen. It is not clear though whether and at which stage flumazenil could be used to improve memory impairment in dementia patients considering the extensive $GABA_A$ergic remodeling (Li et al., 2016). Occasionally, compensatory inhibitory mechanisms may also develop in a subgroup of individuals that experience seizures. Horvath et al. discuss further patterns of interictal or subclinical epileptiform activity, detected in many cognitive disorders, and their primary or secondary role in memory formation through disruption of sleep-memory consolidation. Such information may shape future studies exploring the course of neurodegeneration, shed light on aspects of behavioral and psychiatric dementia symptoms and potentially revolutionize the field of antiepileptic therapy.

In summary, the current Research Topic features the challenges that may appear in view or due to cognitive impairment and the need for comprehensive monitoring and management in the continuum of life, especially when circumstantial pharmacotherapy may be of long-term consequences and advantages.

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All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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MINI REVIEW
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Modeling and Targeting Alzheimer's Disease With Organoids

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Human neurodegenerative diseases, such as Alzheimer's disease (AD), are not easily modeled *in vitro* due to the inaccessibility of brain tissue and the level of complexity required by existing cell culture systems. Three-dimensional (3D) brain organoid systems generated from human pluripotent stem cells (hPSCs) have demonstrated considerable potential in recapitulating key features of AD pathophysiology, such as amyloid plaque- and neurofibrillary tangle-like structures. A number of AD brain organoid models have also been used as platforms to assess the efficacy of pharmacological agents in disease progression. However, despite the fact that stem cell-derived brain organoids mimic early aspects of brain development, they fail to model complex cell-cell interactions pertaining to different regions of the human brain and aspects of natural processes such as cell differentiation and aging. Here, we review current advances and limitations accompanying several hPSC-derived organoid methodologies, as well as recent attempts to utilize them as therapeutic platforms. We additionally discuss comparative benefits and disadvantages of the various hPSC-derived organoid generation protocols and differentiation strategies. Lastly, we provide a comparison of hPSC-derived organoids to primary tissue-derived organoids, examining the future potential and advantages of both systems in modeling neurodegenerative disorders, especially AD.

Keywords: Alzheimer's disease, disease modelling, hPSC-derived brain organoids, pharmacological treatments, primary tissue-derived organoids

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INTRODUCTION

Alzheimer's disease (AD) constitutes the most prominent cause of late-life dementia, affecting over 50 million individuals. Additionally, AD represents one of the leading causes of death worldwide (Collaborators, G.B.D.D, 2019). Although considerable progress has been made in neuroscience, there are currently no available drug treatments curing the disease, thus highlighting that it is accompanied by significant social and economic burden (Vigo et al., 2016; Amin and Pasca, 2018). The majority of AD clinical cases develop symptoms beyond the age of 65 and are collectively referred to as sporadic AD (SAD). Familial AD (FAD) incidents, which pertain only to 2–5% of AD cases, develop early-onset symptoms and have been linked to mutations in genes such as *APP*, *PSEN1*, and *PSEN2* (Holtzman et al., 2011).

AD is caused by neuronal deposition and subsequent toxicity of amyloid-beta (Aβ)- and tau hyperphosphorylation-derived neurofibrillary tangles (NFTs) (Palmer, 2011; Dos Santos Picanco et al., 2018; Yan et al., 2019). In the AD brain, Aβ plaques are formed by aggregation of monomeric Aβ peptides into toxic Aβ oligomers, which subsequently generate the insoluble fibrils. Aβ plaque formation has been shown to trigger inflammatory responses and Reactive Oxygen Species (ROS) production, resulting in neuronal death (Prokop et al., 2013; Heppner et al., 2015; Yan et al., 2019). Additionally, toxic Aβ species may trigger caspase-associated apoptosis, following their transfer into neuronal cells (Prokop et al., 2013; Heppner et al., 2015; Yan et al., 2019). In healthy individuals, β- and γ-secretases proteolyze the amyloid precursor protein (APP) to soluble and non-toxic Aβ monomers, whereas in AD patients, Aβ plaques are formed due to increased production or insufficient removal of Aβ peptides (Bekris et al., 2010). Moreover, extracellular matrix (ECM) components such as heparin sulfate proteoglycans (HSPG) have been shown to foster amyloid plaque formation (van Horssen et al., 2002). Aβ peptide accumulation may synergize with tau-related NFT formation to contribute to AD manifestation, as indicated by a number of studies (Nisbet et al., 2015).

Several limitations accompany the implementation of transgenic mice in elucidating the molecular mechanisms underlying AD pathophysiology, such as the inability to capture tau pathology and the development of AD features early in life (Andorfer et al., 2003; Kitazawa et al., 2012; Sasaguri et al., 2017; Gerakis and Hetz, 2019). Additionally, monolayer neuronal cultures from AD patients lack plaques and tangles and express toxic proteins, which also limit their potential use as model systems (Amin and Pasca, 2018). Thus, novel systems are required to model AD development and serve as platforms for the discovery of effective AD treatments. In this literature review, we aim to provide an overview of recent advances regarding the development of brain organoids as a humanized model system against AD.

iPSCs in AD Modeling

The establishment and optimization of protocols allowing the reprogramming of human somatic cells into induced pluripotent stem cells (iPSC) opened new avenues in disease modeling (Tiscornia et al., 2011). Human pluripotent stem cells (hPSC) include blastocyst-derived human embryonic stem cells (ESC) and hiPSCs reprogrammed from somatic cells. HPSCs display unlimited self-renewal and can differentiate toward mesoderm, endoderm, or ectoderm (Rowe and Daley, 2019). Three methods have been so far established to capture the AD phenotype using hPSCs. The first method pertains to chemical induction with Aβ42 oligomers or Aβ42 inducers, such as aftin5. In this method, neural cells derived from AD-free hPSCs are induced to develop AD phenotypes (Vazin et al., 2014; Pavoni et al., 2018). Although certain pathophysiological features of the disease such as neuronal cytotoxicity can be displayed by implementing this method, induced neuronal cells usually lack other features such as extracellular Aβ plaque formation. The second method is based on the generation of iPSCs from somatic cells carrying known AD mutations and subsequent differentiation of those iPSCs into various types of neuronal cells. iPSCs deriving from FAD patients usually carry *PS1, PS2, or APP* genomic mutations, whereas those deriving from SAD patients carry *APOE4* mutations (Muratore et al., 2014). In the third method, lentiviral transduction or CRISPR-Cas9-mediated genomic editing are implemented in order to induce overexpression or expression of mutant APP, PS1, PS2, and APOE4 proteins in healthy hPSCs (Koch et al., 2012; Huang et al., 2017). Additionally, by utilizing human ESC-derived neurons ectopically expressing APOE2/E3/E4, it was shown that all APOE isoforms could induce Aβ and APP production, albeit to a different extent, with APOE4 being the most potent isoform (Huang et al., 2017). The majority of hPSC-based AD models implemented either two-dimensional (2D) or embryoid body (EB) differentiation methodologies to produce different types of neurons, including forebrain, cortical glutamatergic, GABAergic, and cholinergic neurons (Harasta and Ittner, 2017; Jorfi et al., 2018).

2D cell culture models of FAD and SAD based on patient-derived iPSCs have been shown to resemble some features of AD pathophysiology, such as intracellular accumulation of soluble Aβ species, aggregation of insoluble Aβ species, and tau hyperphosphorylation (Kondo et al., 2013; Freude et al., 2014). Moreover, iPSC-derived neurons from FAD patients can successfully capture important features of AD pathogenesis at early stages (Israel et al., 2012). However, while iPSC- or ESC-derived neurons cultured in monolayer have yielded important findings, they fail to present various morphological and functional properties of the human brain, which poses limitations in their use as model systems for neurodegenerative diseases. Neuronal maturation and development of synapse connections are governed by cell-cell and ligand-receptor signaling, which are not sufficiently established when neurons are cultured in monolayer (Amin and Pasca, 2018). Monolayer cultures do not offer accurate representations of the number, functional interactions, and regulatory functions typically observed in oligodendrocytes, astrocytes, and microglia in the human brain. Additionally, it is difficult to faithfully mimic neuronal maturation in monolayer cultures, as the *in vivo* process takes place on much longer timescales than monolayer cultures can be maintained (Dehaene-Lambertz and Spelke, 2015; Silbereis et al., 2016). In the case of AD, in particular, 2D cultures fail to display aggregation of extracellular β-amyloids, as only low Aβ species levels are produced even in the presence of the most prominent FAD genetic mutations. Moreover, the absence of interstitial compartment is believed to inhibit extracellular β-amyloid aggregation in 2D cultures (Choi et al., 2014).

Modeling AD With hPSC-Derived Organoids

The limitations of monolayer cultures triggered the development of additional *in vitro* model systems capable of resembling human brain architecture and function more accurately than before (Nakano et al., 2012; Muguruma et al., 2015). The

improvement of protocols for *in vitro* iPSC differentiation led to the establishment of "organoids", which are three-dimensional (3D) self-organized structures displaying morphological and functional similarities with complex organs, such as the brain. Brain organoid formation relies on the self-organization ability of hiPSCs, which may be facilitated by additional exogenous components, for example matrigel (Mansour et al., 2018; Pham et al., 2018). Brain organoids develop to display organized structures, resembling distinct regions of the brain, thus maintaining hallmarks of key developmental processes involved in brain formation (Lancaster et al., 2013). Over the past few years, various attempts have been made to model specific brain substructures with the use of organoids. In this context, forebrain, midbrain, hippocampus, and retinal organoids have been developed from hiPSCs (Di Lullo and Kriegstein, 2017). A major point of discussion regarding the optimization of organoid formation protocols is whether cell fate induction should be facilitated through the addition of exogenous morphogens and signaling molecules or not facilitated at all. Several protocols favor spontaneous neural induction by avoiding supplementation of organoid media with exogenous factors, thereby resulting in the acquisition of heterogeneous cell populations, corresponding to various brain regions (Lancaster et al., 2013; Camp et al., 2015; Quadrato et al., 2017). Undirected organoids, often grown in ECM, stochastically give rise to cells corresponding to multiple brain sections ranging from the retina to hindbrain (**Figure 1A**) (Lancaster et al., 2013). One major limitation of spontaneous neural induction is that a proportion of cells are randomly differentiated into non-ectodermal cell types (Camp et al., 2015; Quadrato et al., 2017). Hence, most current efforts are based on protocols optimizing the application of extrinsic cues to induce neuronal differentiation.

In guided brain formation, defined combinations of exogenously applied factors direct the *in vitro* specification of stem cell aggregates into organoids (**Figure 1B**) (Pasca et al., 2011; Mariani et al., 2015;

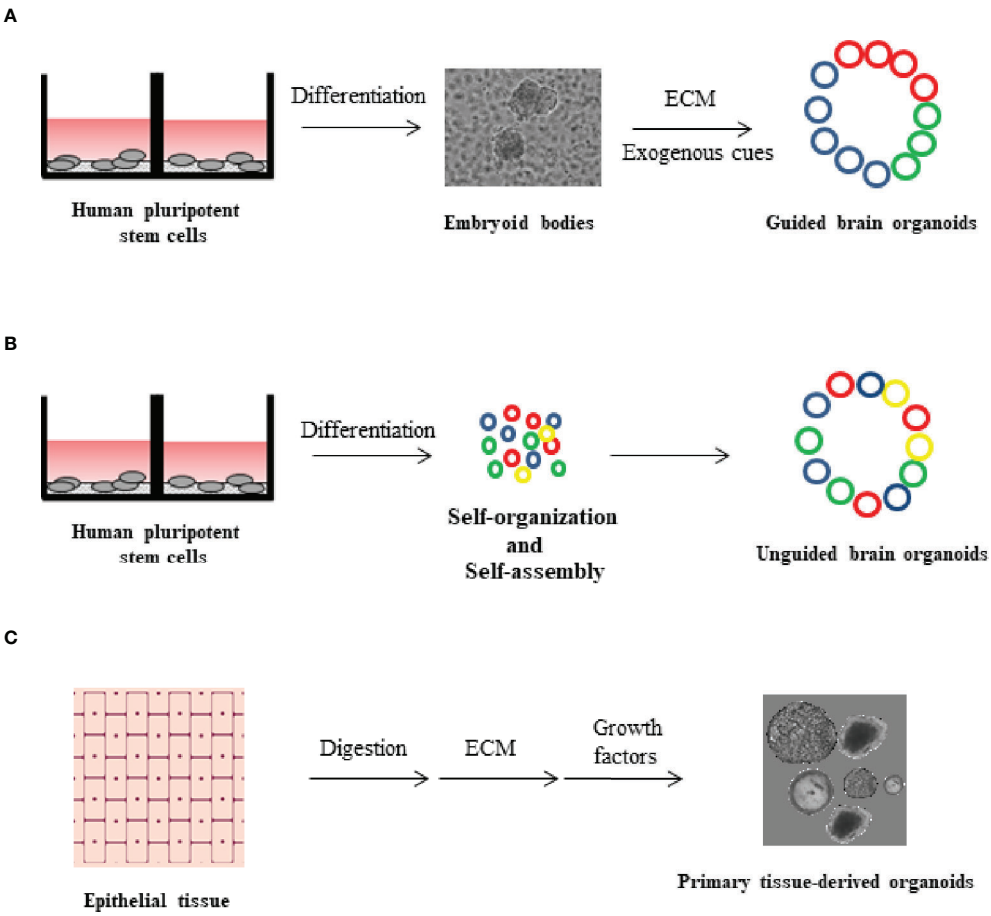

FIGURE 1 | Organoid formation technologies from human pluripotent stem cells (hPSCs) and primary tissue. **(A)** Guided brain organoids generated from hPSCs through embryoid body (EB) formation. The process requires extrinsic factors, such as extracellular matrix (ECM) and exogenous differentiation signals. The presence of different cell types in the organoids is indicated by different colors (blue, red and green) **(B)** Unguided brain organoids generated from hPSCs upon stem cell self-organization and self-assembly, in the absence of extrinsic factors. With this method, non-ectodermal cell types may be incorporated in brain organoids (yellow color) **(C)** Primary tissue-derived organoids are generated by human epithelial tissue of any age. Described protocols include tissue digestion and subsequent use of defined cell culture media supplemented with tissue-specific growth factors. Organoids are embedded in ECM.

Qian et al., 2016; Amin and Pasca, 2018). Guided methodologies for brain organoid generation were first described by the Sasai group, which conceived and optimized targeted 3D differentiation protocols based on culturing EB aggregates in serum-free conditions (Eiraku et al., 2008; Danjo et al., 2011; Muguruma et al., 2015; Sakaguchi et al., 2015). Directed organoid cultures have the advantage of containing different cell lineages at relatively stable proportions, thereby limiting potential variations across different batches and cell lines (Sloan et al., 2017). Organoids mature over a period of many months (Sloan et al., 2017), achieving a diameter of several millimeters, and contain heterogeneous cell types including neuronal subtypes, outer radial glia cells, astrocytes, and oligodendrocytes (Camp et al., 2015; Qian et al., 2016; Birey et al., 2017; Quadrato et al., 2017; Sloan et al., 2017; Amin and Pasca, 2018).

In order to model inter-regional interactions pertaining to brain physiology, several groups have attempted to differentiate hPSCs toward distinct brain region-specific organoids before fusing them together to allow the formation of "assembloids" integrating multiple region identities (Bagley et al., 2017; Birey et al., 2017; Xiang et al., 2017). Along those lines, assembloids have formed *via* fusion of dorsal and ventral forebrain organoids (Birey et al., 2017). In those structures, intraneurons originating from the ventral region translocate to the dorsal region, thus resembling the *in vivo* situation.

Brain organoids generated from hPSCs have been recently implemented to model various neurological disorders such as autism (Mariani et al., 2015; Birey et al., 2017), microcephaly (Tiscornia et al., 2011), Parkinson's disease (Kim et al., 2019), and Zika virus infections (Qian et al., 2016). The first successful attempt in using organoids to model AD was based on human neuronal progenitor cells genetically manipulated to overexpress mutant PS1 and APP (Choi et al., 2014). This methodology allowed the simultaneous presence of β-amyloid- and tau-related features in a single 3D model system. Those 3D structures carrying FAD mutations displayed increased detergent-resistant accumulations of phosphorylated tau, together with filamentous tau.

A sophisticated model of AD cerebral organoids was recently generated from FAD patient- or Down patient-derived iPSCs (Gonzalez et al., 2018). In this model, brain organoids displayed progressive accumulation of amyloidogenic Aβ peptides, accompanied by the development of structures strongly resembling amyloid plaques and NFTs. These phenotypes were absent in cerebral organoids derived from "control" templates such as healthy hiPSC, mouse ESCs, or mouse iPSCs (Gonzalez et al., 2018).

Recently a new 3D human tri-culture model including neurons, astrocytes, and microglia was developed to model AD with the use of microfluidics (Park et al., 2018). The model displayed critical features of AD pathology, such as β-amyloid aggregation, tau hyperphosphorylation, neuroinflammatory activity, microglial recruitment, axonal cleavage resulting from neurotoxic activities, and release of NO with deleterious effects on AD neurons and astrocytes (Park et al., 2018).

Use of hPSC-Derived Organoids as a Treatment Platform for AD

Two studies have implemented AD brain organoids in order to assess the effect of pharmacological agents on various disease features. Both studies used primarily modulators of β- or γ-secretase and were able to observe reductions in Aβ peptide levels, as well as alterations in tau pathology, in line with previous reports involving iPSCs. Choi et al. (2014) developed 3D-differentiated neuronal cells carrying FAD mutations, and importantly, demonstrated that perturbation of β-amyloid generation with β- or γ-secretase inhibitors attenuated both β-amyloid and tau-related pathology, indicating that tau-dependent phenotypes may be driven by excessive accumulation of Aβ species as a result of FAD mutations (Choi et al., 2014). Additionally, the use of glycogen synthase kinase 3 (GSK3) was found to regulate β-amyloid-mediated tau phosphorylation in that system. Thus, that study constituted the first attempt to show that stem cell-derived 3D *in vitro* systems can potentially serve as drug treatment platforms against AD (Choi et al., 2014).

In a more recent study by Raja et al. (2016), hPSC-derived organoids from FAD patients again exhibited AD-like pathophysiological features, including amyloid aggregation, tau hyperphosphorylation, and endosome abnormalities, in an age-dependent fashion (Raja et al., 2016). The authors showed that the 3D system they developed could be easily subjected to experimental manipulation and serve as a potential drug treatment platform. The authors found that treatment of FAD patient-derived organoids with γ-secretase inhibitor compound E or BACE-1 β-secretase inhibitor (β-secretase inhibitor IV) partially reversed both amyloid and tau pathology. Additionally, in contrast to published data not supporting a pivotal role of amyloids in AD manifestation (Takahashi et al., 2015; Kametani and Hasegawa, 2018), the authors showed that inhibition of Aβ species limited tau hyperphosphorylation only after Aβ reduction was observed, suggesting that Aβ accumulation-driven phenotypes in AD may emerge prior to tauopathy (Raja et al., 2016).

Limitations of hPSC-Derived Organoids in Modeling AD

HPSC-derived brain organoids display most of the advantages of 2D cultures, while offering the ability to model complex cell-cell interactions, as they usually contain more than one cell population. Because of their advantages, hPSC-derived brain organoids have been utilized to model AD and examine the impact of pharmacological factors on disease progression, however, serious technical hurdles are still required to be resolved. Additionally, the organoid generation technology applied so far to model neurodegenerative diseases, including AD, needs to be reviewed and updated.

One critical limitation to modeling AD with the use of hPSC-derived organoids relates to aging. Aging constitutes the main risk factor to develop AD, especially in the case of SAD, and the process of aging is accompanied by numerous genetic alterations resulting in changes in the overall cellular transcriptional profile (Lopez-Otin

et al., 2013; Gerakis and Hetz, 2019). However, iPSC-derived neural cells display a transcriptional profile similar to prenatal brain (Camp et al., 2015; Gerakis and Hetz, 2019), thereby making it challenging to recapitulate aging-related phenotypes.

Another important limitation is the lack of complete vascularization. Vascularization is critical to mimic the *in vivo* situation in the brain, as maturation of neuronal cells cannot be accomplished without sufficient oxygen and nutrient supply. Insufficient neuronal cell maturation results in perturbed synapse formation, whereas lack of vascularization overall limits organoid culturing periods (Lancaster et al., 2013; Di Lullo and Kriegstein, 2017). Lack of vascularization additionally prevents modeling important aspects of brain physiology, such as the blood brain barrier (Huch et al., 2017). Along these lines, cerebral organoids produced from AD- or Down patient-derived iPSCs structurally resemble the human cortex, however they contain only neurons and glial cells, lacking oligodendrocytes. Additionally, those organoids fail to establish active synapses (Gonzalez et al., 2018). To overcome vascularization-related hurdles, heterotypic cultures combining mouse brain cells or brain progenitors with endothelial and mesenchymal stem cells have been recently used to generate 3D organ buds (Takebe et al., 2015), however, the functionality of that system has yet to be addressed in mice and humans. Additionally, it has been shown that although brain organoids are able to incorporate exogenous endothelial cells, the resulting endothelial network may not be functional (Pham et al., 2018).

HPSC-derived organoid models are so far challenged by low reproducibility and homogeneity. Organoid differentiation protocols relying on hPSC self-organization, in particular, lead to variable outcomes. Brain organoids differ from each other in size and structure, which are limiting factors in accurately modeling diseases such as AD. The small size, in particular, of hPSC-derived organoids comprises an important limitation in modeling human brain development, especially at later stages (Rambani et al., 2009). Microfluidics, spinning bioreactors and orbital shakers combining new biomaterials and culture methodologies, have been proposed as new avenues to control neural patterning more accurately and improve oxygen and nutrient supply to the organoid interior (Kadoshima et al., 2013; Qian et al., 2016; Lancaster et al., 2017; Yan et al., 2019).

Improvements in culturing conditions and the use of novel biomaterials might also help rectify another important limitation encountered in hPSC-derived organoid cultures, which is insufficient immune cell representation. Several brain organoid systems developed so far are characterized by the presence of astrocytes, but no microglial cells (Yakoub, 2019). The absence of microglial cells could be also attributed to their distinct embryonic origin, as they derive from yolk sac erythromyeloid precursors (Ginhoux and Prinz, 2015; Li and Barres, 2018).

HPSC-derived organoids predominantly rely on the process of somatic cell reprogramming, which has been extensively linked to increased risk of genomic instability, as iPSCs may often carry mutations related to known tumorigenic loci (Mayshar et al., 2010; Hussein et al., 2011; Laurent et al., 2011). This implication poses serious limitations to the use of hPSC-derived organoids in modeling human disease. Additionally, genomic analyses of early passage iPSCs have indicated that they might retain "epigenetic memory" related to their previous fate, by displaying DNA methylation patterns encountered in somatic cells, at regions proximal to CpG islands. Consequently, this leads to variations in gene expression which might affect hPSC usage as organoid generation templates (Doi et al., 2009; Kim et al., 2010; Polo et al., 2010; Bar-Nur et al., 2011; Puri and Nagy, 2012).

Another limitation related to hPSC-derived organoids is that most hPSC cultures are feeder cell-dependent, adding to the complexity of the culturing protocols and increasing the risk of underlying cell culture infections. A shift to feeder-free culturing conditions could increase reproducibility across cell lines and laboratories (Lancaster et al., 2017; Yoon et al., 2019). Due to the above limitations, hPSC-derived organoid cultures need to be constantly compared to independent batches of multiple hPSC lines and adequately assessed for their capacity to produce consistent results, before being put forward as powerful disease model systems.

Future Perspectives of the Organoid Technology

Since 2009, a 3D *in vitro* culture system for several organs, such as small intestine, colon, stomach, prostate, liver, pancreas, breast, lung, and skin has been established (Sato et al., 2009; Barker et al., 2010; Sato et al., 2011; Karthaus et al., 2014; Boj et al., 2015; Huch et al., 2015; Sachs et al., 2018; Wiener et al., 2018; Sachs et al., 2019), based on stimulating the self-renewal capacity of the underlying stem cell populations. Culturing of the above tissues in defined conditioned media results in the formation of 3D mini-tissues, also called organoids. Those primary tissue-derived organoids can be established from mouse and human tissue of any age, they do not require additional cell types to stimulate growth, are genetically stable and can retain the *in vivo* organization and development of the tissue they derive from. More importantly, they do not depend on iPSC technology and their long-term culture has been optimized through various protocols depending on the tissue (**Figure 1C**) (Rossi et al., 2018).

Patient-derived organoids offer a unique model system, as it resembles the *in vivo* situation more closely than any other cell culture so far. All attempts, however, to generate organoids immediately derived from primary material have been focused on epithelial tissue. Given that the study of neurodegenerative disorders requires the establishment and maintenance of non-epithelial cell cultures, one of the most important future challenges is to adapt the current patient-derived organoid technology to model diseases encountered in non-epithelial tissues. Taking into account the numerous advantages of patient-derived organoids, the field is soon expected to expand this cutting edge technology to encompass non-epithelial tissue. In doing so, the biggest challenge would be to define the optimal media composition supporting the *in vitro* generation and maintenance of patient-derived brain organoids. The next step following the establishment of hPSC-free brain organoids would

be to implement means of genetic manipulation and drug delivery, allowing for personalized treatment approaches. Along those lines, and considering the advantages of patient-derived brain organoids with regards to functionality and biosafety, the potential of utilizing the system in regenerative medicine would be greater than any other system so far.

DISCUSSION

Several attempts have been made to model and pharmacologically target neurodegenerative diseases, such as AD, with the use of brain organoids. So far, brain organoid generation attempts have been mostly focused on somatic cell reprogramming, a process in which patient-derived somatic cells are induced to become hPSCs (Amin and Pasca, 2018). HPSCs can be subsequently differentiated into monolayer neuronal cultures or brain organoids, which are 3D neural cell aggregates resembling various brain regions. In the case of AD, there have been several attempts to generate brain organoids using the hPSC technology (Raja et al., 2016; Pavoni et al., 2018; Gerakis and Hetz, 2019; Qian et al., 2019) and a lot of progress has been made both in modeling the disease and assessing the effectiveness of drugs like γ-secretase inhibitors to reverse AD-related phenotypes. With regards to their differentiation pattern, unguided brain organoids have shown suitability in modeling cell-lineage diversity in whole brain development, whereas directed brain organoids may be fused to form assembloids in order to capture and study processes linked to specific brain regions, including the hippocampal loss in AD (Bagley et al., 2017; Birey et al., 2017; Xiang et al., 2017).

HPSC-derived organoids are accompanied by a series of limitations, such as lack of or limited integration of important cell types (e.g. microglial cells and oligodendrocytes), lack of distinct cortical neuronal layer formation, no evidence of gyrification, nor complex neuronal circuitry (Gerakis and Hetz, 2019; Qian et al., 2019). Additionally, the iPSC technology itself poses limitations with regards to safety, genomic stability, and reproducibility.

Current organoid models are majorly derived from the epithelium of various organs. Established protocols for generating primary tissue-derived organoids could overcome the aging-related issues of hPSC-derived organoids, as primary tissue-derived organoids can be established from mammalian tissue of any age. Additionally, primary tissue-derived organoids are based on more stringent differentiation protocols, in contrast to protocols relying on hPSC self-organization. It has been widely reported that stochasticity in the hPSC differentiation process culminates in unpredictable outcomes in brain organoid cultures, adding to reproducibility issues. The challenge of adapting epithelial organoid generation protocols to meet the requirements of non-epithelial tissue culture still remains.

AUTHOR CONTRIBUTIONS

AP conceived the topic and wrote the manuscript. MT, NF, and AAP contributed to writing the manuscript and critically reviewed it.

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Different Doses of Pharmacological Treatments for Mild to Moderate Alzheimer's Disease: A Bayesian Network Meta-Analysis

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Background: Pharmacological treatments play a significant role in treating mild to moderate Alzheimer's disease (AD), but the optimal doses of various drugs used for these treatments are unknown. Our study compared the efficacy, acceptability, and safety of different doses of pharmacological treatments for mild to moderate AD.

Methods: Randomized controlled trials (RCTs) were identified by searching the PubMed, EMBASE, and Cochrane Library databases (all RCTs published from the date of inception of the databases until September 19, 2019). Trials comparing the efficacy, acceptability, and safety of pharmacological interventions involving donepezil, galantamine, rivastigmine, memantine, huperzine A, and *Ginkgo biloba* extract EGb761, alone or in combination, were identified. The primary outcomes were efficacy, acceptability, and safety.

Results: Our meta-analysis included 37 studies involving 14,705 participants. In terms of improving cognitive function, galantamine 32 mg, galantamine 24 mg, donepezil 5 mg, and donepezil 10 mg were more effective than other interventions, with the surface under the cumulative ranking curve (SUCRA) values of 93.2, 75.5, 73.3, and 65.6%, respectively. According to the SUCRA values, EGb761 240 mg was considered to be the optimal intervention in terms of both acceptability and safety. With regard to clinical global impression, rivastigmine 12 mg had the highest probability of being ranked first (83.7%). The rivastigmine 15 cm^2 patch (SUCRA = 93.7%) may be the best choice for daily living. However, there were no interventions that could significantly improve neuropsychiatric symptoms, compared with the placebo.

Conclusions: Different doses of the tested pharmacological interventions yielded benefits with regard to cognition, acceptability, safety, function, and clinical global impressions, but not effective behaviors.

Keywords: Alzheimer's disease, donepezil, network meta-analysis, pharmacological treatment, randomized controlled trial

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BACKGROUND

There were an estimated 50 million dementia patients worldwide in 2018. Although this disease currently represents an enormous public health problem, the number of dementia patients is predicted to rise to 152 million by 2050 (Alzheimer's Disease International, 2018). Alzheimer's disease (AD) is an irreversible neurodegenerative disease that manifests as progressive memory loss and cognitive dysfunction, and is the leading cause of dementia, accounting for 50–75% of all cases globally (International., Alzheimer's disease, 2019). There is currently no cure for AD; the typical pharmacological therapeutic goals are to delay disease progression and to improve the patients' quality of life. Pharmacological treatments approved by the US Food and Drug Administration are mainly grouped into two classes by their differing mechanisms of action: acetylcholinesterase inhibitors (AChEIs), such as donepezil, galantamine, and rivastigmine, which are widely used treatments for mild to moderate disease stages (NICE; Corbett et al., 2012); and N-methyl-D-aspartate receptor antagonists, typically memantine, for moderate to severe disease stages (Kishi et al., 2017).

Donepezil is the primary treatment for mild to moderate AD; it is well tolerated and results in cognitive improvement (Rogers et al., 1998b; Rogers et al., 1998a; Burns et al., 1999). Moreover, evidence suggests that donepezil has dose-dependent effects: with increasing doses, its efficacy improves, although more adverse events also occur. Increased improvements in cognition are indicated for donepezil 10 mg but not donepezil 5 mg, especially at 18 and 24 weeks, based on the meta-analysis of Whitehead et al., which included 10 clinical trials (Whitehead et al., 2004). In routine practice, the variety of different drug preparations and dosages poses a challenge for physicians responsible for decision-making with regard to treatment options for AD.

EGb761, extracted from *Ginkgo biloba*, is a common herbal treatment for AD (Akram and Nawaz, 2017). A previous systematic review and meta-analysis demonstrated that compared with placebo, the *Ginkgo biloba* extract EGb761 appeared to have stronger cognitive effects (standard mean difference [SMD] = −0.58, 95% confidence interval [CI]: −1.14, −0.01) (Weinmann et al., 2010). Although the efficacy of the *Ginkgo biloba* extract EGb761 was confirmed, when compared with donepezil, the results were not conclusive (Mazza et al., 2006; Yancheva et al., 2009; Nasab et al., 2012). In addition, a Cochrane systematic review of six trials suggested that huperzine A, a reversible and selective AChEI, is likely beneficial to AD patients and resulted in no apparent serious adverse events (Li et al., 2008). To date, a direct comparison of huperzine A, EGb761, an AChEI, or memantine has not been conducted in the same study.

It should be noted that a previous network meta-analysis focused on the comparative effectiveness of different anti-dementia treatments by using direct or indirect evidence, but did not consider different drug doses (Thancharoen and Limwattananon, 2019) or include comprehensive pharmacological interventions (Dou et al., 2018). A network meta-analysis allows the summation of direct and indirect evidence from relevant randomized controlled trials (RCTs) and the performance of an integrated analysis to determine the optimal pharmacological therapy for mild to moderate AD (Higgins and Whitehead, 1996). Therefore, this study aimed to comprehensively evaluate the efficacy (i.e., improvements in cognitive function), acceptability (i.e., completion of treatment), and safety (i.e., number of adverse events) of different doses of pharmacological agents used for treating mild to moderate AD, which can be used to inform clinical practice.

Abbreviations: AD, Alzheimer's disease; AChEIs, acetylcholinesterase inhibitors; PRISRM, Preferred Reporting Items for Systematic Reviews and Meta-Analysis; RCTs, randomized controlled trials; DSM, Diagnostic and Statistical Manual of Mental Disorders; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association; MMSE, minimental state examination; ADAS-cog, Alzheimer's Disease Assessment Scale-cognition subscale; ADCS-ADL, Alzheimer's Disease Cooperative Study-Activities of Daily Living; NPI, Neuropsychiatric Inventory; CIBIC-Plus, Clinician's Interview-Based Impression of Change plus Caregiver Input; SMD, standard mean difference; ORs, odds ratios; DIC, Deviance Information Criteria; SUCRA, surface under the cumulative ranking curve.

METHODS

Search Strategy

This network meta-analysis was performed in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) extension for network meta-analysis (Hutton et al., 2015). Relevant RCTs were identified in titles and abstracts in the PubMed, EMBASE, and the Cochrane Library databases. Results were restricted to English language publications from the date of the database inception to September 19, 2019. No restrictions were placed on publication dates or status. We adopted the MeSH and Emtree terms "Alzheimer's disease," "donepezil," "galantamine," "rivastigmine," "memantine," "huperzine A," "*Ginkgo biloba* extract," and "randomized controlled trials" combined with the corresponding free words adapted appropriately for each of the databases in the search algorithm. Additionally, we manually searched the references from the cited articles to identify meta-analyses and RCTs to avoid missing potentially eligible clinical trials. The details of the search strategies involving different databases are described in the **Additional file: Supplementary 1**.

Selection Criteria

The selection criteria were based on the principle of the Population-Intervention-Comparator-Outcomes-Study design (PICOS) (Costantino et al., 2015). The eligible studies were RCTs and had to meet the following criteria: 1) participants were clinically diagnosed with AD in accordance with the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM) or the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984). Mild to moderate AD was classified by a score of 10–26 (inclusive) in the Mini-Mental State Examination

(MMSE) (Folstein et al., 1975); 2) trials compared the effectiveness of pharmacological interventions using donepezil, galantamine, rivastigmine, memantine, huperzine A, or *Ginkgo biloba* extract alone or in combination, and drug dosages were not only within the therapeutic range but were also specific; 3) outcome measures covered at least one of the following outcomes: cognitive, global assessment, behavior, function, acceptability, or safety; and 4) the duration of follow-up was between 12 and 104 weeks. The following exclusion criteria were applied: 1) RCTs that recruited fewer than 10 participants in each group; 2) unavailability of the full text of the study, even with the support of expert librarians; and 3) participants diagnosed with other types of dementia or neurological disorders unrelated to AD, or outcome data for participants with AD that could not be independently assessed apart from data for participants diagnosed with other types of dementia.

Data Extraction

Two investigators (LN and CH) independently extracted the relevant data from all eligible studies published in English using predefined standardized spreadsheets. All extracted data were based on intention-to-treat analysis. Any discrepancies were resolved to consensus by two investigators (LN and CH) or arbitrated by a third investigator (ZT). The following information was documented for every study: first author, publication year, detailed trial information, diagnostic criteria, patient characteristics (i.e., age, gender, race, and baseline MMSE scores), treatment (dose, frequency), sample size, outcomes of the change from baseline (cognitive, global assessment, behavior, function), number of treatment completion, incidences of adverse events, and the duration of follow-up. Finally, all extracted data were cross-checked by two investigators (LN and CH) to ensure accuracy.

Quality Assessment

We evaluated the quality of the included trials using the Cochrane Collaboration's risk of bias assessment tool (Higgins et al., 2011), and the trials were judged to have a low risk of bias, an unclear risk of bias, or a high risk of bias. Any discrepancies between the two authors' evaluations (ZT and LN) were resolved by discussion.

Outcome Measures

We considered the overall mean change in cognitive function from the baseline to the study endpoint, the number of patients who completed the trial during the treatment period, and the number of patients who experienced any adverse events for our primary outcomes, as these were the most consistently reported estimates of efficacy, acceptability, and safety of interventions for mild to moderate AD. Cognitive function was primarily appraised by the Alzheimer's Disease Assessment Scale-cognition subscale (ADAS-cog), and the MMSE. For secondary outcome measures, we also estimated the changes from baseline to the endpoints of cognitive function, behavioral symptoms, and the clinical global impressions of patients, which were assessed by the Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL) scale, the Neuropsychiatric Inventory (NPI), and the Clinician's Interview-Based Impression of Change plus Caregiver Input (CIBIC-Plus) scale, respectively.

Statistical Analysis

First, we estimated the SMD for continuous outcomes and odds ratios (ORs) for dichotomous outcomes along with the corresponding 95% confidence interval (CI) by using a random-effects model, which served as the pooled effect sizes in conventional pair-wise meta-analysis. To assess the statistical heterogeneity of the direct comparison in the quantitative analysis, we used the I^2 statistic and p values. Stata software version 14.0 (Stata Corporation, College Station, TX, USA) was used for all analyses.

Second, for all collected outcomes, we performed a Bayesian network meta-analysis combining direct and indirect comparisons based on a random-effect model considering the smaller deviance information criteria (DIC) value. The data analysis used OpenBUGS software (version 3.2.3), and the network diagram was produced using Stata software (version 14.0). We chose various initial values at random with the run of three Markov chains simultaneously. The total number of iterations was 30,000. The median of the calculated data served as pooled estimated effect sizes (SMD or OR), and the 2.5 and 97.5 percentiles served as the corresponding 95% credible interval (CrI). The statistical significance was evaluated in line with whether the CrI included 0 or 1. Moreover, we also calculated the surface under the cumulative ranking curve (SUCRA) to rank the interventions for each outcome in which the SUCRA value was closely related to the rank of each intervention. In addition, if the network of interventions had closed loops, the node-splitting method and loop-specific method were performed to evaluate the statistical inconsistency (Salanti et al., 2008; Dias et al., 2010; Veroniki et al., 2013). The determination of whether the loop consistency was significant depended on the CI of the inconsistency factor (IF) value containing 0. Finally, for the small-sample effect assessment of intervention networks, we constructed a comparison-adjusted funnel plot and performed a visual assessment.

RESULTS

Literature Search Results

In total, 4,567 citations were identified by searching the PubMed, EMBASE, and the Cochrane Library databases. After 1,133 duplicate citations were removed using Endnote X7 software, the titles and abstracts for 3,434 citations were retrieved. Subsequently, the full text of 121 potentially eligible studies were reviewed further. From these, 85 publications were excluded primarily because they included other diseases (n = 18), did not report the desired intervention agents (n = 20), reported undesired outcomes (n = 5), were not RCTs (n = 9), were duplicate studies (n = 9), were not in English (n = 4), or were conference abstracts without available full texts (n = 20). Finally, 36 eligible studies met the inclusion criteria. In addition, we identified an additional publication from the references.

Overall, 37 studies (Rogers et al., 1998a; Rogers et al., 1998b; Burns et al., 1999; Rosler et al., 1999; Homma et al., 2000; Raskind et al., 2000; Tariot et al., 2000; Wilcock et al., 2000; Wilkinson and Murray, 2001; Winblad et al., 2001; Jones et al., 2004; Seltzer et al., 2004; Brodaty et al., 2005; Karaman et al., 2005; Schneider et al., 2005; Johannsen et al., 2006; Mazza et al., 2006; Peskind et al., 2006; Rockwood et al., 2006; Feldman and Lane, 2007; Winblad et al., 2007; Bakchine and Loft, 2008; Yancheva et al., 2009; Choi et al., 2011; Frolich et al., 2011; Maher-Edwards et al., 2011; Nakamura et al., 2011; Rafii et al., 2011; Cummings et al., 2012; Ihl et al., 2012; Zhang et al., 2012; Hager et al., 2014; Haig et al., 2014; Marek et al., 2014; Gault et al., 2015; Zhang et al., 2015; Zhang et al., 2016) were available for inclusion in the network meta-analysis. The PRISMA flowchart detailing the literature search process is shown in **Figure 1**.

Characteristics of the Eligible Studies

The characteristics of the included studies and details of the patients are shown in **Table 1**. The 37 studies involving 14,705

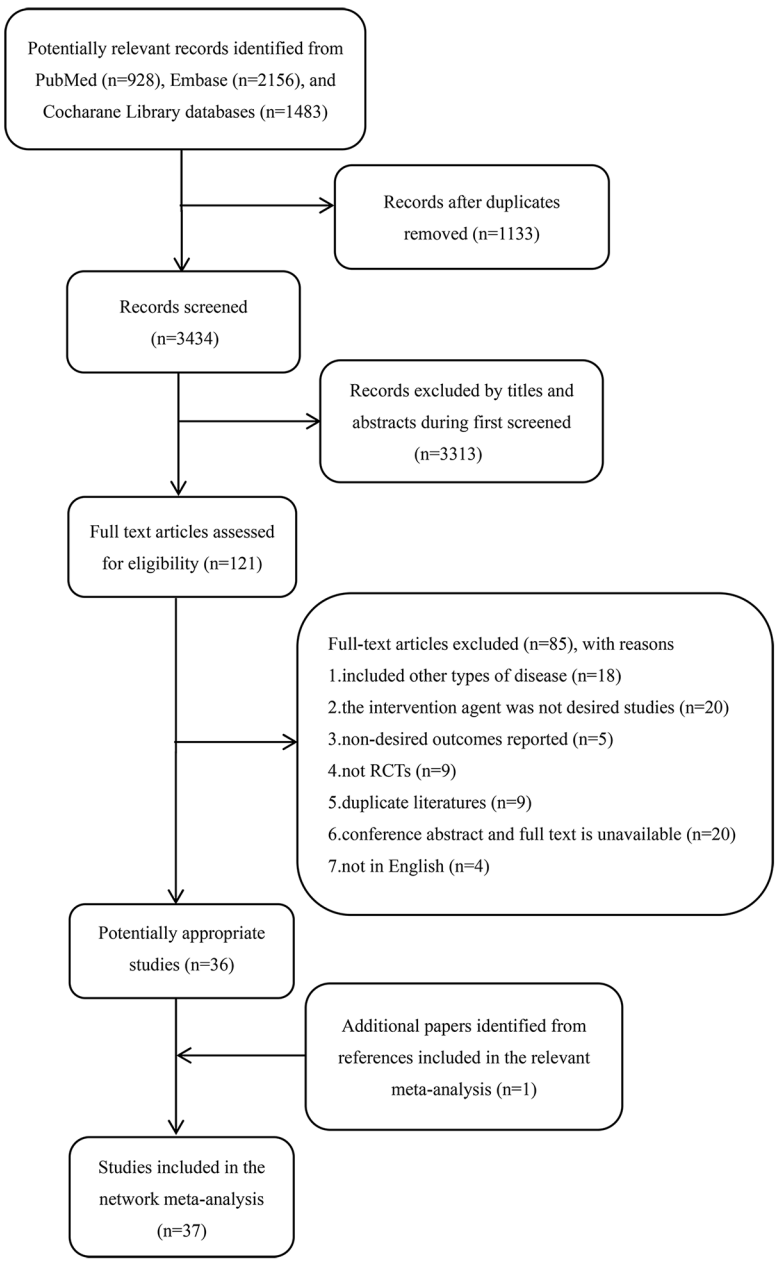

FIGURE 1 | The PRISMA flowchart for detailed search results and selection. RCT, randomized controlled trial.

TABLE 1 | Description of included studies and patient characteristics.

Study	Treatment	N	Age Mean (SD)	Gender (% female)	Baseline MMSE Mean (SD)	Criteria	Duration (weeks)
Zhang et al., 2016	Rivastigmine patch 10 cm^2	248	70.4 (8.02)	56.5	16.0 (3.46)	NINCDS-ADRDA	24
	Rivastigmine 12 mg	253	69.8 (8.20)	54.9	16.6 (3.08)		
Zhang et al., 2015	Memantine 20 mg	80	69.75 (8.06)	61.25	15.88 (4.43)	NINCDS-ADRDA	24
	Donepezil 10 mg	87	70.13 (7.99)	59.77	15.53 (4.22)		
Hager et al., 2014	Galantamine 24 mg	1,024	73.0 (8.9)	65.5	19.0 (4.12)	NINCDS-ADRDA	104
	Placebo	1,021	73.0 (8.7)	64.1	19.0 (4.04)		
Zhang et al., 2012	Galantamine 24 mg	116	73.3 (8.5)	51	18.8 (3.8)	NINCDS-ADRDA	16
	Donepezil 10 mg	117	74.0 (8.4)	55	17.9 (4.1)		
Ihl et al., 2012	EGb761 240 mg	163	64.9 (9.5)	66.9	NA	NINCDS-ADRDA	24
	Placebo	170	64.2 (8.7)	65.3	NA		
Rafii et al., 2011	Huperzine A 400 μg	68	77.57 (8.79)	60.29	19.00 (4.26)	NINCDS-ADRDA	16
	Huperzine A 200 μg	69	78.06 (6.91)	68.12	19.25 (4.20)		
	Placebo	73	78.1 (8.35)	64.38	19.12 (4.00)		
Choi et al., 2011	Rivastigmine patch 10 cm^2 + Memantine 20 mg	88	75.0 (7.3)	75	16.8(4.3)	NINCDS-ADRDA	24
	Rivastigmine patch 10 cm^2	84	74.7 (7.7)	84.34	16.4(4.7)		
Yancheva et al., 2009	EGb761 240 mg	31	69.0 (8.0)	54.8	NA	NINCDS-ADRDA	22
	Donepezil 10 mg	33	66.0 (8.0)	84.4	NA		
	EGb761 240 mg + Donepezil 10 mg	32	68.0 (9.0)	67.7	NA		
Winblad et al., 2007	Rivastigmine patch 10 cm^2	293	73.6 (7.9)	68	16.6 (3.1)	DSM-IV NINCDS-ADRDA	24
	Rivastigmine 12 mg	297	72.8 (8.2)	65.6	16.4 (3.1)		
	Placebo	302	73.9 (7.3)	66.6	16.4 (3.0)		
Winblad et al., 2001	Donepezil 10 mg	142	72.1 (8.6)	69.7	19.37 (4.37)	DSM-IV NINCDS-ADRDA	52
	Placebo	144	72.9 (8.0)	59	19.26 (4.54)		
Wilkinson and Murray, 2001	Galantamine 24 mg	56	72.9 (8.2)	59	18.2 (3.0)	DSM-III-R NINCDS-ADRDA	12
	Placebo	87	74.2 (8.4)	59	18.7 (2.8)		
Wilcock et al., 2000	Galantamine 24 mg	220	71.9 (8.3)	63.18	19.5 (3.4)	NINCDS-ADRDA	24
	Galantamine 32 mg	218	72.1 (8.6)	63.3	19.0 (3.8)		
	Placebo	215	72.7 (7.6)	61.4	19.3 (3.5)		
Tariot et al., 2000	Galantamine 24 mg	273	77.7 (6.6)	67.03	17.7 (3.3)	NINCDS-ADRDA	20
	Placebo	286	77.1 (8.5)	62.24	17.7 (3.4)		
Seltzer et al., 2004	Donepezil 10 mg	96	73.3 (9.6)	50	24.1 (1.7)	DSM-IV NINCDS-ADRDA	24
	Placebo	57	75.1 (8.8)	60	24.3 (1.3)		
Schneider et al., 2005	EGb761 240 mg	170	78.1 (7.0)	56.0	17.9 (4.0)	DSM-IV NINCDS-ADRDA	26
	Placebo	174	77.5 (7.4)	52.0	18.2 (4.1)		
Rosler et al., 1999	Rivastigmine 12 mg	243	72.0	59.0	19.9	DSM-IV NINCDS-ADRDA	26
	Placebo	239					
Raskind et al., 2000	Galantamine 24 mg	212	75.9 (7.3)	65.57	19.5 (4.4)	NINCDS-ADRDA	24
	Galantamine 32 mg	211	75.0 (8.7)	58.77	19.1 (4.4)		
	Placebo	213	75.3 (8.8)	61.5	19.2 (4.4)		
Peskind et al., 2006	Memantine 20 mg	201	78.0 (7.3)	60.2	17.4 (3.7)	NINCDS-ADRDA	24
	Placebo	202	77.0 (8.2)	57.43	17.2 (3.4)		
Nakamura et al., 2011	Rivastigmine patch 5 cm^2	282	74.3 (7.5)	68.8	16.8 (2.9)	DSM-IV NINCDS-ADRDA	24
	Rivastigmine patch 10 cm^2	287	75.1 (6.9)	67.9	16.5 (3.1)		
	Placebo	286	74.5 (7.4)	68.2	16.6 (2.9)		
Mazza et al., 2006	EGb761 160 mg	25	66.2 (6.0)	52.0	18.80 (3.62)	DSM-IV	24
	Donepezil 5 mg	25	64.5 (6.0)	48.0	18.55 (3.47)		
	Placebo	26	69.8 (3.0)	61.0	18.80 (3.63)		
Rockwood et al., 2006	Galantamine 24 mg	64	77.0 (8.0)	64.0	20.8 (3.3)	NINCDS-ADRDA	16
	Placebo	66	78.0 (8.0)	62.0	19.9 (4.2)		
Karaman et al., 2005	Rivastigmine 12 mg	24	74.11 (4.3)	54.17	11.40 (1.0)	DSM-IV NINCDS-ADRDA	52
	Placebo	20	73.40 (4.0)	55	13.20 (0.9)		
Jones et al., 2004	Donepezil 10 mg	64	73.8 (7.4)	51.6	18.3 (3.3)	DSM-IV NINCDS-ADRDA	12
	Galantamine 24 mg	56	75.1 (7.7)	71.4	18.4 (3.7)		
Bakchine and Loft, 2008	Memantine 20 mg	318	74.0 (7.4)	65.0	18.6 (3.3)	DSM-IV NINCDS-ADRDA	24
	Placebo	152	73.3 (6.9)	60.0	18.9 (3.2)		
Brodaty et al., 2005	Galantamine 24 mg	327	76.5 (7.77)	64.0	17.80 (4.14)	NINCDS-ADRDA	26
	Placebo	324	76.3 (8.03)	64.0	18.08 (4.08)		
Cummings et al., 2012	Rivastigmine patch 15 cm^2	280	75.6 (7.4)	66.1	14.1 (4.8)	DSM-IV NINCDS-ADRDA	48
	Rivastigmine patch 10 cm^2	287	75.9 (6.8)	63.4	14.2 (4.6)		
Gault et al., 2015	Donepezil 10 mg	68	72.4 (8.42)	45.6	19.6 (3.82)	NINCDS-ADRDA	12

(Continued)

TABLE 1 | Continued

Study	Treatment	N	Age Mean (SD)	Gender (% female)	Baseline MMSE Mean (SD)	Criteria	Duration (weeks)
	Placebo	68	73.6 (8.23)	61.8	19.7 (3.95)		
Haig et al., 2014	Donepezil 10 mg	60	70.5 (8.31)	60.0	18.1 (4.1)	NINCDS-ADRDA	12
	Placebo	63	70.3 (7.84)	61.9	18.2 (3.9)		
Marek et al., 2014	Donepezil 10 mg	66	71.8 (8.4)	53.0	19.3 (3.7)	NINCDS-ADRDA	12
	Placebo	66	71.7 (9.0)	60.6	19.4 (3.7)		
Rogers et al., 1998	Donepezil 5 mg	154	72.9 (7.5)	63	19.0 (5.0)	DSM-III-R NINCDS-ADRDA	24
	Donepezil 10 mg	157	74. 6 (7.5)	62	18.9 (5.0)		
	Placebo	162	72.6 (7.6)	61	19.2 (5.1)		
Johannsen et al., 2006	Donepezil 10 mg	99	74.1 (7.6)	59.6	18.8 (4.8)	NINCDS-ADRDA	12
	Placebo	103	71.4 (9.3)	63.1	18.5 (4.8)		
Frolich et al., 2011	Donepezil 10 mg	161	73.9 (6.48)	65.8	NA	NINCDS-ADRDA	12
	Placebo	164	73.5 (6.42)	55.2	NA		
Feldman and Lane, 2007	Rivastigmine 12 mg	227	71.4 (7.9)	60	18.3 (4.5)	DSM-IV NINCDS-ADRDA	26
	Placebo	222	71.7 (8.7)	60	18.7 (4.6)		
Rogers et al., 1998	Donepezil 5 mg	157	73.8 (8.4)	69	19.4 (4.9)	DSM-III-R NINCDS-ADRDA	12
	Donepezil 10 mg	158	73.4 (8.2)	61	19.4 (5.0)		
	Placebo	153	74.0 (8.0)	61	19.8 (4.3)		
Burns et al., 1999	Donepezil 5 mg	271	72.0 (8.2)	61	20.0 (4.9)	DSM-III-R NINCDS-ADRDA	24
	Donepezil 10 mg	273	72.0 (8.3)	57	20.0 (3.3)		
	Placebo	274	71.0 (8.3)	55	20.0 (5.0)		
Maher-Edwards et al., 2011	Donepezil 10 mg	67	71.1 (8.39)	63	19.2(3.20)	DSM-IV NINCDS-ADRDA	24
	Placebo	63	71.6 (6.72)	70	18.3(3.36)		
Homma et al., 2000	Donepezil 5 mg	116	70.1 (7.6)	68	17.8 (3.9)	DSM-IV	24
	Placebo	112	69.4 (8.8)	66	16.6 (3.9)		

DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders third edition, revision; NINCDS-ADRDA, The National Institute of Neurological and Communicative Disorders Association and Stroke-AD and Related Disorders Association; MMSE, Mini-mental State Examination; NA, Not available.

participants contributed to the network meta-analysis. Across all trials, the year of RCT publication ranged from 1998 to 2016. The mean study sample size was 175 participants in each group, with a range between 20 and 1,024 patients. The mean (SD) age of participants was between 64.2 (8.4) and 78.1 (8.35) years of age. The minimum percentage of females was 45.6%, and the maximum percentage was 84.4%. Most trials (35 [94.6%] of 37) adopted the NINCDS-ADRDA diagnostic criteria. Follow-up data was available for all patients for a minimum of 12 weeks and a maximum of 104 weeks.

Quality of the Assessment

Detailed information regarding the risk of bias in all 37 studies is presented in **Figure 2** and **Additional file: Supplementary 2**. It was difficult to assess the risk of selection bias in most studies, owing to the absence of adequate details recorded for randomization and allocation concealment. We identified one study with a high risk of bias associated with the blinding of participants and personnel. As for the blinding of the outcome assessment, 29 trials were rated as having an unclear risk of bias, and only eight studies had evidence indicating a low risk of bias. Most studies (36 of 37) had a low risk of bias for incomplete outcome data. The percentage of studies with unclear bias was 70.3. In addition, a high risk of bias was noted in six studies. In total, the overall quality of the studies was judged to be good.

Pair-Wise Meta-Analysis

The tested interventions, except for rivastigmine 12 mg, the rivastigmine 5 cm^2 patch, huperzine A 400 μg, and huperzine A 200 μg, showed statistically significant differences with regard to the ADCS-cog assessment for mild to moderate AD when compared with the placebo. However, in the MMSE, donepezil 10 mg, donepezil 5 mg, rivastigmine 10 cm^2, galantamine 24 mg, huperzine A 400 μg, and huperzine A 200 μg was superior to the placebo. In terms of acceptability, well-tolerated interventions included rivastigmine 12 mg, rivastigmine 10 cm^2 patch, rivastigmine 5 cm^2 patch, galantamine 24 mg, and galantamine 32 mg compared with the placebo. For all interventions, except for donepezil 5 mg, rivastigmine 10 cm^2 patch, memantine 20 mg, and EGb761 240 mg, adverse events occurred more often than that with the placebo. For secondary outcomes, in terms of daily living, either the rivastigmine 10 cm^2 patch or galantamine 24 mg was superior to placebo. Compared with placebo, donepezil 10 mg, donepezil 5 mg, rivastigmine 12 mg, the rivastigmine 10 cm^2 patch, and the rivastigmine 5 cm^2 patch showed statistically significant differences with regard to the clinical global assessment in patients with mild to moderate AD. Compared with the placebo, only galantamine 24 mg and EGb761 240 mg improved behavioral symptoms. Heterogeneity was found only in the direct comparisons of memantine 20 mg vs. placebo (I^2 = 83.1%), galantamine 24 mg *vs.* placebo (I^2 = 78.0%), and rivastigmine 12 mg *vs.* placebo (I^2 = 76.9%), with I^2 values greater than 70%. These results of the pair-wise meta-analyses are outlined in detail in **Additional file: Supplementary 3**.

Network Meta-Analysis—Primary Outcomes

A network diagram of all the eligible comparisons involving 24 trials of cognitive function based on the ADAS-cog scale is

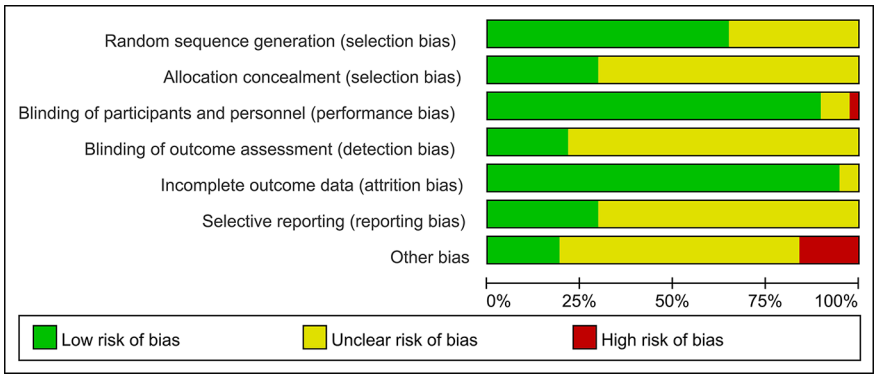

FIGURE 2 | Risk of bias graph presented as percentage across all studies (green represents low risk of bias; red represents high risk of bias; and yellow represents an unclear risk of bias).

presented in **Figure 3A**. As outlined in **Figure 3A**, the placebo was the most common comparator in all interventions comparisons; only the rivastigmine 15 cm^2 patch and the combination of rivastigmine 10 cm^2 and memantine 20 mg were not directly compared with the placebo. Six closed loops existed across all comparisons. Based on the inconsistency factors (IFs) and 95% CIs, we concluded that the direct and indirect evidence was consistent. The relevant inconsistency results and the figures are shown in **Additional file: Supplementary 4**. In terms of improving cognitive function, galantamine 24 mg, galantamine 32 mg, donepezil 10 mg, and donepezil 5 mg were more effective than placebo, with SMDs of −0.39 (95% CrI: [−0.65, −0.12]) for galantamine 24 mg, −0.62 (−1.01, −0.24) for galantamine 32 mg, −0.30 (−0.52, −0.07) for donepezil 10 mg, and −0.37 (−0.69, −0.04) for donepezil 5 mg. Galantamine 32 mg was superior to rivastigmine 12 mg (SMD = −0.65, 95% CrI: [−0.17, −0.20]) and the rivastigmine 10 cm^2 patch (SMD = −0.52, 95% CrI: [−1.06, −0.02]). However, for other interventions, there were no statistically significant differences. In addition, when compared with rivastigmine 12 mg, galantamine 24 mg was more efficacious (SMD = −0.41, 95% CrI: [−0.85, −0.05]). The informative results for mild to moderate AD are shown in **Table 2** (in the top right corner). As shown in **Figure 4A** and **Additional file: Supplementary 5**, the five most efficient interventions were ranked as galantamine 32 mg (SUCRA = 93.2%), galantamine 24 mg (SUCRA = 75.5%), donepezil 5 mg (SUCRA = 73.3%), donepezil 10 mg (SUCRA = 65.6%), and memantine 20 mg (SUCRA = 57.0%). Furthermore, we also assessed cognitive function using the MMSE. The network plot, including a total of 17 studies, is presented in **Figure 3B**. We noted consistent results in both direct and indirect comparisons. In the network meta-analysis, no interventions were associated with statistically significant differences compared with placebo (**Figure 5A**). Furthermore, rivastigmine 12 mg had the highest probability of being ranked first according to SUCRA (72.9%), followed closely by the combination of the rivastigmine 10 cm^2 patch and memantine 20 mg (SUCRA = 63.1%) and the rivastigmine 5 cm^2 patch (SUCRA = 60.7%) (**Additional file: Supplementary 5**).

The network of eligible comparisons for the assessment of acceptability is shown in **Figure 3C**. In total, 33 trials and 16 treatments were included; most treatments were monotherapies, except for the combinations of EGb761 240 mg and donepezil 10 mg and the rivastigmine 10 cm^2 patch and memantine 20 mg. We found no evidence indicating an inconsistency between direct and indirect evidence *via* the IF and 95% CIs of nine closed loops (**Additional file: Supplementary 4**). Our analysis showed that the interventions of rivastigmine 12 mg (OR = 0.52, 95% CrI: [0.34, 0.79]), galantamine 24 mg (OR = 0.72, 95% CrI: [0.53, 0.95]), rivastigmine 10 cm^2 patch (OR = 0.60, 95% CrI: [0.37, 0.95]), and galantamine 32 mg (OR = 0.44, 95% CrI: [0.27, 0.71]) were associated with a significantly increased probability of treatment completion compared with placebo. In addition, EGb761 240 mg was superior to the rivastigmine 10 cm^2 patch (OR = 2.57, 95% CrI: [1.07, 6.50]) and rivastigmine 12 mg (OR = 2.95, 95% CrI: [1.24, 7.30]). Moreover, galantamine 32 mg was inferior to EGb761 240 mg (OR = 0.29, 95% CrI: [0.11,0.70]) (see the left corner of **Table 2**). We also ranked all treatments and found that EGb761 240 mg (SUCRA = 87.5%), donepezil 5 mg (SUCRA = 83.4%), and EGb761 160 mg (SUCRA = 72.5%) were most likely to be ranked first (**Figure 4B**).

A total of 32 trials with 13 interventions presented data on adverse events. The network diagram is presented in **Figure 3D**. The direct and indirect evidence was consistent (**Additional file: Supplementary 4**). Our network meta-analysis demonstrated that only EGb761 240 mg was better tolerated than placebo for safety (OR = 0.66, 95% CrI: [0.43, 0.99]). Rivastigmine 12 mg, galantamine 24 mg, the rivastigmine 10 cm^2 patch, donepezil 10 mg, galantamine 32 mg, and the rivastigmine 15 cm^2 patch were associated with a significantly increased risk of adverse events compared with placebo (OR = 2.69, 95% CrI: [1.96, 3.90], OR = 1.53, 95% CrI: [1.25, 1.98], OR = 1.68, 95% CrI: [1.18, 2.48], OR =

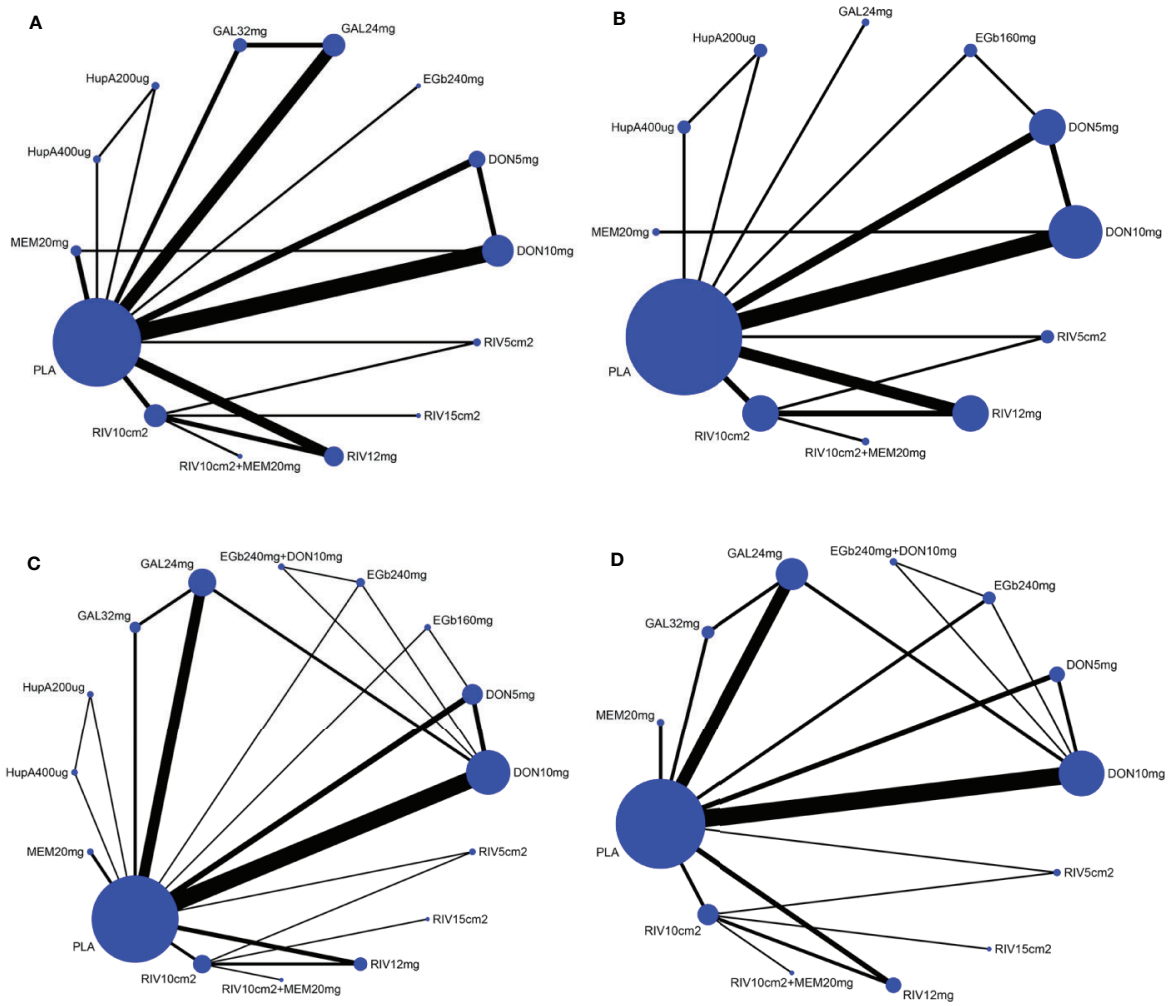

FIGURE 3 | Network of eligible comparisons for all pharmacological treatments included in the analyses [**(A)** according to ADAS-cog scale, **(B)** MMSE results, **(C)** acceptability, **(D)** safety]. Treatments with direct comparisons are linked with a black line; its width is proportional to the number of trials evaluating every pair of the comparison. Blue Nodes represent different treatments. Node size is proportional to the total number of patients for each treatment in the network. MMSE, Mini-Mental State Examination; ADAS-cog, Alzheimer's Disease Assessment Scale-cognition subscale; PLA, Placebo; RIV10cm2, Rivastigmine patch 10 cm^2; RIV10cm2 +MEM20mg, Rivastigmine patch 10 cm^2 + Memantine 20 mg; RIV12mg, Rivastigmine 12 mg; RIV15cm2, Rivastigmine patch 15 cm^2; RIV5cm2, Rivastigmine patch 5 cm^2; DON10mg, Donepezil 10 mg; DON5mg, Donepezil 5 mg; EGb240mg, EGb761 240 mg; GAL24mg, Galantamine 24 mg; GAL32mg, Galantamine 32 mg; HupA200μg, Huperzine A 200 μg; HupA400μg, Huperzine A 400 μg; MEM20mg, Memantine 20 mg; EGb160mg, EGb761 160 mg; EGb240mg+DON10mg, EGb761 240 mg + Donepezil 10 mg.

1.43, 95% CrI: [1.16, 1.76], OR = 2.51, 95% CrI: [1.58, 4.09], OR = 2.34, 95% CrI: [1.19, 4.83], respectively; **Figure 5B**). Other drugs, such as donepezil 5 mg as a monotherapy, and the combinations of the rivastigmine 10 cm^2 patch with memantine 20 mg as well as EGb761 240 mg with donepezil 10 mg showed no statistical differences when compared with placebo. Based on SUCRA values, the optimal acceptable intervention was likely to be EGb761 240 mg (SUCRA = 97.8%). Memantine 20 mg and donepezil 5 mg followed closely behind as the second (SUCRA = 78.9%) and third (SUCRA = 71.7%) most acceptable interventions (**Additional file: Supplementary 5**).

Network Meta-Analysis—Secondary Outcomes

Networks of eligible comparisons of the secondary outcomes are presented in **Additional file: Supplementary 6**, demonstrating predominantly head-to-head comparisons of drugs with active drugs or placebo. Regardless of whether the CIBIC-plus scale, ADCS-ADL, or NPI scales were used, the direct and indirect evidence indicated consistent results. (**Additional file: Supplementary 4**). For the assessment of clinical global impressions *via* the CIBIC-plus scale, memantine 20 mg, donepezil 10 mg, rivastigmine 12 mg, and donepezil 5 mg were

TABLE 2 | Network meta-analysis comparison of 16 pharmacological treatments for mild to moderate Alzheimer's disease.

PLA	0.03 (-0.24,0.36)	**-0.39 (-0.85,-0.12)**	-0.10 (-0.43,0.27)	**-0.30 (-0.52,-0.07)**	-0.16 (-0.74,0.42)	0.00 (-0.68,0.67)	-0.20 (-0.90,0.54)	0.07 (-0.51,0.66)	…	**-0.62 (-1.01,-0.24)**	-0.24 (-0.60,0.12)	-0.09 (-0.61,0.44)	…	**-0.37 (-0.69,-0.04)**	-0.20 (-0.85,0.49)
0.52 (0.34,0.79)	RIV12mg	**-0.41 (-0.85,-0.05)**	-0.13 (-0.49,0.21)	-0.33 (-0.72,0.01)	-0.19 (-0.88,0.43)	-0.03 (-0.80,0.68)	-0.22 (-0.97,0.48)	0.04 (-0.64,0.67)	…	**-0.65 (-0.17,-0.20)**	-0.26 (-0.76,0.17)	-0.12 (-0.70,0.42)	…	-0.40 (-0.87,0.01)	-0.23 (-0.92,0.42)
0.72 (0.53,0.95)	1.39 (0.82,2.28)	GAL24mg	0.29 (-0.14,0.74)	0.09 (-0.26,0.44)	0.22 (-0.42,0.87)	0.39 (-0.34,1.11)	0.19 (-0.57,0.97)	0.46 (-0.19,1.10)	…	0.24 (-0.61,0.15)	0.15 (-0.29,0.59)	0.30 (-0.29,0.89)	…	0.02 (-0.40,0.44)	0.18 (-0.52,0.93)
0.60 (0.37,0.95)	1.15 (0.72,1.83)	0.83 (0.48,1.45)	RIV10cm^2	-0.20 (-0.63,0.19)	-0.06 (-0.76,0.60)	0.09 (-0.68,0.85)	-0.10 (-0.73,0.52)	0.17 (-0.53,0.84)	…	**-0.52 (-1.06,-0.02)**	-0.14 (-0.66,0.35)	0.01 (-0.53,0.52)	…	-0.27 (-0.76,0.20)	-0.10 (-0.68,0.47)
0.82 (0.63,1.07)	1.59 (0.97,2.60)	1.14 (0.80,1.68)	1.38 (0.80,2.39)	DON10mg	0.14 (-0.49,0.76)	0.30 (-0.41,1.01)	0.10 (-0.63,0.87)	0.37 (-0.26,0.99)	…	-0.32 (-0.77,0.12)	0.06 (-0.32,0.44)	0.21 (-0.35,0.78)	…	-0.07 (-0.41,0.28)	0.10 (-0.59,0.83)
0.54 (0.17,1.62)	1.04 (0.31,3.40)	0.75 (0.23,2.35)	0.90 (0.26,3.03)	0.65 (0.20,2.04)	HupA400μg	0.16 (-0.52,0.83)	-0.04 (-0.95,0.91)	0.23 (-0.59,1.06)	…	-0.46 (-1.16,0.24)	-0.08 (-0.76,0.61)	0.07 (-0.71,0.86)	…	-0.21 (-0.88,0.47)	-0.04 (-0.91,0.87)
0.82 (0.24,2.66)	1.58 (0.44,5.41)	1.14 (0.33,3.85)	1.38 (0.38,4.94)	1.00 (0.29,3.35)	1.52 (0.52,4.75)	HupA200μg	-0.20 (-1.16,0.81)	0.07 (-0.82,0.97)	…	-0.62 (-1.40,0.15)	-0.24 (-1.00,0.52)	-0.09 (-0.95,0.77)	…	-0.37 (-1.12,0.38)	-0.20 (-1.14,0.76)
0.84 (0.26,2.81)	1.63 (0.51,5.37)	1.17 (0.35,4.05)	1.42 (0.49,4.22)	1.03 (0.31,3.53)	1.58 (0.31,8.34)	1.03 (0.20,5.74)	RIV10cm^2+ MEM20mg	0.27 (-0.88,1.19)	…	-0.43 (-1.25,0.38)	-0.04 (-0.86,0.75)	0.11 (-0.72,0.92)	…	-0.17 (-0.97,0.61)	-0.01 (-0.87,0.84)
1.53 (0.71,3.35)	**2.95 (1.24,7.30)**	2.12 (0.95,5.00)	**2.57 (1.07,6.50)**	1.87 (0.85,4.19)	2.86 (0.74,11.84)	1.87 (0.46,8.11)	1.81 (0.45,7.45)	EGb240mg	…	-0.69 (-1.40,0.01)	-0.31 (-1.00,0.38)	-0.16 (-0.95,0.63)	…	-0.44 (-1.11,0.23)	-0.27 (-1.15,0.64)
0.87 (0.17,5.50)	1.68 (0.31,11.08)	1.22 (0.23,7.77)	1.47 (0.27,9.86)	1.06 (0.21,6.66)	1.63 (0.22,15.03)	1.07 (0.14,10.07)	1.05 (0.13,9.39)	0.57 (0.10,3.72)	EGb240mg+ DON10mg	…	…	…	…	…	…
0.44 (0.27,0.71)	0.84 (0.43-1.59)	**0.61 (0.37,0.99)**	0.74 (0.37,1.45)	0.53 (0.31,0.92)	0.82 (0.24,2.82)	0.53 (0.15,1.94)	0.51 (0.14,1.83)	**0.29 (0.11,0.70)**	0.50 (0.07,2.83)	GAL32mg	0.38 (-0.14,0.91)	0.53 (-0.11,1.19)	…	0.25 (-0.25,0.75)	0.42 (-0.33,1.21)
0.76 (0.42,1.38)	1.48 (0.70,3.05)	1.06 (0.55,2.06)	1.29 (0.60,2.77)	0.93 (0.48,1.77)	1.42 (0.41,5.17)	0.93 (0.25,3.52)	0.90 (0.24,3.36)	0.50 (0.18,1.31)	0.88 (0.12,5.05)	1.75 (0.81,3.75)	MEM20mg	0.15 (-0.48,0.79)	…	-0.13 (-0.59,0.34)	0.03 (-0.72,0.82)
0.58 (0.29,1.18)	1.13 (0.53,2.40)	0.81 (0.39,1.75)	0.98 (0.49,1.97)	0.71 (0.34,1.50)	1.10 (0.30,4.13)	0.71 (0.18,2.85)	0.70 (0.19,2.49)	0.38 (0.13,1.07)	0.66 (0.09,4.05)	1.34 (0.58,3.16)	0.77 (0.31,1.92)	RIV5cm^2	…	-0.28 (-0.90,0.33)	-0.11 (-0.89,0.68)
1.26 (0.34,5.35)	2.44 (0.61,10.99)	1.75 (0.46,7.66)	2.12 (0.53,9.76)	1.53 (0.41,6.60)	2.35 (0.43,14.79)	1.53 (0.26,10.43)	1.48 (0.25,9.82)	0.82 (0.18,4.15)	1.42 (0.15,13.33)	2.87 (0.71,13.38)	1.65 (0.39,7.90)	2.15 (0.49,10.78)	EGb160mg	…	…
1.26 (0.83,1.96)	**2.44 (1.34,4.47)**	**1.75 (1.06,3.00)**	**2.12 (1.13,4.07)**	**1.54 (1.01,2.37)**	2.36 (0.72,7.94)	1.54 (0.44,5.59)	1.50 (0.42,5.16)	0.82 (0.34,2.00)	1.44 (0.22,7.88)	**2.89 (1.53,5.62)**	1.65 (0.81,3.54)	2.16 (0.96,4.91)	1.01 (0.24,3.73)	DON5mg	0.16 (-0.57,0.93)
0.70 (0.29,1.67)	1.35 (0.56,3.20)	0.97 (0.38,2.45)	1.17 (0.55,2.48)	0.85 (0.33,2.11)	1.31 (0.31,5.54)	0.85 (0.19,3.85)	0.83 (0.22,3.04)	0.46 (0.14,1.43)	0.80 (0.10,5.08)	1.60 (0.57,4.35)	0.91 (0.32,2.67)	1.20 (0.43,3.29)	0.56 (0.10,2.69)	0.55 (0.20,1.45)	RIV15cm^2

The results are presented as the OR and 95% CrI for acceptability (lower left quarter) and as the SMD and 95% CrI for cognitive function in ADAS-cog (upper right quarter). For acceptability, ORs higher than 1 favor the column-defining treatment. For cognitive function, SMDs lower than 1 favor the row-defining treatment. OR, odds ratio; CrI, credible interval; SMD, standard mean difference; ADAS-cog, Alzheimer's Disease Assessment Scale-cognition subscale; PLA, placebo; RIV12mg, Rivastigmine 12 mg; GAL24mg, Galantamine 24 mg; RIV10cm2, Rivastigmine patch 10 cm^2; DON10mg, Donepezil 10 mg; HupA400μg, Huperzine A 400 μg; HupA200μg, Huperzine A 200 μg; RIV10cm2+MEM20mg, Rivastigmine patch 10 cm^2 + Memantine 20 mg; EGb240mg, EGb761 240 mg; EGb240mg+DON10mg, EGb761 240 mg + Donepezil 10 mg; GAL32mg, Galantamine 32 mg; MEM20mg, Memantine 20 mg; RIV5cm2, Rivastigmine patch 5 cm^2; EGb160mg, EGb761 160 mg; DON5mg, Donepezil 5 mg; RIV15cm2, Rivastigmine patch 15 cm^2.

Bold texts in the table mean ORs and SMDs are statistcaly significant. Light blue in the lower left corner represented the odds ratio (OR) and 95% credible interval (CrI) for acceptability, orange in the upper right corner represented the standard mean difference (SMD) and 95% CrI for cognitive function in ADAS-cog, and dark blue represented different interventions.

significantly superior to placebo (SMD = −0.27, 95% CrI: [−0.48, −0.07]; SMD = −0.34, 95% CrI: [−0.50, −0.17]; SMD = −0.40, 95% CrI: [−0.62, −0.18]; SMD = −0.29, 95% CrI: [−0.53, −0.06]) (**Additional file: Supplementary 7**). The SUCRAs ranged from 83.7% for the highest-ranked treatment strategy (rivastigmine 12 mg) to 40.3% for the lowest-ranked agent (rivastigmine 5 cm^2) (**Additional file: Supplementary 5**). In the assessment for improvements daily living using the ADCS-ADL scale, donepezil 10 mg, galantamine 24 mg, and the rivastigmine 15 cm^2 patch were statistically more efficacious than placebo, with SMDs and 95% CrIs of 0.21 (0.02, 0.40) for donepezil 10 mg, 0.22 (0.06, 0.37) for galantamine 24 mg, and 0.51 (0.17, 0.81) for the rivastigmine 15 cm^2 patch (**Additional file: Supplementary 7**). As shown in **Additional file: Supplementary 5**, the rank of the three most efficient interventions was the rivastigmine 15 cm^2 (SUCRA = 93.7%), the combination of rivastigmine 10 cm^2 and memantine 20 mg (SUCRA = 71.1%), followed by galantamine 24 mg (SUCRA = 60.3%). Twelve studies assessed neuropsychiatric symptoms using the NPI scale for nine different treatment interventions and placebo. However, in our network meta-analysis, there were no interventions that significantly improved neuropsychiatric symptoms compared with placebo.

Publication Bias

We produced comparison-adjusted funnel plots, with different colors representing different comparisons. Through a visual inspection, we found that the funnel plots presented an essentially symmetrical distribution, indicating that there were no small-sample effects for any outcomes (**Additional file: Supplementary 8**).

DISCUSSION

This comprehensive network meta-analysis was based on 37 trials, which included 14,705 patients with mild to moderate AD randomly assigned to currently available active agents or placebo, and compared the efficacy, acceptability, and safety of various regimens. The magnitude of intervention ranking varied enormously across different cognitive enhancers and doses, especially in different assessment outcomes. The results suggested that for patients with mild to moderate AD, galantamine 32 mg, galantamine 24 mg, donepezil 5 mg, donepezil 10 mg, and memantine 20 mg were more efficacious for cognitive improvements than other pharmacotherapies. The EGb761 240 mg treatment appeared to be the most optimal in terms of both acceptability and safety. Moreover, of the current treatment therapies, rivastigmine 12 mg offered a more favorable profile with benefits in the clinical global impression. The rivastigmine 15 cm^2 patch, another rivastigmine dosage form, had the highest probability of functional improvement. However, we did not find any effective interventions resulting in behavioral improvements. This project extends a previous network meta-analysis that addressed ten interventions with data for direct and indirect

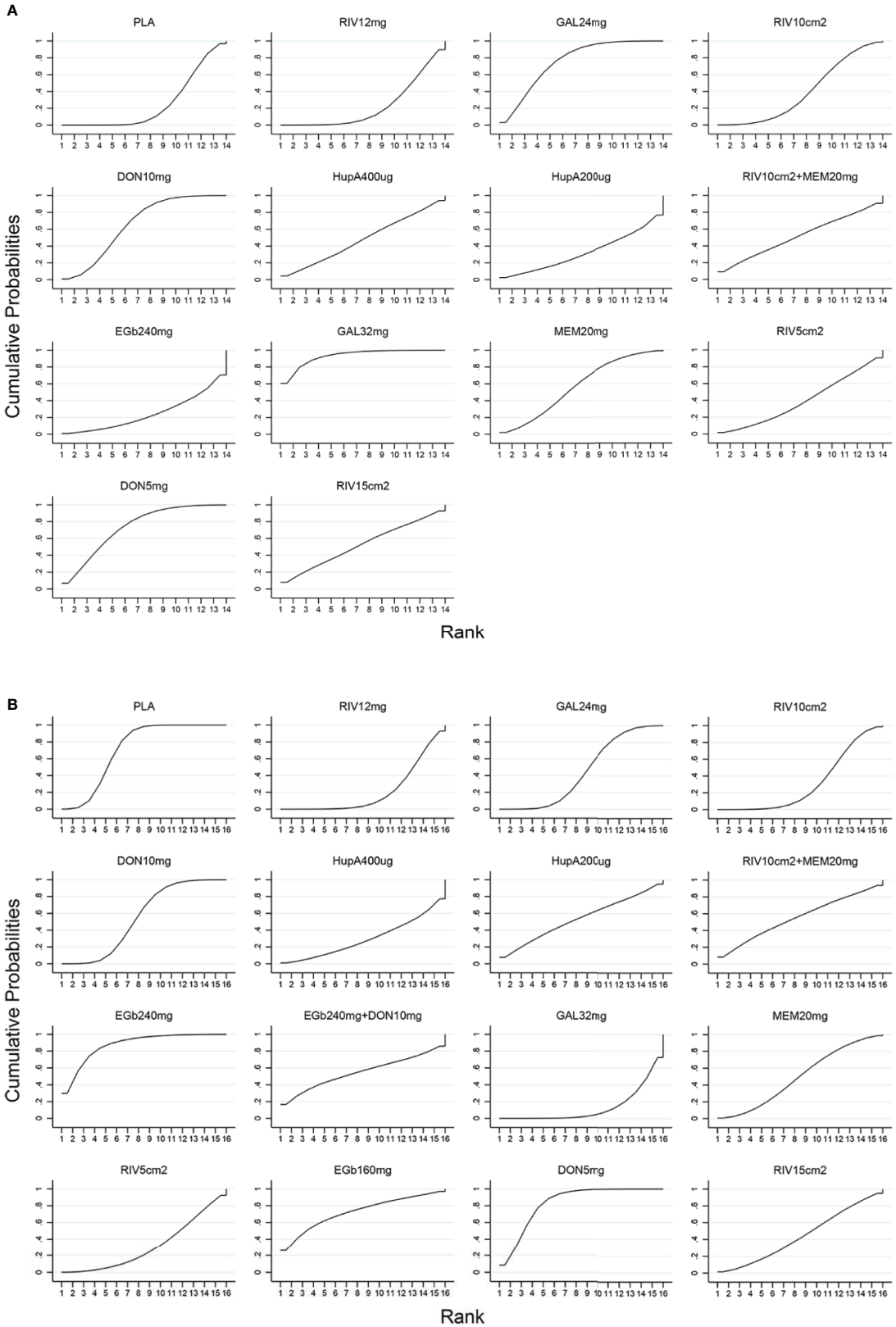

FIGURE 4 | Continued

FIGURE 4 | SUCRA for cognitive function based on ADAS-cog scale **(A)** and acceptability **(B)**. The larger the SUCRA, the higher the ranking. ADAS-cog, Alzheimer's Disease Assessment Scale-cognition subscale; SUCRA, surface under the cumulative ranking curve; PLA, Placebo; RIV12mg, Rivastigmine 12 mg; GAL24mg, Galantamine 24 mg; RIV10cm2, Rivastigmine patch 10 cm^2; DON10mg, Donepezil 10 mg; HupA400μg, Huperzine A 400 μg; HupA200μg, Huperzine A 200 μg; RIV10cm2+MEM20mg, Rivastigmine patch 10 cm^2 + Memantine 20 mg; EGb240mg, EGb761 240 mg; GAL32mg, Galantamine 32 mg; MEM20mg, Memantine 20 mg; RIV5cm2, Rivastigmine patch 5 cm^2; DON5mg, Donepezil 5 mg; RIV15cm2, Rivastigmine patch 15 cm^2; EGb240mg+DON10mg, EGb761 240 mg + Donepezil 10 mg; EGb160mg, EGb761 160 mg.

comparisons (Dou et al., 2018). Our study can assist in the provision of relevant options for clinical pharmacotherapies for patients with mild to moderate AD.

Galantamine is a reversible and competitive AChEI (Bores et al., 1996). A previous meta-analysis concluded that galantamine was an effective therapeutic agent and was a preferred treatment for AD compared with donepezil, memantine, and rivastigmine (Li et al., 2019). Galantamine 32 mg was associated with a significant improvement in cognitive function; however, owing to poor acceptability and adverse events, its practical use may be limited. Based on the overall evidence, galantamine 24 mg may therefore, be the optimal treatment option for patients with mild to moderate AD. In addition, the major therapeutic effect of EGb761 240 mg

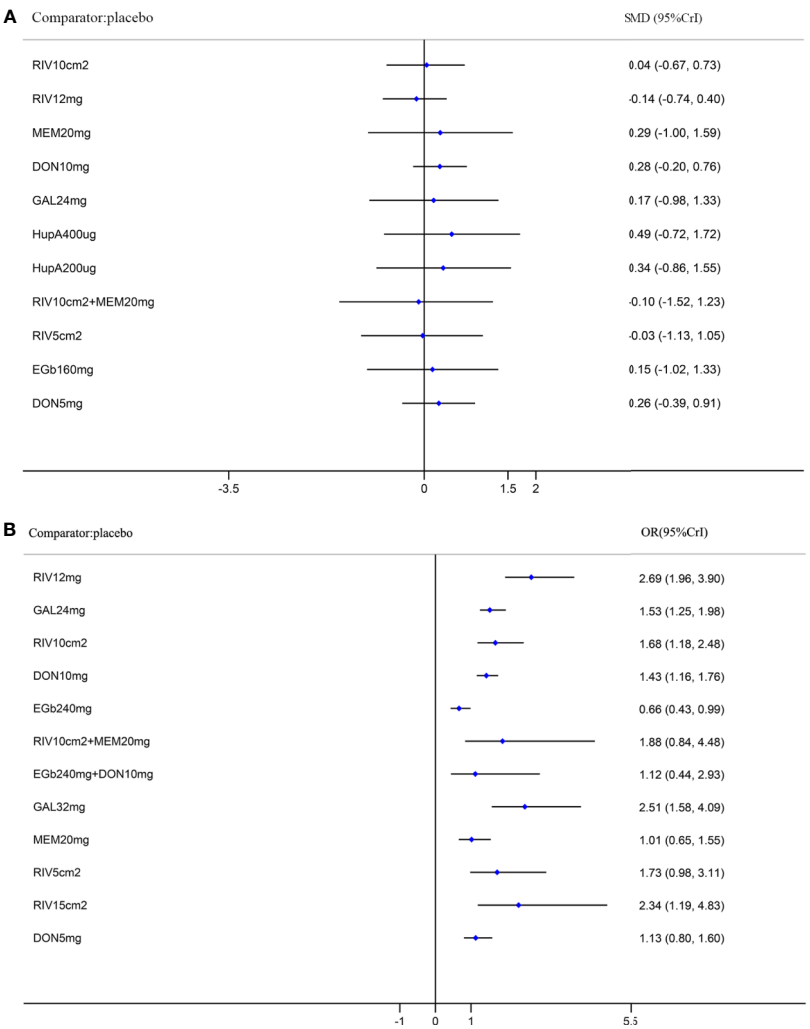

FIGURE 5 | Forest plots of the results of network meta-analysis for function in the MMSE **(A)** and for safety **(B)** compared with placebo. SMD, standardized mean difference; OR, odds ratio; CrI, credible interval; MMSE, Mini-Mental State Examination; RIV10cm2, Rivastigmine patch 10 cm^2; RIV12mg, Rivastigmine 12 mg; MEM20mg, Memantine 20 mg; DON10mg, Donepezil 10 mg; GAL24mg, Galantamine 24 mg; HupA400μg, Huperzine A 400 μg; HupA200μg, Huperzine A 200 μg; RIV10cm2+MEM20mg, Rivastigmine patch 10 cm^2 + Memantine 20 mg; RIV5cm2, Rivastigmine patch 5 cm^2; EGb160mg, EGb761 160 mg; DON5mg, Donepezil 5 mg; EGb240mg, EGb761 240 mg; EGb240mg+DON10mg, EGb761 240 mg + Donepezil 10 mg; GAL32mg, Galantamine 32 mg; RIV15cm2, Rivastigmine patch 15 cm^2.

is based on its acceptability and fewer associated adverse events. Although some studies have shown that EGb761 was favorable for cognitive, behavioral, and functional improvements, and clinical global impressions (Yancheva et al., 2009; Ihl et al., 2012; Yang et al., 2016), their sample sizes were much smaller, and the results were mixed. Thus, we propose that EGb761 should be researched further in large-scale randomized controlled trials. It has been reported that huperzine A is a well-tolerated intervention leading to improvements in cognitive impairment; however, until now, the evidence from our network meta-analysis did not recommend its use (Xing et al., 2014). A secondary analysis showed that regardless of dosage form and dose, rivastigmine produced a relatively marked improvement in both clinical global impression and daily living. The rivastigmine patch is frequently used in patients with mild to moderate AD because the adverse events associated with the patch are greatly reduced compared with that of the capsule form (Winblad et al., 2007). It is a novel drug delivery method that allows continuous drug administration.

We carefully monitored quality between the included trials and found that the majority of trials were considered to be unclear with regard to selection bias, especially, allocation concealment. Additionally, open-label trials were included. Nevertheless, our analysis could still be powered to provide objective evaluations for unclear factors given the even distribution of patient characteristics and the objective method adopted in each treatment group. Through the node-splitting method and loop-specific method, we noticed no significant differences between consistency in terms of the concerned evaluated outcomes. To assess the bias of small-sample effects, we also produced a comparison-adjusted funnel plot, and the findings were reassuring.

We are aware of three studies associated with AD that also integrated direct and indirect comparisons simultaneously in one network meta-analysis (Dou et al., 2018; Thancharoen and Limwattananon, 2019; Tsoi et al., 2019). In contrast to these previous studies, our study included new interventions and integrated all available high-quality RCTs with regard to the effectiveness, acceptability, and safety of cognitive enhancers in treating mild to moderate AD in one analysis, while examining different doses of treatments as independent interventions.

As with any network meta-analysis, our study has some limitations. Although we tried our best to include all eligible literature through comprehensive and systematic review, the sample size was still small for some interventions in individual RCTs. Furthermore, not all studies reported data for each outcome measure. However, it is essential to include all eligible studies in a network meta-analysis to reduce potential biases. Finally, this study primarily compared the efficacy, acceptability, and safety of pharmacological treatments for mild to moderate AD but did not include an analysis of cost-effectiveness. It is known that AD poses an enormous economic burden, and it is necessary to consider the balance of the therapeutic effects and costs. However, there was a lack of primary data involving cost-effectiveness in the included studies.

CONCLUSIONS

In summary, our network meta-analysis findings suggested that galantamine (32 mg and 24 mg) and donepezil (5 mg and 10 mg) were the most effective strategies for improving the cognitive symptoms of patients with mild to moderate AD. We posit our findings, which we believe can support clinical decision-making. When taking acceptability and safety into account, EGb761 240 mg may be the optimal therapeutic choice. Rivastigmine 12 mg achieved the highest level of clinical global impression, and in terms of function, rivastigmine 15 cm^2 patch is likely to be the best intervention. Nevertheless, none of the interventions effectively improved behavior. We hope that our study contributes markedly to the process of making accurate and efficient clinical decisions with regard to AD treatment.

DATA AVAILABILITY STATEMENT

All datasets for this study are included in the **Supplementary Material**.

AUTHOR CONTRIBUTIONS

TZ and HL were involved in the concept and design of the study. TZ drafted the manuscript. All authors were involved in acquisition, analysis, and interpretation of the data, revised the manuscript, and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.00778/full#supplementary-material

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REVIEW
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Gene Polymorphisms Affecting the Pharmacokinetics and Pharmacodynamics of Donepezil Efficacy

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Donepezil (DNP) is the first-line drug used for Alzheimer's disease (AD). However, the therapeutic response rate of patients to DNP varies from 20 to 60%. The main reason for the large differences in the clinical efficacy of DNP therapy is genetic factors, some of which affect pharmacokinetics (PK), while others affect pharmacodynamics (PD). Thus, much emphasis has been placed on the investigation of an association between PK- and PD-related gene polymorphisms and therapeutic response to DNP, but a consistent view does not yet exist. In this review, we summarize recent findings regarding genetic factors influencing the clinical efficacy of DNP, including substantial differences in individual responses as a consequence of polymorphisms in Cytochrome P450 (CYP) 2D6, CY3A4, CY3A5, APOE, ABCA1, ABCB1, ESR1, BCHE, PON-1, CHRNA7, and CHAT. We also discuss possible strategies for the evaluation of the clinical efficacy of DNP, with a specific focus on possible biomarkers of PK/PD parameters, and provide perspectives and limitations within the field, which will also be beneficial for understanding the multiple mechanisms of DNP therapy in AD.

Keywords: donepezil, clinical efficacy, gene polymorphisms, pharmacogenetics, pharmacodynamics

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INTRODUCTION

Donepezil (DNP) is a cholinesterase inhibitor widely used for the treatment of mild to moderate Alzheimer's disease (AD) in the past 20 years. Recently, an increasing number of randomized case–control studies have confirmed the clinical value of DNP in the treatment of mild to moderate Alzheimer's disease (Cacabelos et al., 2016; Birks and Harvey, 2018). Due to its good liposolubility, it can easily pass through the blood–brain barrier. DNP binds to cholinesterases and has a strong affinity for acetylcholinesterase, especially in the cerebral cortex (Prvulovic and Schneider, 2014). Thus, DNP has the beneficial therapeutic effect of inhibiting acetylcholinesterase in the brain and few adverse effects (which are mainly gastrointestinal reactions) (Noetzli et al., 2014). It is the first-line treatment for mild to moderate AD in more than 75 countries worldwide (Birks and Harvey, 2018).

However, the clinical response of AD patients to DNP varies largely, and the therapeutic efficacy ranges from 20 to 60% [5–9] (Matsui et al., 1999; Raskind et al., 2000; Yang et al., 2011; Albani et al., 2012; Barth et al., 2012). Pharmacogenetic factors account for 60–90% of drug variability in drug disposition and pharmacodynamics (Cacabelos, 2008). A large number of related studies have shown that the main reason for the large differences in the clinical efficacy of DNP therapy may be closely related to genetic factors (Raskind et al., 2000; Noetzli et al., 2014). In this review, we attempt to (1) summarize the genetic variants that may impact the response to DNP treatment in AD patients according to pharmacogenetic and pharmacodynamic effects as well as (2) provide an overview of possible PK/PD biomarkers of DNP efficacy and perspectives and limitations within the field.

DATA SOURCES AND SEARCH STRATEGY

A comprehensive search of studies about genes related to the pharmacokinetics (PK) and pharmacodynamics (PD) of DNP treatment in AD patients published up to April 2020 was performed. Publications were restricted to the English language, and well-designed studies were included. Studies were identified through an electronic search of two databases: PubMed and Web of Science. For the search strategy, we used the subject words "donepezil", "clinical efficacy", "gene polymorphisms", "pharmacogenetics", "pharmacodynamics", and "Alzheimer's disease" and their free words to search the two databases for articles written in English. Relevant reference lists were also searched. We included studies involving quantitative analysis if they met the following criteria: (1) research papers of randomized case–control studies considering the association between gene polymorphism and efficacy of DNP in AD; (2) studies reporting sufficient information on inclusion criteria and exclusion criteria for patients; and (3) studies reporting the numbers of enrolled patients and genotype frequencies in patients. We excluded (1) duplicates within and between the databases, (2) studies of animals, (3) studies not related to dementia of AD, (4) studies with no analysis of donepezil when it was used as the basic treatment or a positive control, and (5) articles that were not research papers, such as letters to the editor, case reports, or review articles. Studies used to analyze the efficacy of donepezil in patients with AD mostly included Caucasian and Mongoloid populations.

EFFICACY-RELATED PHARMACOKINETIC GENES

Metabolism is one of the major causes leading to variability in the clinical response to DNP (Rogers and Friedhoff, 1996; Winblad et al., 2001). DNP is mainly metabolized by hepatic enzymes, and 6-deoxy-DNP (6-O-DNP) is the main active metabolite (Tiseo et al., 1998; Cascorbi, 2003; Suh et al., 2005; Prvulovic and Schneider, 2014; Adlimoghaddam et al., 2018). Cytochrome P450 (CYP) 2D6, CYP3A4, CYP3A5, and CYP2C9 are thought to be involved in the metabolism of DNP (Noetzli et al., 2014). It has been reported that donepezil is mainly metabolized by CYP2D6 and CYP3A4 in the liver (Noetzli and Eap, 2013); thus, in recent years, a number of studies have reported the association between CYP2D6 and CYP3A4 polymorphisms and the clinical efficacy of DNP, which we will elaborate in the following paragraphs.

CYP3A4 and CYP3A5

CYP3A4 and CYP3A5 are genes encoding metabolic enzymes related to the efficacy of DNP in AD patients (McEneny-King et al., 2015). Italian scholar Laura Magliulo et al. studied the clinical effects of the CYP3A genes on DNP efficacy in 54 AD patients and 285 control patients in Italy. They found that the genetic polymorphisms in CYP3A4 and CYP3A5 did not significantly affect DNP metabolism and patients. However, AD patients with the CYP3A5*1 allele had better clinical outcomes than patients with the CYP3A5*3/*3 allele, but the results were not significant (Magliulo et al., 2011). Another study carried out in Chinese patients with AD also indicated that the CYP3A4 gene does not influence the efficacy of DNP (Ma et al., 2019).

The CYP3A allele does not affect the pharmacokinetics of DNP *in vivo*, which may be the reason why the CYP3A allele is not significantly associated with the efficacy of DNP. We demonstrated that CYP3A4 contributes much less to the metabolism of DNP *in vivo*, while CYP2D6 mostly contributes to the metabolism of DNP (Lu et al., 2015). Our findings were also confirmed by the Swiss team Muriel Noetzli and colleagues. They studied the effect of CYP3A on DNP clearance in patients. Among the 129 Swiss AD patients, there were 5 CYP3A variants: CYP3A4*1B (rs2740574), CYP3A4 (rs4646437), CYP3A4*22 (rs35599367), CYP3A5*3 (rs776746), and CYP3A7*1C (−262T > A and −270T > G), and these variants did not affect the pharmacokinetics of DNP *in vivo* (Noetzli et al., 2014).

CYP2D6

CYP2D6 Is the Main Enzyme Metabolizing DNP

Orally administered DNP has an approximately 95% plasma protein binding rate (Adlimoghaddam et al., 2018). After oral administration of DNP, it is metabolized by the liver P450 enzymes, and 6-O-DNP is the most active metabolite (Matsui et al., 1999; Pilli et al., 2011; Barth et al., 2012). Lu et al. performed an *in vivo* study and identified CYP2D6 as the predominant metabolic enzyme of DNP (Noetzli et al., 2014; Lu et al., 2015). Data show that CYP2D6 is involved in more than 25% of drug metabolism, and this gene has more than 90 allelic variations (Cascorbi, 2003). CYP2D6 gene polymorphisms affect the efficacy of DNP in AD patients, who either experience a therapeutic effect from DNP at the prescribed drug dose or no response to DNP (Honghao, 2013). The mechanism may involve the association between CYP2D6 gene polymorphisms and the plasma concentration of DNP. Studies have been conducted on the relationship between CYP2D6 gene polymorphism, DNP

plasma concentration, and effect of DNP in Caucasian and Mongoloid populations.

The Association Between CYP2D6 Polymorphisms and the Efficacy of DNP

CYP2D6 rs1080985 is the main mutation in the Caucasian population. CYP2D6 rs1080985 is the CYP2D6*2A variant, which confers a normal phenotype to Caucasian people. Studies have reported CYP2D6*2A (rs1080985) polymorphism influences the clinical efficacy of DNP. Studies have explored the relationship between the rs1080985 polymorphism and the efficacy of DNP; however, the results are not consistent.

Alberto Pilotto et al. studied 127 patients with Alzheimer's disease in Italy (Pilotto et al., 2009). It has been reported that there is an association between the CYP2D6*2A (rs1080985) G allele and patient responses to DNP. The rs1080985 G allele is associated with a faster rate of drug metabolism, resulting in DNP being less effective in patients (Zanger et al., 2001; Gaedigk et al., 2003), and Alberto Pilotto's study confirms this conclusion from a clinical perspective (Pilotto et al., 2009). Diego Albani et al. studied 415 patients with Alzheimer's disease in Italy (Albani et al., 2012). By using logical linear regression analysis, the rs1080985 G allele was indeed associated with an ineffective therapeutic effect of DNP.

Muriel Noetzli et al. believe that different alleles of CYP2D6 influence the metabolic behavior of DNP, which may be the main reason for the differences in DNP treatment efficacy in AD patients. The CYP2D6 gene polymorphism caused a difference in the clearance rate of DNP in patients. In this study, 129 AD patients treated with DNP therapy were enrolled, and the researchers genotyped the relevant CYP2D6*3, CYP2D6*4, CYP2D6*5, and CYP2D6*6 alleles in patients. The researchers obtained the pharmacokinetic parameters of DNP *in vivo* by establishing a population pharmacokinetic model. The results showed that the CYP2D6 alleles had different effects on the clearance of DNP *in vivo*. Poor metabolizers had a 32% slower rate of *in vivo* clearance of DNP and a 67% slower metabolism rate than ultra-rapid metabolizers (Noetzli et al., 2014). The CYP2D6 gene polymorphism affects the metabolic behavior of DNP in patients, which may lead to inconsistencies in the efficacy of DNP.

At the same time, studies have found that the metabolic behavior of CYP2D6 *in vivo* can be changed. R. Cacabelos et al. simultaneously analyzed the APOE and CYP2D6 genes and found that patients with the APOE4/4 genotype may be complete metabolizers of CYP2D6*1/*1, suggesting that patients with a homozygous APOE-4 have highly potent CYP2D6 drug metabolism (Cacabelos and Martinez-Bouza, 2011). Similarly, the distributions of the APOE-4/4 gene in extensive metabolizers and poor metabolizers (as dichotomized based on CYP2D6) were also different (Carson et al., 2008). However, it is not clear whether the effects of APOE polymorphism on the CYP2D6 gene can influence DNP efficacy. Lu et al. reported a trend toward a combined effect of APOE and the CYP2D6 rs1065852 polymorphisms on the clinical efficacy of DNP in Han Chinese patients with AD. The study identified that the patients who were APOE E3 noncarriers and who had the CYP2D6*10/*10 allele showed the best clinical response to DNP (Lu et al., 2016), and the authors believe the mechanism may be related to the effect of APOE on P450-related enzymes (Lu et al., 2016).

However, in Asians rs1065852 polymorphism is the most common mutant allele: it is reported that 37.9% of the Chinese population carries the CYP2D6*1 variant, 51.3% carries the CYP2D6*10 variant (Sakuyama et al., 2008; Saito et al., 2018). For the CYP2D6 rs1065852 polymorphism, Yuan Zhong et al. investigated the relationship of the CYP2D6*1/*1, CYP2D6*1/*10, and CYP2D6*10/*10 alleles in 106 Asian patients with mild to moderate Alzheimer's disease with DNP efficacy. The study found that patients with the CYP2D6*10/*10 allele had better efficacy than those with the other CYP2D6 genotypes, and the steady-state plasma concentration (Cp/dose) of DNP in patients with the CYP2D6*10/*10 allele was significantly higher than those of the other two groups. It is predicted that the peripheral blood concentration may also be a factor related to the efficacy of DNP treatment. This study was limited by the number of samples, and a larger sample size will be needed in the future (Zhong et al., 2013). Thitipon Yaowaluk et al. also reported that CYP2D6*10 carriers have a better therapeutic response to DNP than patients with other CYP2D6 genotypes because they have a higher Css of DNP (Yaowaluk et al., 2019).

However, other studies reported that the efficacy of DNP was influenced by the concentration of DNP but not by CYP2D6 polymorphisms. Miranda, L F et al. followed patients for 12 months and found that a good response was influenced by the concentration of DNP, which was associated with efficacy (Miranda et al., 2017).

On the other hand, there are studies reporting that the correlation between the rs1080985 G allele and efficacy is not significant. Aleksandra Klimkowicz Mrowiec et al. studied 88 Caucasian patients who received DNP for 10 months and concluded that the GG, CG, and CC alleles of rs1080985 were not associated with the efficacy of DNP (Pilotto et al., 2009).

Discussion of CYP2D6 and the Efficacy of DNP

CYP2D6 (rs1065852), CYP2D6 (rs1080985), CYP2D6*3 (rs35742686, 2549delA, P/N: 4312554), CYP2D6*4 (rs3892097, 1846G > A, P/N: 4312555), CYP2D6*6 (rs5030655, 1707delT, P/N: 4312556), CYP3A4*1B (rs2740574, −392A > G), and CYP2D6*10 have been studied for their association with DNP efficacy. However, the results are not consistent. We believe the main reasons for the inconsistent results include the following: 1) these studies were conducted in different ethnic groups. Research in different ethnic groups will have bias in enrollment, for example, most studies are not multicenter for collection of samples; in addition, the distribution frequency of genes in different ethnic groups is different, leading to different statistically significant results; and part of the reason may be: 2) they investigated concentrations of racemic DNP as opposed to S-DNP, which is the pharmaco-effective enantiomer of DNP. Thus, the inconsistency may have resulted from the use of racemic-DNP and the differences in the metabolism of each enantiomer, including the effective S-enantiomer, in the liver,

resulting in the Cp/dose of racemic DNP being unable to be used to evaluate the clinical outcome of the drug.

Based on the above findings, our group has focused on the efficacy of S-DNP. We have shown that CYP2D6*1/*10 and CYP2D6*10/*10 (rs1065852) are the two alleles with the highest mutation frequency in Han Chinese populations (Lu et al., 2016). We further explored whether the plasma concentrations of S-DNP (based on CYP2D6 polymorphisms) were significantly associated with therapeutic responses. The findings suggest that plasma concentrations of S-DNP influence the therapeutic outcomes following treatment with DNP in Han Chinese patients with Alzheimer's disease. Therefore, the results suggest that determining a patient's steady-state plasma concentration of S-DNP in combination with their CYP2D6 genotype might be useful for clinically monitoring the therapeutic efficacy of DNP (Lu et al., 2015; Lu et al., 2016) and further exploring the association of CYP2D6 and APOE. We confirmed that both CYP2D6 and APOE have an influence on therapeutic response to DNP (Lu et al., 2016). Our study may explain the inconsistent results of other studies.

CYP2D6 may be an important genetic marker for the clinical efficacy of DNP.

The plasma concentration of S-DNP is strongly associated with DNP efficacy. However, studies aimed at specific populations with larger samples will be needed in the future to confirm this conclusion.

CYP2C9

Laura Magliulo et al. studied the clinical effects of the CYP2C9 gene on DNP efficacy in 54 AD patients and 285 control patients in Italy. They found that genetic polymorphisms of CYP2C9 did not significantly affect DNP metabolism and patients (Magliulo et al., 2011). These results are consistent with Lu's study, which identified that CYP2C9 contributes little to the metabolism of DNP by exploring the kinetic parameters of the 6-ODD metabolite of DNP from the perspective of cDNA-expressed P450 enyzmes (Lu et al., 2015).

ABCB1

ABCB1, which regulates the movement of compounds across the blood–brain barrier (BBB), may influence the transport of DNP (McEneny-King et al., 2015). Some studies have focused on the association between ABCB1 polymorphisms and the efficacy of DNP, and the results suggest that ABCB1 polymorphism is not one of the main reasons for the variations in the therapeutic response to DNP. Muriel et al. reported that in a study of a total of 129 patients, no association was found between ABCB1 polymorphism and DNP efficacy (Noetzli et al., 2014). Laura Magliulo et al. reported that ABCB1 (3435C > T, 2677G > T/A, and 1236C > T) polymorphisms had no impact on the clinical outcome in 54 Italian patients (Magliulo et al., 2011). The same conclusion was obtained by Thitipon Yaowaluk et al. in patients in Thailand. No significant association of ABCB1 3435C > T or ABCB1 1236C > T with the Cp of DNP or the clinical efficacy of DNP was found in this study (Yaowaluk et al., 2019).

We determined that both (R)- and (S)-DNP were not P-gp substrates (Lili et al., 2013), which may be the reason for the negative outcome of DNP treatment in patients with ABCB1 polymorphisms. McEneny-King et al. also reported that DNP is not a substrate of P-gp but a weak inhibitor of DNP (McEneny-King et al., 2015), which is in agreement with our previous report.

PHARMACODYNAMIC-RELATED GENES

Studies have shown that DNP efficacy is influenced by polymorphisms in pharmacodynamic genes. Apolipoprotein E (APOE), which is believed to be associated with AD pathogenesis, has been reported to modulate the response to DNP treatment; ABCA1, which plays a key role in cholesterol transport and APOE metabolism in the brain, has been reported to be related to Alzheimer's disease. Thus, some studies have focused on the association between ABCA1 polymorphisms and DNP efficacy (**Table 1**).

Since DNP functions as an acetylcholinesterase inhibitor, the related BCHE, PON-1, CHRNA 7, and ChAT polymorphisms have been well studied. Due to sex differences, the ESR1 gene is another popular topic of related studies (**Table 1**).

APOE

Apolipoprotein E (APOE) is a polymorphic protein with three alleles: E2, E3, and E4. APOE is mainly involved in the transformation and metabolism of lipoproteins (Uddin et al., 2019). In recent years, several studies have shown that APOE polymorphism is associated with the efficacy of DNP in the treatment of Alzheimer's disease.

It is widely believed that the E4 allele is a "risk factor" for AD, and patients with at least one E4 allele in the APOE gene are defined as carriers of the E4 gene (Josefsson et al., 2017). In the study of the relationship between DNP efficacy and the APOE gene, the results have been inconsistent.

(1) Some studies have shown that patients with AD carrying the E4 allele have the best DNP efficacy (Bizzarro et al., 2005; Choi et al., 2008; Cacabelos and Martinez-Bouza, 2011); (2) other studies have shown that E4 noncarriers responded better to DNP than E4 carriers (Poirier et al., 1995; Borroni et al., 2002). In addition, (3) some studies have shown that APOE E4 has no impact on DNP efficacy (Nozawa et al., 2009; Pilotto et al., 2009; Santoro et al., 2010; Chianella et al., 2011; Zhong et al., 2013; Waring et al., 2015; Yaowaluk et al., 2019). (4) We reported a significant difference in the frequency of APOE E3 alleles between DNP responders and nonresponders: E3 noncarriers showed a better response to DNP treatment than E3 carriers; however, we did not find a significant difference in APOE E4 frequency between responders and nonresponders (Lu et al., 2016). This may partially result from the differential Aβ peptide production, which is associated with the APOE E2 and E4 (the alleles in E3 noncarriers), between carriers of different alleles, which may be compensated for by DNP-induced sAPP production (Choi et al., 2008; Xiao et al., 2016).

Moreover, the combined effects of APOE and CYP2D6 genotype on DNP efficacy have been reported. In one

TABLE 1 | Differential relationships between DNP efficacy and the related PK/PD gene polymorphisms in patients with Alzheimer's disease in different populations.

Genes	polymorphisms	Association/ no association and Population	number of patients	Scale type	Follow-up time period	references
CYP2D6	CYP2D6 (rs1065852)	Y,Chinese;	77;96;85	MMSE;MMSE; TMSE	3m;6m;36m	Zhong et al., 2013; Lu et al., 2015; Yaowaluk et al., 2019
	CYP2D6 (rs1080985)	Y,Italian; American; German	415;115;203	MMSE;MMSE;/	6m;6m;/	Gaedigk et al., 2003; Pilotto et al., 2009; Albani et al., 2012
	CYP2D6*3 (rs35742686, 2549delA, P/N: 4312554), CYP2D6*4 (rs3892097, 1846G > A, P/N: 4312555), CYP2D6*6 (rs5030655, 1707delT, P/N: 4312556), CYP3A4*1B (rs2740574, –392A > G)	Y,Swiss	129	/	1–96m	Noetzli et al., 2014
	CYP2D6*3	N,Italian	92	MMSE	12m	Chianella et al., 2011
	CYP2D6 (rs1080985)	N,Polish	116	MMSE,CDT,IADL	1m	Klimkowicz-Mrowiec et al., 2013
CYP3A4	CYP3A4*1B(rs2740574);CYP3A4*3(rs4986910), CYP3A4*4(C:30634211_30)	N,Italian	42	MMSE,CDR,ADL, CIBIC-plus	3m	Magliulo et al., 2011
	CYP3A4*1B (rs2740574), CYP3A4 (rs4646437), CYP3A4*22 (rs35599367),	N,Swiss	129	/	1m-96m	Noetzli et al., 2014
CYP3A5	CYP3A5*3 (rs776746)	N,Swiss; N, Thailand	129;85	/;TMSE	1m-96m	Noetzli et al., 2014; Yaowaluk et al., 2019
	CYP3A5*1,CYP3A5*2(C:30633862_10); CYP3A5*6(C:30203950_10);	N,Italian	42	MMSE,CDR,ADL, CIBIC-plus	3m	Magliulo et al., 2011;
CYP2C9	CYP2C9 (rs1057910 and rs4918758),	N,Chinese	179	CDR,ADAS-cog, MMSE	12m	Ma et al., 2019
ABCB1	ABCB1 2677G > T (rs2032582), ABCB1 3435C > T (rs1045642), ABCB1 1236C > T (rs1128503)	N,Swiss	129	/	1m-96m	Noetzli et al., 2014
	ABCB1(1236C > T,3435C > T,2677G > A/T)	N,Italian;N, Thailand	42;85	MMSE,CDR,ADL, CIBIC-plus;TMSE	3m;36m	Magliulo et al., 2011; Yaowaluk et al., 2019
	ABCB1(rs1045642,rs2032582,rs1128503)	N,Chinese	88	MMSE	3m	Lu et al., (data not shown)
ABCA1	ABCA1(rs2230806)	Y,Chinese	88	MMSE	3m	Lu et al., 2018
	ABCA1 (rs2230808)	N,Chinese	88	MMSE	3m	Lu et al., 2018
APOE	APOE E4 carriers have the best response to DNP	Iberian;Italian; Korean	155;81;51	MMSE;MMSE; ADAS-cog	12m;12–16m;12m	Bizzarro et al., 2005; Choi et al., 2008; Cacabelos and Martinez-Bouza, 2011
	APOE E4 noncarriers have the best response to DNP	Italian; Canadian	25;40	MMSE;ADAS-cog, MMSE	1m;8m	Poirier et al., 1995; Borroni et al., 2002
	APOE E4 carriers have no response to DNP	Chinese; Italian; Janpanese; American; Thailand	96; 115; 171;61;938; 165; 85	MMSE;MMSE; MMSE; MMSE, HDS-R; MMSE, ADAS-Cog;ADAS-Cog;TMSE	6m; 6m; 12m;1-6m;9m;3m;36m	Pilotto et al., 2009; Nozawa et al., 2009; Santoro et al., 2010; Chianella et al., 2011; Zhong et al., 2013; Waring et al., 2015; Yaowaluk et al., 2019
	APOE E3 noncarriers have the best response to DNP	Chinese	85	MMSE	3m	Lu et al., 2016
ESR1	ESR1	Y,Italian	157	MMSE	15m	Scacchi et al., 2014
BCHE	BCHE(rs1803274, rs1355534, rs1803274)	N,Italian; Spainish	101; 114	MMSE; SIB, ADCS-ADL	15m; 24m	Blesa et al., 2006; Scacchi et al., 2009
	BCHE (rs1803274)	Y, American	145	MMSE	36m	Sokolow et al., 2017
		N,Italian	92	MMSE	12m	Chianella et al., 2011
PON-1	PON-1	Y,Italian	42	MMSE	9m	Pola et al., 2005
CHRNA7	CHRNA7 (rs8024987)	Y,Chinese Taiwanese;	204	MMSE	6m	Weng et al., 2013;
	CHRNA7 (rs6494223)	Y/N,Brazilian	177	MMSE	6m	Braga et al., 2015
ChAT	ChAT (rs2177369)	Y,Italian	101	MMSE	15m	Scacchi et al., 2009

mechanism, the APOE-related DNP response involves CYP2D6-related effects on liver metabolism (Lu et al., 2016). APOE-CYP2D6 interactions might influence the therapeutic response in AD *via* changes in lipid metabolism and liver function (Cacabelos and Martinez-Bouza, 2011).

ABCA1

ABCA1 is a cholesterol transporter that neutralizes the Aβ aggregation capacity in an APOE-dependent manner. ABCA1 enables the clearance of amyloid β (Aβ) peptide from the brain in mouse models through its role in the lipidation of APOE. DNP

treatment reduced cholesterol accumulation in adult neural stem cells *in vitro*. ABCA1 gene polymorphisms may influence the efficacy of DNP (Lu et al., 2018).

There are few studies on ABCA1, but one of our studies has reported the association between ABCA1 and the efficacy of DNP: patients with the ABCA1 rs2230806 GG genotype responded better to DNP treatment than those with the AA and AG genotypes. We consider that the probable reasons for the ABCA1 rs2230806 genotype influencing DNP efficacy may be the result of DNP-induced sAPP production (Choi et al., 2008). Other probable reasons may include the following: 1) DNP has been shown to induce sAPP production (Mori et al., 1995; Choi et al., 2008). 2) ABCA1 works as a transporter that transports Aβ from the brain into the blood, eventually causing Aβ to be cleared from the brain and reducing the level of Aβ in the brain. The mechanism may be related to ABCA1's ability to reduce β-secretase activity. In addition, ABCA1 promotes cholesterol efflux to the cerebrospinal fluid, thereby improving cognitive decline in AD patients (Yassine et al., 2016; Marchi et al., 2019). These mechanisms suggest that ABCA1 can reduce the production of Aβ by regulating cholesterol efflux and reducing the intracellular content of cholesterol, thereby improving cognitive decline, possibly in an APOE-dependent manner, while the cholesterol transporter ABCA1 neutralizes the Aβ aggregation capacity in an APOE-dependent manner (Lupton et al., 2014). 3) Based on the above analysis, ABCA1 influences DNP efficacy *via* Aβ aggregation, but this mechanism requires further study.

We also found that patients who were APOE E3 noncarriers and had the ABCA1 rs2230806 GG genotype tended to have a better clinical response to DNP therapy than other patients, which indicated that there may be crosstalk between APOE E3 and ABCA1. The transcription of APOE is regulated by LXR-α, and the expression of ABCA1 mRNA is regulated by LXR-α and increases in parallel with APOE transcription during apoptosis (Cacabelos, 2008), suggesting a potential mechanism.

ESR1

The gene encoding estrogen receptor alpha (ERα) is reported to be involved in cognitive function. One of the potential mechanisms by which estrogen modulates cognitive function is *via* the cholinergic system (Tinkler and Voytko, 2005).

Some studies have suggested that gene polymorphism in estrogen receptor alpha (ESR1) is related to the efficacy of DNP. Animal experiments have shown that the cholinergic system may be regulated by estrogen and that estrogen affects cognitive ability (Tinkler and Voytko, 2005). Some researchers have studied the association between AD and ESR1 (the gene encoding the ER gene) (Corbo et al., 2006; Sundermann et al., 2010). However, whether genetic variation of ESR1 plays a role in drug response in AD has not been studied thus far.

A study by Renato Scacchi et al. examined whether ESR gene polymorphisms affect the therapeutic effects of acetylcholinesterase inhibitors. There are two variant sites for ESR1: rs2234693 and rs9340799. The allelic types are PPXX, PPXx, PpXX, and PpXx. A total of 184 Caucasians participated in one study. The study found that PX carriers had a higher drug response to DNP than noncarriers (Scacchi et al., 2014).

The study also found that women were more sensitive to DNP treatment than men. Since estrogen may affect the biosynthesis of acetylcholine, it mainly functions *via* ERa by regulating the activity of acetyltransferases. This may be the reason why women are more sensitive to DNP treatment. At the same time, the *in vitro* study showed that patients carrying the P allele had increased transcription of ESR1 and thus the activity of estrogen compared with patients carrying other alleles. This has also been confirmed by other clinical trials: in menopausal women carrying the Px allele, the concentration of estradiol in plasma was higher than that in women carrying other alleles (Scacchi et al., 2014). The P and X alleles in ESR1 promote the biosynthesis of acetylcholine, thereby enhancing the inhibition of drug-related acetylcholinesterase. Thus, the total amount of effective acetylcholinesterase is increased, and the effect of cognitive reduction is reduced (Scacchi et al., 2014).

BCHE

Butyrylcholinesterase (BCHE) belongs to the cholinergic enzyme family. One single nucleotide polymorphism generally reported is BChE rs1803274 (the so-called K allele), and another is BChE rs1355534. The correlation between the K variant and AD has been extensively studied, and several studies have performed case–control comparisons. However, the results are not consistent. Lehmann et al. concluded that there was no significant association between the K variant and the onset of AD, and the K variant was not a risk factor for AD. However, their substudy showed a significant increase in the risk of AD in the group with men over the age of 75 who carried the K and E4 genes compared with the control group (Lehmann et al., 2001).

Sophie Sokolow et al. reported that BChE rs1803274 (K allele) is associated with a poor response to donepezil therapy after a 3-year observation in 145 patients with MCI (Caucasian), which indicated that BChE rs1803274 (K allele) may be a genetic marker of donepezil efficacy (Sokolow et al., 2017). However, Italian scholar Renato Scacchi et al. studied the efficacy of DNP in patients with BChE rs1355534 and BChE rs1803274 (K allele) and delayed-onset AD, and they concluded that there was no significant association between the BChE gene and DNP efficacy (Scacchi et al., 2009). Similar results were reported by Blesa et al., who studied the efficacy of DNP and Lismin in the treatment of AD patients and the relationship between the K allele and rs1803274 allele. They did not find a statistically significant difference (Blesa et al., 2006).

De Beaumont L et al. reported that carriers of the APOE E4 and/BCHE-K∗ variants responded better to donepezil therapy than other patients after a three-year observation. They reported that APOE E4- and BCHE-K∗-positive subjects had reduced brain cholinergic activity, which may be the reason for their better response to donepezil therapy (De Beaumont et al., 2016).

PON-1

Paraoxonase (PON-1) is a versatile biologically active arylesterase that hydrolyzes surrounding neurotoxins. In addition, it is also a potent exogenous acetylcholinesterase inhibitor (Kondo and Yamamoto, 1998; Costa et al., 2005).

Roberto Pola et al., from Italy, explored the relationship between genetic polymorphisms and the efficacy of acetylcholinesterase inhibitors (DNP and rivastigmin) in AD patients. QQ, QR, and RR are three alleles of the 192 site of the PON-1 gene. The responsive group had a significantly higher frequency of the R allele than the unresponsive group, which suggests that the 192 Q/R gene polymorphism of PON-1 affects the efficacy of acetylcholinesterase inhibitors in patients. A total of 73 Brazilian AD patients were enrolled in the study. Among all of the patients taking acetylcholinesterase inhibitors, the proportion of patients who carried the R genotype in the group with superior efficacy was significantly higher than that in the ineffective group. There were no significant differences in DNP efficacy between the rivastigmin and other treatment groups. Studies have shown that AD patients with the PON-1 gene carrying the R allele are more susceptible to treatment with acetylcholinesterase inhibitors than AD patients with the QQ allele (Pola et al., 2005).

CHRNA7

Acetylcholine receptor subunit $\alpha 7$ (CHRNA7) plays a role in the pathogenesis and prevention of AD. There are a few studies on whether the CHRNA7 gene polymorphism affects the efficacy of DNP in patients with AD. Researchers in Brazil posited that the CHRNA7 gene polymorphism affects the efficacy of DNP in patients with AD. The researchers followed up patients for 2 years to explore the association between the efficacy of acetylcholinesterase inhibitors and the T allele of rs6494223. After 6 months of observation, in 77 patients receiving DNP, there was a significant association between the T allele of CHRNA7 and the efficacy of acetylcholinesterase inhibitors in patients with MMSE >20. However, after 24 months of treatment, the T allele of CHRNA7 was not significantly associated with treatment efficacy (Braga et al., 2015).

CHRNA7 gene polymorphism has been thought to be associated with schizophrenia and AD (Joo et al., 2010; Ancin et al., 2011). The T allele of rs6494223 is associated with a progressive decrease in mild cognitive decline and a reduction in mental disorder syndrome (Carson et al., 2008). The T allele may be indicative of deeper choline dysfunction, confusion, and a better response to acetylcholinesterase inhibitors. This hypothesis has been observed in patients with dementia caused by dementia with Lewy bodies and Parkinson's disease (Court et al., 2001). One Brazilian study was the first to study the efficacy of acetylcholinesterase inhibitors in patients with AD (Braga et al., 2015).

Another study by Chinese scholars in Taiwan concluded that female AD patients with the rs8024987 allele had better efficacy with acetylcholinesterase inhibitors than male patients with this allele. These carriers have better efficacy with galantamine than noncarriers who use DNP (Weng et al., 2013).

A probable mechanism is that acetylcholinesterase inhibitors increase the concentration of acetylcholine, which binds to a7 nAChR, encoded by CHRNA7. The effect of CHRNA7 polymorphism on the effects on cognitive function induced by acetylcholine inhibitors in humans may be accomplished by the following: 1. regulation of the release of presynaptic neurotransmitters; 2. enhancement of memory function *via* regulation of cholinergic neurotransmission; 3. neuroprotection *via* a7 nAChR; 4. upregulation of a7 nAChR by an acetylcholinesterase inhibitor; and 5. positive allosteric regulation of a7 nAChR associated with galantamine (Weng et al., 2013).

Taken together, these results show that CHRNA7 gene polymorphism may be one of the genetic markers for the efficacy of DNP therapy.

ChAT

Choline acetyltransferase is encoded by the ChAT gene located on chromosome 10q 11.2 (Francis et al., 1999; Li et al., 2012). The ChAT rs2177369 polymorphism plays an important role in the formation of acetylcholine. DNP works on the cholinergic system, and thus, it is thought to be related to variability in drug efficacy. Italian scholar Renato Scacchi et al. studied the association between the ChAT rs2177369 polymorphism and the efficacy of DNP in the treatment of late-onset AD. Their study concluded that the G/G genotype was considered a risk gene relative to the G/A+A/A gene. Eighty-seven patients (27.7% males, 72.3% females; age range 56–93 years) took a daily dose of 5 mg DNP, and 14 patients took a daily dose of 10 mg DNP. The ChAT rs2177369 polymorphism was analyzed. The study showed that compared with patients with AD with the G/A +A/A genotypes, AD patients carrying the ChAT rs2177369 G/G genotype had a poorer response to DNP, suggesting that the ChAT gene is a risk gene (Scacchi et al., 2009).

CONCLUSIONS AND FUTURE PERSPECTIVES

DNP plays an important role in the treatment of Alzheimer's disease, but its individual efficacy varies widely, leading to treatment failure and economic waste in clinical therapy (Francis et al., 1999). Gene polymorphisms affect the pharmacokinetics and pharmacodynamics of donepezil efficacy. Therefore, genetic factors are closely related to individual variations in efficacy. The discovery and development of genetic biomarkers provide individualized medicine based on a patient's genetic markers. Studies on the efficacy of DNP and related gene polymorphisms in various ethnic groups and various countries are summarized above. To our knowledge, this is the first review which summarized gene polymorphisms which affect the pharmacokinetics and pharmacodynamics of donepezil efficacy, providing new ideas and new targets for the DNP treatment of AD.

Among the analyses we mentioned, CYP2D6 and APOE genes were the most explored genes. CYP2D6 polymorphisms certainly influence the efficacy of DNP among different people. According to current studies, CYP3A4, CYP3A5, and ABCB1 have no significant influence on DNP efficacy. However, with regard to CYP2D6, ABCA1, APOE, ESR1, BCHE, PON-1, CHRNA7 and CHAT and their mechanism, further research is needed. Additionally, it is noteworthy that variations in a single gene probably have a limited impact on drug efficacy; multiple

gene variations may have a greater impact on drug efficacy. There are some reports that have studied the combined effect of two genes on DNP efficacy, such as the CYP2D6 and APOE genes or the ABCA1 and APOE genes (Lu et al., 2016; Lu et al., 2018), but more comprehensive clinical analyses of genes and corresponding in-depth studies of multiple genes are lacking. In addition, experimental studies of the mechanisms of action are needed. More studies on the relationship between multiple genes will lead to more accurate prediction of DNP clinical efficacy and are also conducive to the discovery of the pharmacological effects of DNP. There is a lack of studies on the combined impact of multiple genes on DNP efficacy. The mechanisms by which these genes affect DNP are largely unknown and merit further investigation.

There were limitations of the above studies, and the following are some points that should be included in future studies: (1) a long period for observation (more than 12 months); (2) a more specific focus, such as a focus specifically on the DNP efficacy in different stages of AD; (3) larger sample sizes; and (4) regulation of ethical and social issues. Numerous studies are needed before DNP treatment can be successfully translated into the clinic.

The reasons for the limited role of genomics in the clinical efficacy of DNP may be as follows: First, at present, the research on the clinical efficacy of DNP is mostly focused on the study of single gene, and the lack of comprehensive analysis of multiple genes, which is one of the reasons why the conclusions of these studies have limited clinical hints. Second, among the influencing factors of DNP's drug efficacy, what is the proportion of genes, this is also an unresolved question, and it is worth further research. However, several biomarkers might be promising to assess the treatment response of DNP. There is no doubt that genomics has absolutely important implications for the clinical efficacy of drugs. Some other studies suggest the significance of genomics for clinical treatment, such as VKORC1 (−1639G/A) and CYP2C9 (1075A/C) SNP, which greatly affect the clinical efficacy of warfarin (Zhenghong Qin, 2010); this conclusion has been very mature. At present, as far as we know, there have been research and development of related gene kits, which can be better applied to personalized medicine of warfarin.

In conclusion, the results of future studies will provide possible strategies for the evaluation of the DNP clinical efficacy and will also be beneficial for understanding the multiple mechanisms by which DNP produced a therapeutic effect in AD.

AUTHOR CONTRIBUTIONS

JL and XW were responsible for writing the manuscript, JL, LW, JF, and YZ were responsible for the literature review, YH was responsible for critical revision of the manuscript, CG was responsible for the manuscript review.

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Detoxification Improves Multidomain Cognitive Dysfunction in High-Dose Benzodiazepine Abusers

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Purpose: High-dose benzodiazepines (BZDs) abuse has been documented to cause multidomain cognitive dysfunction. We explored whether cognitive abnormalities to high-dose BZD abuse might be reversed by detoxification with slow subcutaneous infusion of flumazenil.

Methods: We recruited 96 patients consecutively admitted to the Department of Internal Medicine, Addiction Medicine Unit, Verona University Hospital, Italy for detoxification from high-dose BZD dependence. After selection for inclusion and exclusion criteria, 50 patients (23 men, 27 women; age 42.7 ± 10.3 years) were included. They underwent a comprehensive neuropsychological battery to explore verbal memory, visuospatial memory, working memory, attention, and executive functions 28–30 days prior to admission for detoxification (T0) and at the end of detoxification, i.e., 7 days after admission (T1). A group of 50 healthy adults (24 men, 26 women; mean age 44.5 ± 12.8 years) matched for age, sex, and education served as controls.

Results: At T0, patients scored significantly worse than healthy controls in all the neuropsychological tests. Depression and anxiety scores were associated with impaired verbal memory at T0 in patients. T1–T0 comparison showed improved performances in all neuropsychological tests after the end of detoxification in patients.

Conclusion: We confirmed that all neuropsychological domains were significantly and profoundly impaired by high-dose BZD abuse and documented that cognitive abnormalities improved after detoxification with slow subcutaneous infusion of flumazenil.

Keywords: benzodiazepine, cognition, detoxification, neuropsychology, substance use disorders, treatment

INTRODUCTION

Benzodiazepines (BZDs) and related Z-drugs (Zs) are gamma-amino-butyric acid type A (GABA-A) positive allosteric modulators, which are prescribed for anxiety and insomnia and represent one of the most widely used groups of pharmaceuticals worldwide (Soyka, 2017). Among patients on BZDs or Zs, 6–76% become long-term users, 15–44% experience moderate-to-severe

withdrawal symptoms and 3–4% show misuse or dependence (Faccini et al., 2016).

High-dose BZD dependence is a specific substance use disorder (Tamburin et al., 2017a) associated with reduced quality of life (Lugoboni et al., 2014; Tamburin et al., 2017b) and difficult treatment (Stevens et al., 2014; Liebrenz et al., 2015). A cross-sectional telephone survey carried out in France, Germany, Italy and the United Kingdom estimated that 0.14 and 0.06% of the general population took higher-than-recommended dose of anxiolytics and hypnotics, respectively (Ohayon and Lader, 2002). These data are in accordance with the estimated prevalence of 0.16% of high-dose BZD users in Switzerland (Petitjean et al., 2007) and suggest the number of high-dose BZD/Z abusers to be around 1.5 million in Europe and 600,000 in the United States.

Long-term BZD use was reported to be associated with abnormalities in cognitive functions, including attention, memory and learning (Boeuf-Cazou et al., 2011; Barker et al., 2004a; Puustinen et al., 2014; Helmes and Østbye, 2015; Fond et al., 2018), and higher risk of delirium, cognitive decline, falls, fractures, injuries, and road accidents (Finkle et al., 2011; van der Sluiszen et al., 2017; Kok et al., 2018; Picton et al., 2018; Wedmann et al., 2019). However, most of these reports were from people at higher risk of cognitive decline, such as elderly people (Finkle et al., 2011; Helmes and Østbye, 2015; Picton et al., 2018), intensive care unit patients (Kok et al., 2018), or patients with schizophrenia (Fond et al., 2018), whereby separating side effects of BZDs from symptoms of aging or a pathological state may be troublesome. Furthermore, BZD use was suggested to increase the risk of dementia, but studies reported contrasting data on this point, possibly because the presence of sleep disorders or neuropsychiatric symptoms in patients with preclinical dementia may lead to an increased probability of being prescribed a BZD (Gray et al., 2016; Islam et al., 2016; Zhang et al., 2016). Neuroimaging reports yielded conflicting findings, also, in that BZD use was reported to be associated either with brain volume reduction in schizophrenia (Huhtaniska et al., 2017), or lower cortical β-amyloid levels in non-demented elderly people (Chung et al., 2016).

High-dose BZD users offer a unique chance to explore the effect of BZD/Z on cognition, because of their relatively young age, and the absence of significant comorbidity in many of them (Federico et al., 2017). We have previously shown profound multidomain dysfunction involving all cognitive domains in a group of young adults (age 44.2 ± 9.7) with high-dose BZD/Z abuse, no neurological or psychiatric comorbidity, except depression and anxiety disorders, and no concurrent substance use disorders (Federico et al., 2017).

Different treatments have been proposed for BZD detoxification (Kawasaki et al., 2012; Soyka, 2017). Low-dose slow subcutaneous infusion of flumazenil, a GABA-A negative allosteric modulator, has been proposed for the detoxification from BZD dependence (Hood et al., 2014; Soyka, 2017), and is currently given to patients with high-dose BZD/Z abuse to achieve rapid detoxification (Faccini et al., 2016; Tamburin et al., 2017a). Human data on the cognitive effects of flumazenil are lacking, but the chronic administration of flumazenil may have a protective role against cognitive decline in rats (Colas et al., 2017). In addition, the short-term administration of flumazenil was reported to improve long-term memory in a mouse model of Down's syndrome (Marczynski et al., 1994).

The present study is aimed to explore whether cognitive changes to high-dose BZD abuse might be reversed by detoxification with flumazenil slow subcutaneous infusion (Faccini et al., 2016; Tamburin et al., 2017a). To achieve this aim, we assessed a group of high-dose BZD abusers who underwent a thorough neuropsychological testing before and after flumazenil slow infusion.

MATERIALS AND METHODS

Patients and Controls

From January to December 2017, we recruited 96 patients consecutively admitted to the Department of Internal Medicine, Addiction Medicine Unit, Verona University Hospital, Italy for detoxification from high-dose BZD dependence, defined as BZD dependence according to DSM-IV-TR criteria (American Psychiatric Association [APA], 2000), with abuse lasting more than 6 months, daily BDZ intake exceeding at least five times the maximum daily recommended dose (i.e., >50 mg diazepam/day) (Faccini et al., 2016), and problematic use, such as mixing BZDs, escalating dosage, using BZDs for recreational purposes, or obtaining BZDs illegally (Lugoboni et al., 2014; Liebrenz et al., 2015; Tamburin et al., 2017a).

The BZD/Z dose was standardized as daily diazepam dose equivalent (DDDE, mg) according to conversion tables (Faccini et al., 2016; Tamburin et al., 2017a).

The inclusion criteria were: (a) age ≥18 years, (b) formal education ≥ 8 years, (c) Italian as mother language, (d) normal or corrected-to-normal vision, (e) no hearing loss, (f) no acute drug intoxication, (g) no neurological diseases that might interfere with cognition, (h) normal overall cognition documented by a Mini Mental State Examination score >24/30, (i) no psychiatric diseases except depression and/or anxiety disorders, and (j) no documented concurrent alcohol or other substance use disorder (Federico et al., 2017).

After selection, 50 patients (23 men, 27 women; age 42.7 ± 10.3 years, median 42; education 12.8 ± 4.9 years, median 13) were included (**Figure 1**). A group of 50 age, sex, and education-matched healthy subjects not assuming BZDs served as controls (24 men, 26 women; age 44.5 ± 12.8 years, median 44; education 13.1 ± 3.4 years, median 13; n.s. for all comparisons vs. patients). Baseline demographic variables in patients and controls are shown in **Table 1**.

The study was conducted according to the Declaration of Helsinki and approved by the ethics committee of the Verona University Hospital (approval code 683CESC). Patients and controls gave written informed consent to the study and to off-label administration of flumazenil (patients only).

Neuropsychological Assessment

Patients and controls underwent a comprehensive neuropsychological battery to explore verbal, visuospatial and working memory, attention, and executive functions (Federico

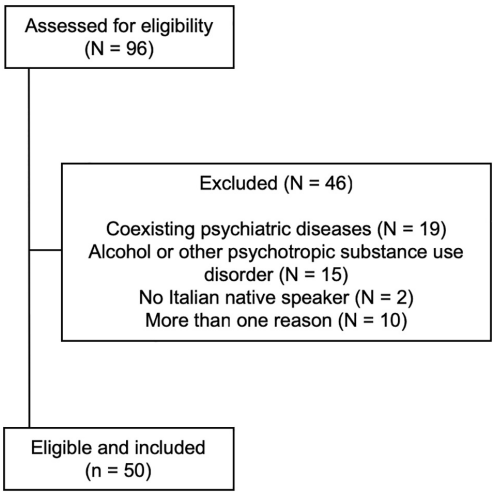

FIGURE 1 | Flow diagram of the study and reasons for patients' exclusion.

TABLE 1 | Baseline demographic variables in patients and controls.

	Patients	Controls	*p* value
Age[a]	42.7±10.3	44.5±12.8	n.s.
Sex (M/F)	23/27	24/26	n.s.
Education[a]	12.8±4.9	13.1±3.4	n.s.
Smoke (yes/no)	27/23	24/26	n.s.
Alcohol (yes/no)	2/48	0/50	n.s.

[a]*Data reported as mean ± S.D.*

et al., 2017; Cecchini et al., 2019). Neuropsychological assessment was performed at T0 (i.e., 28–30 days prior to admission for detoxification) and T1 (i.e., at the end of detoxification, 7 days after admission). BDZs could be taken more than 8 h prior to the T0 neuropsychological assessment, which was performed 28–30 days before the detoxification treatment. The BZD of abuse was stopped 7 days before T1 neuropsychological assessment. From the first day of detoxification, patients received oral clonazepam in the morning (around 8 a.m.) at progressively decreasing dosage (range: 0.5–2 mg). The T1 neuropsychological testing was administered in the afternoon (around 4 p.m.).

To avoid the potential bias of learning/practice effect at T1, neuropsychological tests that have been demonstrated not to be influenced by learning, and/or parallel/alternate forms of the test previously administered at T0, were used (Carlesimo et al., 1996; Amodio et al., 2008; Casarotti et al., 2014; Goretti et al., 2014; Zucchella et al., 2018a).

Verbal Memory

Verbal memory was assessed with the Italian versions of the Digit Span Forward Test (DSFT) and the Rey Auditory Verbal Learning Test (RAVLT), which is divided into immediate recall (IR) and delayed recall (DR) tests. DSFT measures short-term memory. Subjects are asked to repeat progressively longer digit series starting from three up to the longest series they can remember (Monaco et al., 2013). RAVLT explores verbal learning and memory. Subjects are asked to repeat all words they can remember from a list of 15 unrelated words the examiner read aloud previously (IR test, five trials) and to recall the previously presented words after 10-min delay (DR test) (Carlesimo et al., 1996).

Visuospatial Memory

Visuospatial memory was assessed with the Rey-Osterrieth Complex Figure Test (ROCF), where subjects are asked to copy a complex bidimensional figure (IR) and then redraw it after a 10-min delay (DR) (Caffarra et al., 2002).

Working Memory

Working memory was assessed with the Digit Span Backward Test (DSBT), which is the same as DSFT, but subjects are asked to recall the digit series in reverse of the presented order (Monaco et al., 2013).

Attention

Attention was assessed with the Trail Making Test Part A (TMT-A) and the Symbol Digit Modalities Test (SDMT) (Amodio et al., 2008; Goretti et al., 2014). TMT-A explores selective attention and visuospatial exploration, by asking the subject to draw lines sequentially connecting 25 encircled numbers. The time required to complete the task and the number of errors are recorded. SDMT is a measure of psychomotor speed. Subjects are required to transcribe symbols to numbers in the shortest time possible. The SDMT score is the number of correct answers in 90 s.

Executive Functions

Executive functions were evaluated with the Trail Making Test Part B (TMT-B), the Stroop test and the Phonemic Verbal Fluency Test (PVFT). TMT-B is similar to TMT-A, except that the task evaluates mental flexibility and task switching by asking the subjects to alternate between numbers and letters (Amodio et al., 2008). The Stroop test is a measure of inhibitory control. The subjects are asked to read color-related words printed in black type, name the color in which words are typed, and read color-related words typed in a different color (i.e., the word "blue" written in red type). The time to complete the task and the number of errors were recorded (Brugnolo et al., 2016). The PVFT measures lexical access, mental flexibility and abstract thinking by asking the subjects to generate as many words beginning with three test letters as possible in a given time (60 s for letter). The PVFT score is the total number of words reported (Carlesimo et al., 1996).

Depression and Anxiety

Depression was explored with the Beck Depression Inventory II (BDI-II), a 21-item self-administered questionnaire (score 0–3 for each item, cut-off for moderate to severe depression 28) to measure the severity of depressive symptoms during the previous 2 weeks (Federico et al., 2017). The internal consistency and test-retest reliability for the Italian version range from 0.76 to 0.87 (Sica and Ghisi, 2007).

Anxiety was assessed with the State Trait Anxiety Inventory form Y (STAI-Y) that is composed of two 20-item self-applied

questionnaires to measure state and trait anxiety. Each item is scored on a 1–4 Likert-type format; the cut-off for mild anxiety is 40 (Federico et al., 2017). The test-retest reliability for the STAI-Y state scale and the trait scale is 0.49 and 0.82, respectively (Pedrabissi and Santiniello, 1989). The internal coherence (Cronbach's alpha) varies from 0.91 to 0.95 for the state scale and from 0.85 to 0.90 for the trait scale (Pedrabissi and Santiniello, 1989).

Flumazenil Infusion

All patients underwent slow subcutaneous infusion of flumazenil (40.5 μg/hour for 24 h/day for 7 days) through an elastomeric pump (Faccini et al., 2016). They also received oral clonazepam at decreasing dosage from 5–6 mg on the first day to 0.5–2 mg on last day of flumazenil infusion, and prophylactic antiepileptic treatment to reduce the risk of seizures. The antiepileptic treatment was administered during the whole detoxification period (Faccini et al., 2016; Tamburin et al., 2017a). The mean dosage of levetiracetam (N = 27 patients) was 979.2 ± 70.6 mg, and the mean dosage of valproate (N = 23 patients) was 1025.0 ± 111.8 mg.

Statistical Analysis

Data were analyzed with SPSS version 21.0 (SPSS, Chicago, IL, United States). Fisher's exact test was applied to categorical variables. For continuous variables, normality of distribution was tested with the Shapiro-Wilks test. Differences between patients and controls for baseline variables and neuropsychological scores at T0 were analyzed with Student's t-test in case of normal distribution, or the non-parametric Mann-Whitney U test when the distribution was not normal. The potential confounder effect of sex, age, and education was explored by comparing patients (T0) vs. controls with a multivariate generalized linear model with sex, age and education as covariates (Federico et al., 2017). The effect of clinical variables (BDI-II; STAI-Y state and trait; DDDE; high-dose BZD abuse duration; prophylactic antiepileptic treatment) on neuropsychological tests was explored by first entering them into univariate analysis (continuous variables: non-parametric Spearman's rho correlation coefficient; categorical variables: Kruskal-Wallis H rank test), then variables that were significant in the univariate model were entered as covariates into linear regression multivariate models with neuropsychological scores as dependent outcomes. Within-subject T1–T0 differences in neuropsychological scores were explored with paired t-test when the distribution was normal, or the non-parametric Wilcoxon signed-rank order test for non-normal distributions. Neuropsychological scores were reported as Z-scores according to the formula: Z-score = (measured value – mean value according to age and education)/standard deviation according to age and/or education. Negative and positive values indicated worse and better performance than the normal population, respectively. Z-scores was computed for scores with normal distribution in the normative sample, i.e., DSFT and TMT-A/B time (sec), DSBT, ROCF-DR (Carlesimo et al., 2002; Mondini et al., 2011; Monaco et al., 2013). $P < 0.05$ (two-tailed) was the significance threshold for all the tests.

RESULTS

The abused BZD was lormetazepam in 34 patients (68%), zolpidem in 7 (14%), alprazolam in 4 (8%), lorazepam in 2 (4%), triazolam in 1 (2%) and clonazepam in 1 (2%), while 1 patient abused of lormetazepam and zolpidem (2%). The DDDE was 436.7 ± 397.3 mg (median 250, interquartile range, IQR 225–600). The duration of high-dose BZD abuse was 119.7 ± 96.7 months (median 96, IQR 42–180).

The BDI-II score at T0 was 29.7 ± 8.9/63 (median 31, IQR 24–35.5), which indicated moderate-to-severe depression. At T0, the STAI-Y state anxiety score was 39.6 ± 5.8/80 (median 39, IQR 34–44), and the trait anxiety score was 44.0 ± 9.4/80 (median 44, IQR 39–52), which indicated mild anxiety.

Prophylactic antiepileptic treatment during flumazenil infusion (Tamburin et al., 2017a) was levetiracetam in 26 patients, valproate in 21, lamotrigine in 2 and topiramate in 1. There were neither seizures nor adverse effects related to the detoxification with slow subcutaneous infusion of flumazenil. There were no drop-outs.

At T0, the patients group scored significantly worse than healthy controls group in all the neuropsychological tests (**Table 2**).

Multivariate linear regression model showed a significant positive effect (i.e., the higher the anxiety score, the better the performance) of STAI-Y state score on RAVLT-IR (β = 0.58; 95% confidence interval, CI: 0.13, 1.02; p = 0.012) and RAVLT-DR (β = 0.14; 95% CI: 0.01, 0.26; p = 0.03). BDI-II score had a significant negative effect on DSFT (β = −0.03; 95% CI: −0.06, −0.01; p = 0.023). High-dose BZD abuse duration had a significant negative effect on SDMT (β = −0.04; 95% CI: −0.06, −0.01; p = 0.004).

T1–T0 comparison showed that the patient group significantly improved performances in all neuropsychological tests after the end of detoxification period (**Table 3**). Z-scores at T0 and T1 are reported in **Figure 2**.

DISCUSSION

The new finding of this study is that cognitive abnormalities were significantly ameliorated after BZD detoxification by slow subcutaneous infusion of flumazenil. Our data also confirmed that all neuropsychological domains were significantly impaired by high-dose BZD abuse (Federico et al., 2017).

The cognitive changes we found are in keeping with previous studies and a meta-analysis showing moderate-to-large abnormalities in all cognitive domains to long-term BZD use (Barker et al., 2004a; Boeuf-Cazou et al., 2011; Puustinen et al., 2014; Helmes and Østbye, 2015; Fond et al., 2018). In particular, an updated meta-analysis found statistically significant impairment of many neuropsychological domains (i.e., working memory, divided attention, processing speed, visuoconstruction, recent memory and expressive language) to long-term BZD use (Crowe and Stranks, 2018).

Some pharmacological lines of reasoning may explain the neuropsychological abnormalities we found. BZDs act at an

TABLE 2 | Neuropsychological measures in high-dose BZD abusers (T0) and healthy controls.

Neuropsychological test	High-dose BZD abusers (*N* = 50)[a]	Healthy controls (*N* = 50)[a]	*p* value
Verbal memory			
DSFT	5.6 ± 0.8, 6, 5 – 6	6.2 ± 0.5, 6, 6 – 6.5	0.00028
RAVLT-IR	37.6 ± 9.8, 39.5, 30.5 – 44.5	50.5 ± 5.0, 51, 4.75 – 55	<0.0001
RAVLT-DR	7.6 ± 2.7, 8, 5 – 9	13.8 ± 1.5, 14, 13 – 15	<0.0001
Visuospatial memory			
ROCF-IR	31.2 ± 6.5, 34, 29 – 36	35.8 ± 0.6, 36, 36 – 36	<0.0001
ROCF-DR	11.1 ± 6.6, 11.5, 5 – 15.75	26.2 ± 3.3, 27, 24 – 29	<0.0001
Working memory			
DSBT	3.2 ± 1.0, 3, 2 – 4	4.7 ± 0.6, 5, 4 – 5	<0.0001
Attention			
TMT-A (time, s)	52.7 ± 23.3, 48, 37 – 66	23.1 ± 4.9, 23.5, 19 – 27	<0.0001
TMT-A (errors, N)	0.6 ± 1.0, 0, 0 – 1	–[b]	<0.0001
SDMT	28.7 ± 8.5, 29, 20.5 – 33	44.9 ± 9.2, 48, 38 – 53	<0.0001
Executive functions			
TMT-B (time, s)	131.5 ± 57.8, 115, 78.5 – 179	47.5 ± 9.2, 47, 41.75 – 52.25	<0.0001
TMT-B (errors, N)	2.8 ± 2.3, 3, 0 – 5	–[b]	<0.0001
Stroop test (time, s)	32.5 ± 9.2, 31, 28.5 – 36	19.2 ± 3.6, 19.5, 16.5 – 22.125	<0.0001
Stroop test (errors, N)	1.9 ± 2.2, 1, 0 – 4	0.02 ± 0.1, 0, 0 – 0	<0.0001
PVFT	29.7 ± 11.1, 29.5, 21 – 35.5	42.5 ± 5.3, 43, 39 – 46	<0.0001

DR, delayed recall; DSBT, Digit Span Backward Test; DSFT, Digit Span Forward Test; BZD, benzodiazepine; IR, immediate recall; PVFT, Phonemic Verbal Fluency Test; RAVLT, Rey Auditory Verbal Learning Test; ROCF, Rey-Osterrieth Complex Figure Test; SDMT, Symbol Digit Modalities Test; T0, 28–30 days before admission for detoxification with flumazenil slow subcutaneous infusion; TMT-A/B, Trail Making Test Part A/B. [a]Data reported as mean ± S.D., median, interquartile range. [b]None of the healthy controls made any error in this test.

TABLE 3 | Comparison of neuropsychological measures in high-dose BZD abusers at T0 and T1.

Neuropsychological test	T0[a]	T1[a]	*t*/*Z* value[b]	*p* value
Verbal memory				
DSFT	5.6 ± 0.8, 6, 5 – 6	5.9 ± 0.8, 6, 5 – 7	*Z* = –2.97	0.003
RAVLT-IR	37.6 ± 9.8, 39.5, 30.5 – 44.5	42.7 ± 8.1, 43, 35 – 48	*Z* = –5.03	<0.0001
RAVLT-DR	7.6 ± 2.7, 8, 5 – 9	9.2 ± 2.8, 9, 7 – 11	*Z* = –5.21	<0.0001
Visuospatial memory				
ROCF-IR	31.2 ± 6.5, 34, 29 – 36	32.7 ± 5.5, 36, 32 – 36	*Z* = –3.47	0.001
ROCF-DR	11.1 ± 6.6, 11.5, 5 – –15.8	13.1 ± 5.5, 12.5, 9.5 – 16	*Z* = –4.15	<0.0001
Working memory				
DSBT	3.2 ± 1.0, 3, 2 – 4	3.6 ± 0.9, 4, 3 – 4	*Z*=–4.20	<0.0001
Attention				
TMT-A (time, s)	52.7 ± 23.3, 48, 37 – 66	42.7 ± 14.3, 40.5, 30 – 51	*Z*=–5.03	<0.0001
TMT-A (errors, N)	0.6 ± 1.0, 0, 0 – 1	0.06 ± 0.3, 0, 0 – 0	*Z* = –3.60	<0.0001
SDMT[c]	28.7 ± 8.5	35.6 ± 7.0	*t* = –11.76	<0.0001
Executive functions				
TMT-B (time, s)	131.5 ± 57.8, 115, 78.5 – 179	92.5 ± 35.4, 85.5, 67 – 112	Z = –5.68	<0.0001
TMT-B (errors, N)	2.8 ± 2.3, 3, 0 – –5	0.6 ± 1.4, 0, 0 – –1	*Z* = –4.68	<0.0001
Stroop test (time, s)	32.5 ± 9.2, 31, 28.5 – 36	26.7 ± 5.7, 25 – 31	*Z* = –5.24	<0.0001
Stroop test (errors, N)	1.9 ± 2.2, 1, 0 – 4	0.3 ± 0.7, 0, 0 – 0	*Z* = –4.82	<0.0001
PVFT[c]	29.7 ± 11.1	39.5 ± 9.6	*t* = –14.55	<0.0001

DR: delayed recall; DSBT: Digit Span Backward Test; DSFT: Digit Span Forward Test; BZD: benzodiazepine; IR: immediate recall; PVFT: Phonemic Verbal Fluency Test; RAVLT: Rey Auditory Verbal Learning Test; ROCF: Rey-Osterrieth Complex Figure Test; SDMT: Symbol Digit Modalities Test; T0: 28–30 days before admission for detoxification with flumazenil slow subcutaneous infusion. T1: at the end of flumazenil slow subcutaneous infusion, 7 days after admission; TMT-A/B: Trail Making Test Part A/B. [a]Data reported as mean ± S.D., median, interquartile range (mean ± S.D. for variables with normal distribution). [b]Paired t-test in case of normal distribution, or Wilcoxon signed-rank order test (Z-value) for non-normal distributions. [c]Variables with normal distribution.

allosteric modulator site on the GABA-A receptor channel complex, which is composed by 5 (usually 2 α, 2 β, 1 γ) subunits surrounding a chloride pore and modulate cerebral functions through α subunits, which show distinct expression patterns in the brain (Tan et al., 2011). α_1 is responsible for sedation, anterograde amnesia, anticonvulsant effects and

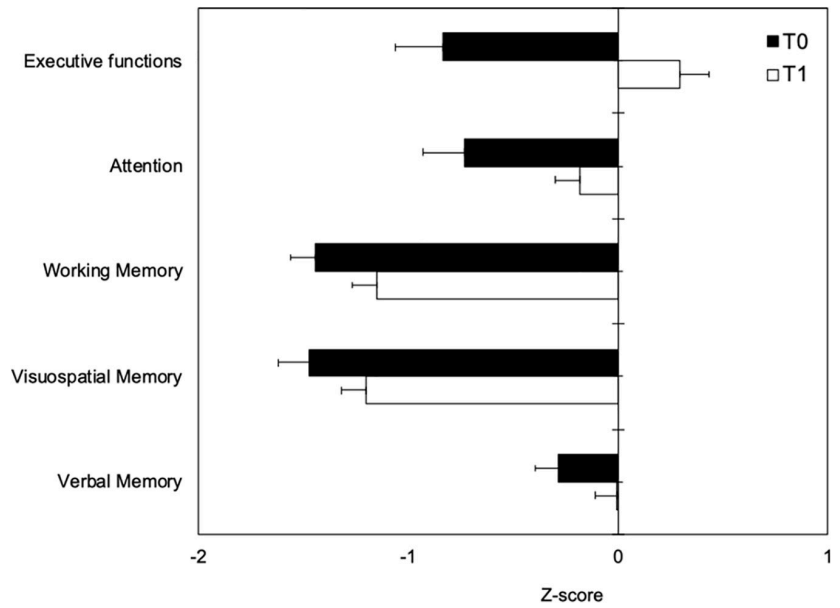

FIGURE 2 | Neuropsychological measures at T0 (black boxes) and T1 (white boxes) represented as Z-scores. Negative values indicating worse performance and positive values indicating better performance than the average value of the normal population.

BZD dependence, α_2 and α_3 are associated with anxiolytic and myorelaxant actions, and α_5 is related to cognition, learning and memory (Tan et al., 2011; Möhler, 2015). The GABA-A receptor channel complex has been suggested to contribute to the cognitive dysfunction in traumatic brain injury (Sun and Feng, 2014).

Zolpidem, which displays α_1 selective affinity, but almost no effect on the α_5 subunit, may produce more memory and cognitive impairment than triazolam, an agonist of all α subunits (Roehrs et al., 1994), suggesting that α_1 plays a major role in the amnestic effect of BZDs. We hypothesize that the severe memory dysfunction we found at T0 in patients may be ascribed to the larger number of them abusing lormetazepam and/or zolpidem, which have a remarkable selectivity for the α_1 subunit (Crestani et al., 2000).

Partial α_5 knockdown in the mice hippocampus improves trace fear conditioning (Crestani et al., 2002), appetitive conditioning and novel object recognition (Yee et al., 2004), and complete α_5 deficit causes improved spatial performance and trace fear memory (Martin et al., 2010). The α_5 subunit is located extrasynaptically in the hippocampal pyramidal cell dendrites, where it mediates tonic inhibition (Möhler, 2015). Excessive activation of α_5 GABA-A receptors by high-dose BZDs may inhibit glutamate-mediated excitatory transmission and worsen cognitive performance in BZD abusers.

Long-term BZD administration is associated with changes in GABAergic and dopaminergic neurons in the ventral tegmental area and other brain regions (Tan et al., 2011). Animal models indicate that prolonged α_1 stimulation induces a shift in the expression of α isoforms, causing reduction of α_1, α_2, increase of α_3, α_4 and α_6, and reduction or increase in α_5 subunits (Tan et al., 2011). α_4 and α_6 subunits are nearly insensitive to BZDs, and the changes in the composition of the GABA-A receptor result in BZD-receptor decoupling, a compensatory mechanism that contributes to BZD tolerance (Cheng et al., 2018). While tolerance to sedative and anticonvulsant effects builds quickly in humans and animal models, cognitive effects of BZDs seem to lack tolerance (Cheng et al., 2018).

The anticholinergic activity of BZDs might contribute to cognitive dysfunction, in particular in persons aged 55 years or older (Coupland et al., 2019), or with concomitant neurological disorders (Forgacs and Bodis-Wollner, 2004), but this mechanism seems unlikely in our patients because of their young age and the absence of neurological comorbidities that rule out the hypothesis of subclinical brain cholinergic damage (Risacher et al., 2016).

Benzodiazepine dosage, expressed as DDDE, did not have any effect on cognition in our sample, probably because the high dose resulted in a ceiling effect (Federico et al., 2017). Abuse duration had a significant negative effect on psychomotor speed assessed by the SDMT, suggesting a possible neuroplasticity effect causing worse performance with longer high-dose BZD intake (Möhler, 2015; Ruparelia et al., 2012).

Different hypotheses can explain the improvement of the neuropsychological outcomes at T1. In keeping with a meta-analysis reporting that long-term BZD users show partial cognitive recovery after withdrawal (Barker et al., 2004b), discontinuation of high-dose BZD and its replacement by low-dose clonazepam is the most likely reason for neuropsychological improvement.

In accordance with experimental evidence of reversal of BZD-induced cognitive impairment by flumazenil (Wesensten et al., 1995), flumazenil infusion could have ameliorated cognition through negative allosteric modulation of α_1 and α_5

GABA-A receptor function. Pharmacological blockade of α_5 subunit function has been suggested to enhance learning and memory (Ballard et al., 2009) in animal models of Down's syndrome that is supposed to be characterized by reduced long-term potentiation and excessive long-term inhibition in the hippocampus (Ruparelia et al., 2012). A short-term course of flumazenil was demonstrated to restore long-term object memory in a mouse model of Down's syndrome (Colas et al., 2017). Flumazenil may have contributed to reverse α isoform changes associated with prolonged BZD exposure through α_6 agonist effect (Tamburin et al., 2017a). This hypothesis is in keeping with animal models of autism spectrum disorders, where rebalance of α_2, α_3, and α_5 GABA-A receptor activity has been reported to improve cognitive and behavioral disturbances (Han et al., 2012; Möhler, 2015).

We excluded patients with dementia or other neurodegenerative conditions, major psychiatric diseases, and concurrent alcohol or other substance use disorder, which may contribute to cognitive impairment in patients taking BZDs and represented a bias to demonstrate a direct link between BZD intake and neuropsychological deficits in previous studies (Verdoux et al., 2005; Billioti de Gage et al., 2014).

Depression and anxiety, which may influence cognition (Krysta et al., 2015) were not ruled out in our sample, because they are frequently comorbid in high-dose BZD abusers. The BDI score was, on average, moderate-to-severe, it was found to have a significant negative effect on DSFT only, but no influence on other neuropsychological outcomes. Anxiety was mild on average, and had significantly positive effect (i.e., the higher the anxiety score, the better the performance) on RAVLT scores. Taken together, these results indicate a potential mild bias effect of psychiatric comorbidity on verbal memory test scores.

The main limitation of this study is the absence of a control group not undergoing BZD detoxification (e.g., people taking clonazepam only at decreasing dosage), but such a design would have raised ethical issues. In addition, the presence of another group of BZD users not requiring flumazenil treatment would have been an important control. Another limitation stems from the relatively short time between T0 and T1 that might have resulted in a learning effect. To reduce this potential source of bias, we chose neuropsychological tests that have been demonstrated not to be influenced by learning, and/or we used parallel/alternate forms (Zucchella et al., 2018a). Indeed, cognitive re-testing of healthy controls at T1 would have strengthened our results. Furthermore, the prophylactic antiepileptic treatment may have influenced cognitive outcome at T1, but its effect was eventually to worsen cognition, and this treatment was necessary to reduce the risk of seizures. The impact of coexisting psychiatric comorbidities (i.e., depression, anxiety disorders) on neuropsychological measures, despite being probably less severe than that of high-dose BZD abuse, could not be completely ruled out. Finally, we did not include further follow-ups at longer times from the end of flumazenil infusion and this point is a limitation of the study. Future studies should assess the long-term outcomes to slow subcutaneous flumazenil infusion. Also, functional neuroimaging or evoked related potential data would have offered evidence on underlying brain changes related to BDZ intake.

CONCLUSION

In conclusion, we found detoxification to significantly ameliorate the severe and multidomain neuropsychological dysfunction in high-dose BZD abuse. The standard treatment for BZD detoxification is slow tapering that may last months in case of high-dose abuse (Soyka, 2017). Our results strengthen the clinical significance of slow subcutaneous flumazenil infusion for high-dose BZD detoxification, because cognitive impairment is one of the main reasons to seek medical assistance (Federico et al., 2017) and results in poorer quality of life (Tamburin et al., 2017b) in this substance use disorder, thus requiring rapid treatment.

Even in the presence of the abovementioned limitations, these findings could be of interest in that they suggest that 7 days of slow subcutaneous infusion of flumazenil may, at least partially, improve BZD-related cognitive deficits. Further randomized controlled studies with long-term follow-up are needed before flumazenil slow cutaneous infusion can be considered as a standard treatment for high-dose benzodiazepine abusers.

The present data may also indicate future research lines. Animal studies indicate that chronic administration of flumazenil increases the life span and protects rats from cognitive worsening during aging, suggesting that age-related excessive BDZ/GABAergic activity may promote neurodegeneration (Colas et al., 2017). Whether flumazenil might have a therapeutic role in age-related neurodegenerative conditions leading to dementia in humans is an interesting research topic, given the absence of disease-modifying treatments (Zucchella et al., 2018b) that may be used early in the course of the disease to block or delay neurodegeneration (Emery, 2011).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Verona University Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AF, FL, and ST designed the study. AF, FL, EM, AM, LM, RC, MF, and ST collected the data. AF, EM, AM, and ST analyzed the data and conducted the statistical analysis. AF, FL, EM, AM, and ST drafted the original version of the manuscript, which was revised critically by LM, RC, and MF. All authors contributed to the interpretation of the data and approved the final version of the manuscript to be published.

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REVIEW
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Inhibiting Epileptiform Activity in Cognitive Disorders: Possibilities for a Novel Therapeutic Approach

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Cognitive impairment is a common and seriously debilitating symptom of various mental and neurological disorders including autism, attention deficit hyperactivity disorder, multiple sclerosis, epilepsy, and neurodegenerative diseases, like Alzheimer's disease. In these conditions, high prevalence of epileptiform activity emerges as a common pathophysiological hallmark. Growing body of evidence suggests that this discrete but abnormal activity might have a long-term negative impact on cognitive performance due to neuronal circuitries' remodeling, altered sleep structure, pathological hippocampo–cortical coupling, and even progressive neuronal loss. In animal models, epileptiform activity was shown to enhance the formation of pathological amyloid and tau proteins that in turn trigger network hyperexcitability. Abolishing epileptiform discharges might slow down the cognitive deterioration. These findings might provide basis for therapeutic use of antiepileptic drugs in neurodegenerative cognitive disorders. The aim of our review is to describe the data on the prevalence of epileptiform activity in various cognitive disorders, to summarize the current knowledge of the mechanisms of epileptic activity in relation to cognitive impairment, and to explore the utility of antiepileptic drugs in the therapy of cognitive disorders. We also propose future directions for drug development and novel therapeutic interventions targeting epileptiform discharges in these disorders.

Keywords: neurocognitive disorder, epileptiform activity, electroencephalography, cognitive decline, memory consolidation, antiepileptic drugs

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INTRODUCTION

Cognitive impairment is a common symptom of various neurological and psychiatric disorders including autism spectrum disorder (ASD), schizophrenia, attention deficit hyperactivity disorder (ADHD), multiple sclerosis (MS), and major neurocognitive disorders (NCDs). The cumulative prevalence of these conditions is ∼50% in developed societies creating prominent medical and social burden. While the mentioned diseases differ significantly in their symptoms and pathological background, diminished memory function is a common characteristic.

The association between epilepsy and the above-mentioned diseases evoked a remarkable interest in the medical literature highlighting various hypotheses and explanations of bidirectional connections. Seemingly, all these syndromes increase the risk for epileptic seizures.

In ASD, reports agree that patients have increased incidence for epileptic seizures ranging from 5 to 38% (Hara, 2007). Symptoms of ADHD are highly common in children affected by epilepsy, and epilepsy is predominantly associated with inattentive type of ADHD (Plioplys et al., 2007). Other studies proposed that children with attention problems have a two–threefold increase for unprovoked seizure occurrence (Austin and Caplan, 2007).

Recently, it has been established that epilepsy is a frequent comorbidity in various forms of NCD (Horváth et al., 2016). Studies on familial AD steadily demonstrate that seizures affect approximately half of patients (Zarea et al., 2016). A study of Beagle et al. demonstrated ∼15% cumulative probability of developing seizures by patients with diffuse Lewy-body dementia (DLB) and 3% by patients with frontotemporal degeneration (FTD) (Beagle et al., 2017). Furthermore, epileptic patients also have a higher chance for late life neurocognitive disorders (Subota et al., 2017).

While numerous studies investigated the link between epileptic seizures and cognitive disorders, reports on epileptiform activity between seizures [interictal epileptiform activity (IEA)] or without seizure activity [subclinical epileptiform activity (SEA)] are underrepresented. While classic epileptology focused on the accurate control of seizures, in recent years, growing body of evidence suggests that IEA might have harmful effect on cognitive functions (Glennon et al., 2016; Hu et al., 2016; Meekes and Jennekens-Schinkel, 2018). It is intriguing to analyze the potential role of SEA as well, since SEA shows similar electrographic features as IEA and the above-mentioned cognitive disorders share another hallmark: the prevalence of SEA is elevated in all conditions.

The aim of our opinion review is to describe the results of studies on the prevalence of IEA and SEA in the various forms of cognitive disorders, to summarize the current knowledge on the effect of epileptiform discharges on cognitive functions, and to propose new directions for therapeutic interventions targeting cognitive decline. To increase the accuracy and novelty of our research, we analyzed studies published later than 2000, and in the prevalence and therapy sections, we included reports on humans only.

PREVALENCE OF SEA AND IEA IN COGNITIVE DISORDERS

Major Neurocognitive Disorders

NCDs represent 80–100 various conditions with progressive neurodegenerative process. AD is the leading cause of cognitive decline by the elderly affecting 37.5 million people worldwide, and this number is expected to triple by 2050 (Abbott, 2011). The first symptoms of AD—as the impairment of episodic memory and difficulty in spatial orientation—occur usually at age of 60–70. During the 6–8 years of disease course, patients lose other cognitive skills including orientation, communication, and language skills and finally the ability of self-care (Cummings and Cole, 2002). The pathological hallmark of AD is the accumulation and progressive spread of misfolded amyloid and tau proteins (Ittner and Götz, 2011). Since we are not able to significantly slow down the progression of cognitive deterioration (Cummings and Cole, 2002), there is a clear need to find possibly modifiable factors of AD, especially in the early phases of the disease. A recently recognized contributor to AD progression is epileptic activity. Numerous human studies highlighted that AD patients have a higher chance to develop epileptic seizures (Horváth et al., 2016). IEA was analyzed in three studies with routine electroencephalogram (EEG) identifying interictal epileptiform activity in third of AD patients who presented with epileptic seizure (Rao et al., 2009; Cretin et al., 2016; Sarkis et al., 2016). In two sleep EEG studies, IEA rate was 62% (Vossel et al., 2013) and 80% (Horváth et al., 2018b) in patients with clinical history of seizures. In these studies, IEA appeared mainly over the frontotemporal areas with a left-side dominance (Rao et al., 2009; Vossel et al., 2013; Cretin et al., 2016; Sarkis et al., 2016; Horváth et al., 2018b). Temporal occurrence of IEA was analyzed only in two studies: in the study of Vossel et al. (2013), 10% of IEA was detected during wakefulness and 64% appeared exclusively in stage2 or deeper sleep, while in our previous report, 82% of IEA was associated with sleep and 55% was detected in deep sleep (Horváth et al., 2017b).

There are only a few studies analyzing the occurrence of SEA in AD. Liedorp at al. found epileptiform discharges in only 3% of 1,674 AD patients (Liedorp et al., 2010) using 30 min long daytime EEGs. Vossel et al. revealed SEA in 6% of 113 AD and MCI patients evaluating daytime routine EEGs in 91% and serial or long-term EEGs in 7% of the patients (Vossel et al., 2013). In another study of Vossel et al. using magnetoencephalography and sleep EEG, SEA was found in 42% of AD patients who have never experienced epileptic seizure before (Vossel et al., 2016). They analyzed the temporal distribution of SEA as well showing that epileptic activity occurs almost completely (90%) during sleep and mainly over the temporal regions. This is in line with our previous reports showing the important role of sleep EEG in the detection of SEA in AD (Horváth et al., 2017b, 2018a). It should be noted that in Vossel's study from 2016, SEA was associated with faster deterioration of cognition determined by Mini-Mental Score Examination (Vossel et al., 2016). Moreover, studies also suggest that AD and mild cognitive impairment (MCI) patients with SEA have an earlier onset of cognitive decline being usually associated with more aggressive forms of AD that show faster progression (Vossel et al., 2016; Horváth et al., 2018b). **Table 1** summarizes the AD studies on the prevalence of IEA and SEA.

DLB is the second most common type of dementia accompanied by changes in behavior, cognition, movement, sleep, and the autonomic functions (Savica et al., 2013). The major symptoms are the rapid eye movement sleep (REM) sleep behavior disorder, memory loss, and visual hallucinations (McKeith, 2002). Furthermore, marked fluctuations in attention or alertness, parkinsonism (slowness of movement, troubled walking, or rigidity), and dysfunction of autonomic nervous

TABLE 1 | Prevalence of epileptiform discharges in Alzheimer's disease.

References	N	Study design	EEG-type	ED (%)
Rao et al., 2009	39	Retrospective, epileptic AD patients	Routine (74%) or no EEG (26%)	38% (IEA)
Cretin et al., 2016	13	Retrospective, epileptic MCI patients	Routine	100% (IEA)
Sarkis et al., 2016	77	Retrospective, epileptic AD patients	Routine	22% (IEA)
Vossel et al., 2013	54	Retrospective, MCI + AD patients	Routine and serial	62% (IEA), 6% (SEA)
Horváth et al., 2018b	42	Prospective	24 h	20% (IEA), 28% (SEA)
Liedorp et al., 2010	1,674	Retrospective	–	3% (SEA)
Vossel et al., 2016	33	Prospective, non-epileptic AD patients	24 h + magnetoencephalography	42% (SEA)

N, number of patients; MCI, mild cognitive impairment; ED, epileptiform discharge; IEA, interictal epileptiform activity; SEA, subclinical epileptiform activity

system (orthostatic hypotonia, constipation) are also present (McKeith, 2002). An important diagnostic hallmark is the hypersensitivity for antipsychotic drugs (McKeith, 2002). The major pathological finding is the widespread accumulation of alpha-synuclein protein (Hishikawa et al., 2003). Reports on DLB-related epilepsy are less frequent compared to AD; however, a recent paper depicts that DLB patients are susceptible for seizures similarly to AD patients (Beagle et al., 2017). Another study using postmortem approach identified myoclonus with the retrospective analysis of clinical records in 21.7% of DLB patients, and it was associated with earlier onset of cognitive decline (Morris et al., 2015). While reports on IED or SEA in DLB are absent, considering that DLB patients might have a similar prevalence of seizures than AD patients, analyzing IED/SEA in DLB is an important future direction.

FTD is a heterogeneous condition encompassing five types of dementia including behavior and language-dominant lobar degenerations (behavioral variant, semantic variant primary progressive aphasia, and non-fluent variant primary progressive aphasia) and motor dominant disorders (corticobasal syndrome, progressive supranuclear palsy) (Bang et al., 2015). Initial symptoms usually appear by adults in their fifth or sixth decade of life (Bang et al., 2015). The histological finding is the progressive accumulation of tau, tdp-43, and fus proteins (Bang et al., 2015). FTD patients tend to have also higher risk for epileptic seizure (Beagle et al., 2017; Arnaldi et al., 2020); however, there is only one case in the literature focusing on the importance of epileptic activity in FTD. With the help of foramen ovale electrodes, SEA was detected in a seizure-free patient with FTD that could have caused the daily variability in her cognitive behavior (Horváth et al., 2017a). Since the number of reported cases on IEA/SEA on FTD is still small, further investigation is necessary (Chan et al., 2004).

Huntington disease is an autosomal dominant neurodegenerative disorder that is characterized by involuntary movements, cognitive decline, and personality changes (Bates et al., 2015). Reports on patients with adult onset showed that prevalence of seizures is similar to the general population (Sipilä et al., 2016). However, epileptic seizures and epileptiform activity occur in 30–40% of patients in the rarer juvenile type (J-HD), which appears in young persons under 21 years of age (Cloud et al., 2012). Currently, there are only a few studies in the literature solely investigating SEA or IEA in Huntington disease. A review of Landau and Cannard (2003) analyzed 23 previously published cases of J-HD patients. Epileptiform abnormalities were noted in 17 (74%). In 10 cases, they were associated with overt epileptic seizures, so the prevalence of IEA was 44%. In seven cases (30%), SEA was detected. Nine patients showed generalized discharges having polyspike and wave activity, while eight others had focal or multifocal epileptiform discharges with posterior predominance. The limitation of this study is that the diagnosis of J-HD was not genetically confirmed. Another study analyzed the pattern of IEA of a J-HD patient with epileptic seizures and described the occipital intermittent rhythmic delta activity as the major hallmark of epileptic activity (Ullrich et al., 2004).

To conclude, patients with various forms of NCD tend to be more vulnerable for epileptic seizures, however, prevalence data show high variability. While SEA is detectable in approximately 17% of AD patients, studies on other NCD forms are scarce. The role of SEA in the accelerated progression of AD draws attention to the need for further investigations.

Multiple Sclerosis

MS is a heterogeneous demyelinating disease of the central nervous system involving inflammatory processes not only of the white matter but also the juxtacortical and cortical areas. Recent studies also highlight that MS should be also considered as a neurodegenerative disorder (Ziemann et al., 2011). Attention has been mostly focused on clinical seizures, as seizures might occur at any stage of MS. Sponsler and Kendrick-Adey conducted the most extensive review on assessing prevalence of seizures among MS patients by compiling results of 25 scientific papers (Sponsler and Kendrick-Adey, 2011). They found that about 2% of MS patients experienced seizures. A study of 36 patients found that early-onset MS frequency was significantly higher in patients with epileptic seizures as compared to those without epilepsy (Durmus et al., 2013). Epileptic events might be a consequence of edema surrounding the lesions, disease-modifying drugs lowering the epileptic threshold, or the reduced cortical thickness as a result of disease course (Geurts et al., 2005; Cheng et al., 2012; Calabrese et al., 2017). A study by Calabrese et al. (2008) reported intracortical lesions in 90% of epileptic patients with relapsing–remitting MS (RRMS), whereas only in 48% with RRMS without epilepsy. In another study by his group, the most affected gray matter lesions in RRMS epileptic patients were the hippocampus (14.2%), the lateral temporal lobe (13.5%), the cingulate (10.0%), and the insula (8.4%) (Calabrese et al., 2017). Lund et al.

suggested that epilepsy in MS should be classified as symptomatic focal epilepsy due to the nature of cortical lesions (Koch et al., 2008; Lund et al., 2014).

Available data on prevalence and background of IEA and SEA in multiple sclerosis are limited. SEA could potentially be a major reference point in guiding a clinician, however, no studies exist that focus solely on SEA in MS patients. EEG abnormalities reported in MS can be diffuse asynchronous theta activity, synchronous rhythmic slow waves, focalized flattened EEG patterns (Striano et al., 2003), or less frequently periodic lateralized epileptiform discharges, which are mostly seen in acute exacerbations of the disease (Lawn et al., 2001; Nyquist et al., 2001; Gandelman-Marton et al., 2003). **Table 2** lists some of the studies that looked at EEG abnormalities distinguishing based on epileptiform and non-epileptiform pathological EEG events. However, most of these studies had varying methodology and looked at alterations in MS patients who already were known to have at least one seizure when they all analyzed IEA. IEA was found in 3.9–86.9% of the patients representing the great variability of the study methods (e.g., EEG technique and length of recording, retrospective vs. case–control studies, sample sizes of 23 patients vs. 29,165 patients). Only three studies analyzed SEA independently, suggesting ∼7–8% prevalence. Bustuchina postulated a bidirectional relation between MS and epileptic activity and suggested that MS might be a network disease, and so emphasis should be put on both entities for best therapeutic outcome (Bustuchina Vlaicu, 2019).

Autism Spectrum Disorder

ASD is an umbrella term for several neurodevelopmental conditions defined by the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V) classification, which share clinical manifestations in varying degrees. Such manifestations are impairment in sociability, communication deficits, non-verbal interaction issues, restricted range of interest, repetitive behavior, and impairment of intellectual and behavioral flexibility (Tuchman and Rapin, 2002; American Psychiatric Association, 2013). Pathophysiological background of this heterogeneous syndrome originates in the neural circuit disconnection between the association cortex of the frontal lobe and the higher-order multimodal temporal lobe (Assaf et al., 2010; Belger et al., 2011). SEA and IEA might be one of the biomarkers of malfunction of these involved intrinsic connectivity networks. **Table 3** summarizes studies that assessed epileptiform discharges in patients diagnosed with ASD. Prevalence of epileptiform activity is reported in 21–75% of patients. Epilepsy has also been associated with ASD, with a rate of 5–39.2% (Hara, 2007; Ghacibeh and Fields, 2015). A study by Clarke et al. found that 32% of their epileptic subjects met the criteria of ASD, however, authors used questionnaires only, and confirming clinical diagnostic tests were not applied (Clarke et al., 2005).

Several studies have suggested that increasing severity of autistic symptoms may be associated with higher likelihood of epileptic abnormalities (Elsayed and Sayyah, 2012; Mulligan and Trauner, 2014). EEG abnormalities have also been associated with autistic regression, lower intellect, delayed motor, and social development in the first year of life (Hrdlicka et al., 2004). This hypothesis is supported by Nicotera et al. as well (Gennaro Nicotera et al., 2019). In their study, epileptiform discharges were also significantly associated with hyperactivity, aggressive behaviors, self-harm behavior, and severe language impairment. Giannotti specifically investigated sleep patterns of ASD children and found that 64.42% of the patients had active sleep problems and also that disrupted sleep was associated with more severe disease course (Giannotti et al., 2008). Regarding the prevention of the syndrome, a 10-year follow-up study conducted by Hara showed that although 18% of the non-epileptic group exhibited SEA on EEG, 68% of epileptic group revealed SEA findings before the onset of epilepsy (Hara, 2007). He suggested that routine EEGs could predict developing epilepsy in the future.

When we consider treating SEA, Chez et al. found that regimental administration of valproic acid normalized the EEG in 46.6% of ASD diagnosed with SEA (Chez et al., 2006). However, we lack studies on EEG changes of ASD patients following ASD therapy, and studies on behavioral aspects could not prove that use of anticonvulsants provided better outcome than placebo (Hirota et al., 2014).

Based on the above, we are still not confident what SEA means on an EEG regarding pathodevelopment of ASD patients, but there are correlations and associations made. Currently EEG screening and prophylactic anticonvulsant treatment is not recommended in ASD (Swatzyna et al., 2019), as we are not certain about the clinical importance of these epileptiform alterations seen on EEG and how clinical outcome would be affected by such medication regime. However, clinicians could

TABLE 2 | Prevalence of epileptiform discharges in multiple sclerosis.

References	N	Study design	EEG type	ED (%)
Dagiasi et al. (2018)	62	Retrospective	Routine	38 (IEA)
Benjaminsen et al. (2017)	431	Retrospective	Routine	3,9 (IEA)
Calabrese et al. (2017)	23	Case–control	Routine	86,9 (IEA)
Kelley and Rodriguez (2009)	168	Review	–	32,7 (IEA)
Nyquist et al. (2001)	43	Retrospective	Sleep–awake	44.2 (IEA)
Sponsler and Kendrick-Adey (2011)	29,164	Review	–	1.95% (SEA)
Cheng et al. (2012)	93	Retrospective	Routine	8.6% (SEA)
Lund et al. (2014)	364	Retrospective	Routine	7.4% (SEA)

N, number of patients; ED, epileptiform discharge; IEA, interictal epileptiform activity; SEA, subclinical epileptiform activity.

TABLE 3 | Prevalence of subclinical epileptiform activity (SEA) in autism spectrum disorder (ASD).

References	N	Study design	EEG-type	ED (%)	Localization
Hughes and Melyn (2005)	59	Case–control	Routine + photic stim	75	Generalized, 59% bilateral spikes and 54% slow-wave complexes
Kim et al. (2006)	32	Prospective cohort	Video-EEG	59	Focal/multifocal sharp waves, generalized paroxysmal fast activity
Hrdlicka et al. (2004)	77	Prospective cohort	Polysomnography	38.1	–
Akshoomoff et al. (2007)	60	Prospective cohort	Routine	32	–
Yasuhara (2010)	1014	Prospective cohort	Routine polysomnography	85.8	Frontal spikes 65.6%, multifocal spikes < 10%
Gennaro Nicotera et al. (2019)	69	Routine	Routine	26.08	Focal spikes, 55.55%; multifocal and diffuse spikes, 44.44%
Mulligan and Trauner (2014)	101	Retrospective	Routine	59.4	–
Giannotti et al. (2008)	104	Prospective cohort	Routine polysomnography + photic stim	40.55	–
Hara (2007)	130	Retrospective follow-up	Routine	21	–
Chez et al. (2006)	889	Retrospective	24-h	60.7	Right temporal spikes, 21.5%; bilateral temporal spikes, 20.2%; generalized spike wave, 16.2%
Elsayed and Sayyah (2012)	47	Case–control	Routine	51.1	Focal frontal, occipital, temporal spikes
Hartley-McAndrew and Weinstock (2020)	123	Retrospective	Routine	30	–

N, number of patients; ED, epileptiform discharge.

consider obtaining a longer EEG examination and overnight EEG video monitoring. Certainly, applying long-term EEG is crucial, as Chez et al. showed that 5% of EEG abnormalities may have been missed in patients who had a negative, routine EEG previously. Chez et al. (2006) and Gennaro Nicotera et al. (2019) found that, when present, EEG abnormalities were detectable predominantly during sleep. For quality assessment prospective, randomized trials are needed, with clear methodology, and with choices of instrumentation that maximize the amount of data gained from the study population.

ADHD

ADHD is a syndrome defined by the American Psychiatric Association DSM-V as a persistent pattern of inattention and/or hyperactivity–impulsivity that interferes with functioning or development. In its presentation, we distinguish predominantly hyperactive–impulsive, inattentive, or combined subtypes (American Psychiatric Association, 2013). Worldwide the syndrome affects around 5% of children and 2.5% of adults (Polanczyk et al., 2014). EEG and functional imaging research on anatomical aspect of the disease shows involvement of the frontal cortex (Parisi et al., 2010; Schulz et al., 2012; Zaimoglu et al., 2015), particularly the dorsal anterior cingulate cortex manifested by decreased function of this brain area during inhibitory task control (Bush et al., 2005) and EEG paroxysmal abnormalities (Kanemura et al., 2013). Data suggest a pathophysiological and comorbid overlap between ADHD and epilepsy (Dunn and Kronenberger, 2006; Kaufmann et al., 2009; Salpekar and Mishra, 2014), a study of 76 children with epilepsy found that 31% of them had ADHD compared to 6% in the healthy control group (Hermann et al., 2007). Some studies found that in epileptic children, inattentive subtype is dominating while the combined type in those without epilepsy (Dunn and Kronenberger, 2006; Hermann et al., 2007; Socanski et al., 2010; Kanemura et al., 2013); however, others failed to show such relation (Lee et al., 2016). Although there is a challenge of distinguishing EEG abnormalities of ADHD and epilepsy in the same patient, there has been emerging focus on investigating SEA and their relation to transient cognitive impairment in this subgroup of children (Aldenkamp and Arends, 2004; Schubert, 2005).

Table 4 summarizes the prevalence of SEA among studies, which varies from 4.9 to 53.1%. Most studies are retrospective and used routine EEG. Epileptic activity in ADHD is commonly detected as generalized 3-Hz spike-and-wave discharges and paroxysmal abnormalities such as focal spikes (frontal, midtemporal, rolandic or parietal, occipital) (Holtmann et al., 2003; Schubert, 2005; Kanemura et al., 2013). A review by Salpekar et al. pointed out that an increase in theta waves in frontal regions seems to be a consistent EEG abnormality in this subgroup of patients and that alpha wave asymmetry and higher theta-to-beta ratio have also been reported (Salpekar and Mishra, 2014). The effect of antiepileptic drugs (AED) in patients with SEAs seems to show behavioral improvement in those children with frontal spikes but less so in case of the age-dependent Rolandic spike abnormalities (Holtmann et al., 2003; Schubert, 2005; Kanemura et al., 2013). Furthermore, SEA also had a positive predictive value of 14% for developing seizures in a group of 347 ADHD children (Richer et al., 2002). It should be noted that SEA was only seen in some of the patients after photic stimulation or hyperventilation in the study of Richer et al. (2002).

In **Table 4**, we collected the most recent studies on prevalence of epileptiform events. The study of Silvestri et al. (2007) reported the highest prevalence of SEA (53.1%) in their prospective cohort of 42 patients. It is noteworthy that this was the only study that used polysomnography for the evaluation. SEAs are

TABLE 4 | Prevalence of subclinical epileptiform activity (SEA) in attention-deficit hyperactivity disorder (ADHD).

References	N	Study design	EEG type	ED (%)	Localization
Kanemura et al. (2013)	46	Prospective cohort	Routine + photic stim 20 min	34.8	100% focal
Lee et al. (2016)	180	Retrospective	Routine	16.1	8.3% general 7.7% focal–frontal, Rolandic
Hughes et al. (2000)	176	Prospective	Routine 1 h with stimulation	30	24% focal 13% bifrontal
Hemmer et al. (2001)	234	Retrospective	Routine awake	15.4	60% focal, (5,6% Rolandic overall)
Millichap et al. (2011)	612	Retrospective	Routine	26.1	42.9% focal 41.7% generalized
Richer et al. (2002)	347	Retrospective	Routine 20 min + photic stim	6.1	–
Zaimoglu et al. (2015)	148	Prospective	Routine 1 h wake–sleep	26.4	Frontal, centrotemporal
Silvestri et al. (2007)	42	Prospective cohort	Sleep EEG (polysomnograpy)	53.1	28.2% centrotemporal, 12.5% frontal
Matoth et al. (2002)	126	Prospective cohort	Routine with stimulation	5	–
Socanski et al. (2010)	517	Retrospective cohort	Routine	7.5	53.9% generalized, 41% focal, 1.7% Rolandic

N, number of patients; ED, epileptiform discharge.

known to be more frequent during sleep, however, the capture of these abnormalities is extremely problematic on a routine 20–30 min long EEG. It is an open question whether or not the subgroup of ADHD patients with SEA would benefit from AEDs by preventing progression of disease and decline of cognitive function. To conclude, several studies have suggested that EEG can be used in specific populations to exclude more crude pathology, albeit others did not support this view (Hemmer et al., 2001; Matoth et al., 2002; Socanski et al., 2010; Millichap et al., 2011). Clearly, there is an important role of investigating SEAs in central nervous system (CNS) pathology, such as ADHD. Until a consensus emerges, there is much room to expand further research.

MECHANISM OF COGNITIVE IMPAIRMENT

Excitotoxity-Mediated Neurodegeneration

Neurodegeneration is a progressive loss of function and structure of neural cells leading to the death of neurons and glial cells (Spillantini and Goedert, 2013). The progressive decline of cognitive functions in neurodegenerative disorders is in line with the spreading of the accumulated misfolded proteins that is the major neuropathological hallmark of these disorders. The toxic proteins are different in the various forms of dementia (taupathies, amyloidopathies, synucleinopathies, etc.), however, they all have harmful effect on cellular membranes, mitochondrial functions, axonal transport, synaptic strength, and on neural survival in oxidative stress (Taylor et al., 2002). Misfolded proteins also change the physiological neuroinflammatory processes activating proinflammatory and neurotoxic mediators (Giovannini et al., 2002). As a summary of induced changes, protein misfolding associates with rapid neuronal death. Spatial distribution of pathological proteins varies among neurodegenerative disorders leading to different clinical presentations (e.g., entorhinal cortex is first to degenerate in AD, and substantia nigra is first in DLB and Parkinson's disease). In MS, neurodegeneration also occurs in an interaction with autoimmune inflammatory responses targeting myelin and oligodendrocytes (Ellwardt and Zipp, 2014). Neurodevelopmental factors ending in decreased neural survival are crucial in the pathogenesis of ASD because of genetic mutations of synaptogenic, inflammatory moderator and axon mobility factors (Kalkan et al., 2016; Rani, 2019). Some studies demonstrated that neurodegeneration occurs in epilepsy, too (Frantseva et al., 2000; Rao et al., 2006). While the typical histopathological hallmark of temporal lobe epilepsy is the neural loss and gliosis detected in the hippocampus, amygdala, and entorhinal cortex, novel examinations report the presence of misfolded tau and amyloid proteins as well (Tai et al., 2016). Furthermore, neuroimaging and physiology data show progressive gray matter atrophy in the structures of epileptic network (Bernhardt et al., 2010).

A common feature among epilepsy and all neurodegenerative disorders is the increased cortical excitability (Di Lazzaro et al., 2004; Gilbert et al., 2004; Takarae and Sweeney, 2017). Growing body of evidence supports that increased excitability precedes neurodegeneration in various diseases. Vucic and Kiernan (2006) and Vucic et al. (2008) reported reduced short-interval intracortical inhibition prior to the symptom onset in patients with amyotrophic lateral sclerosis and with other motoneuron disorders using transcranial magnetic stimulation. According to the studies of Vossel et al. (2013), the occurrence of seizures is increased years before the initial symptoms of AD.

Elevated cortical excitability might contribute to neurodegeneration through excitotoxicity (Mehta et al., 2013). It refers to a toxic effect, resulting from prominent and prolonged activation of excitatory neural receptors causing cell death (Bano et al., 2005). Under normal conditions, glutamate acting on its postsynaptic receptors [*N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)] causes depolarization and permits the increase in intracellular calcium. If depolarization is prolonged or glutamate reaches an excessive concentration in the synaptic cleft, it turns into a neuron-killing toxin causing the disruption of cellular osmotic equilibrium (Ong et al., 2013).

The glutamate neurotransmitter system is affected in many diseases with cognitive symptoms (**Figure 1**). High level of calcium permeable AMPA receptors was identified

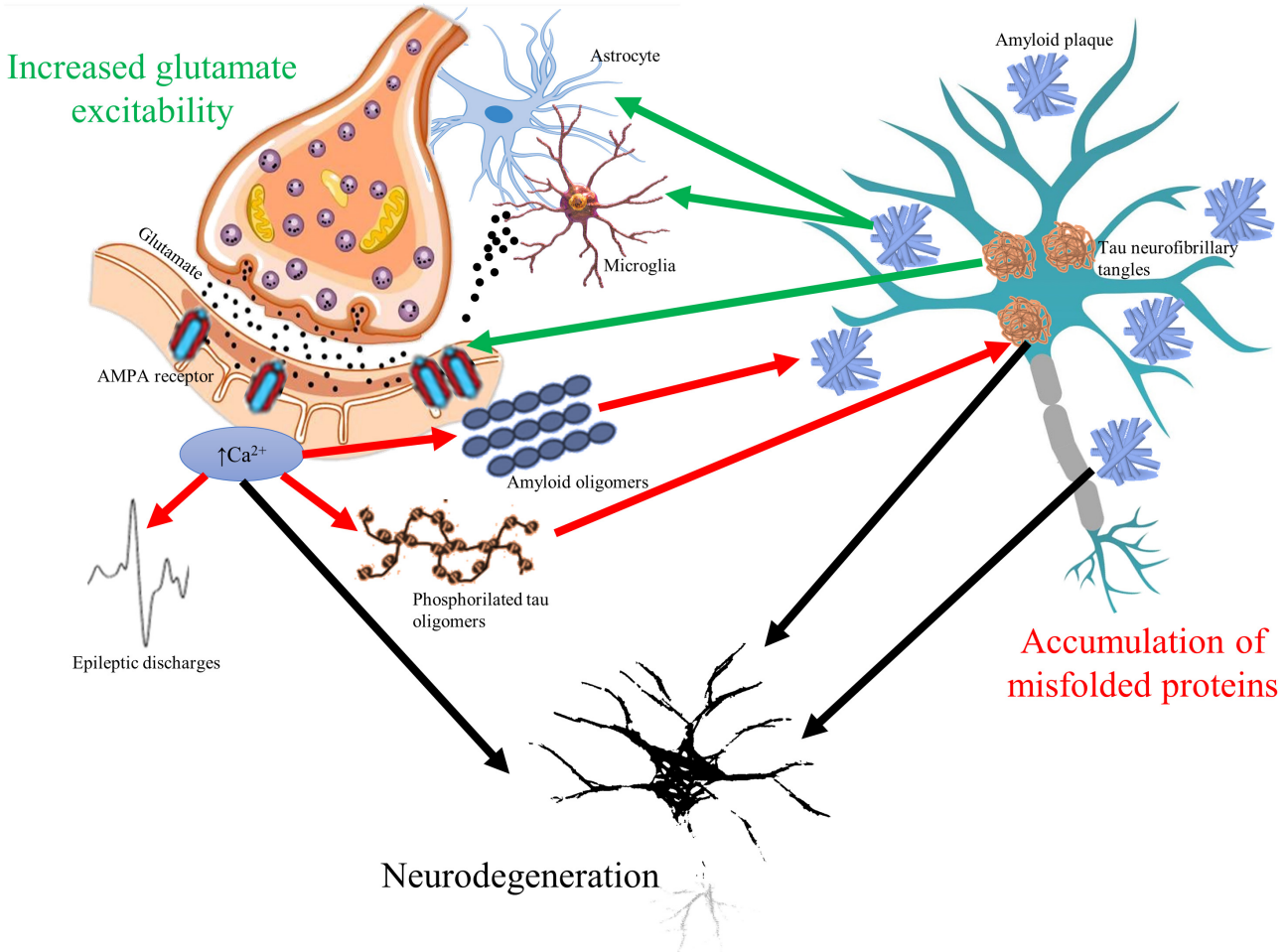

FIGURE 1 | The vicious circle of glutamate mediated hyperexcitability and accumulation of misfolded toxic proteins in cognitive disorders. Glutamate neurotransmitter is altered in all cognitive disorders resulting in overexpression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and elevated intracellular calcium signaling. Elevated calcium signal associates to higher release of amyloid oligomers to extracellular space and to increased phosphorylation of tau oligomers (red arrows). Increased firing of neurons represented by epileptic discharges is a consequence of glutamate-related hyperexcitability as well. On the other hand, accumulation of amyloid plaques and tau neurofibrils change glutamate receptor expression and induce excessive release of glutamate from microglial cells and astrocytes (green arrows). The bidirectional pathologic relationship could result in progressive neurodegeneration (black arrows), which is common hallmark of cognitive disorders.

in amyotrophic lateral sclerosis (Leal and Gomes, 2015). Inflammation induced, microglia driven excitotoxicity is a central event in MS (Gonsette, 2008). Elevated cortical glutamate concentration is a common finding in ASD (Brown et al., 2013). In AD, amyloid induces excessive glutamate release from astrocytes (Esposito et al., 2013), blocks the glutamate transporters of astrocytes responsible for reuptake (Zott et al., 2019), elevates calcium influx with the increase in depolarization (Fu et al., 2012), and activates NMDA receptors (Ferreira et al., 2010). Furthermore, tau might enhance the presynaptic glutamate release (Decker et al., 2016). Normal apoE function is essential in the attenuation of glutamate effect; however, its genetic mutation is the most known risk factor of AD (Aono et al., 2002). On the other hand, prolonged activation of NMDA receptors results in elevated production and secretion of amyloid-beta (Lesné et al., 2005) and in hyperphosphorylation of tau (Liang et al., 2009). It might explain the elevated phospho-tau level in surgical samples of temporal lobe epilepsy patients (Tai et al., 2016).

Since epileptic activity associates to excessive stimulation of NMDA receptors, it is intriguing to speculate that epileptic seizures might contribute to the neural loss presented in various forms of cognitive disorders. Indeed, the presence of epileptic seizures associates with faster progression of symptoms in AD (Vossel et al., 2013). However, short-lasting excitations (IEA and SEA) also associate with increased glutamate release (Kang et al., 2005), so harmful effect of epileptic discharges is predictable. It is reinforced by a study of Dolev et al. (2013), showing that even a 20 Hz burst activity could increase amyloid burden; by the study of Bero et al. (2011), showing that neuronal hyperactivity associates to increased amyloid burden; and by a report of

Vossel et al. (2016), demonstrating the role of SEA in the accelerated progression of AD.

Remodeling of Neural Circuitry

Balance between excitatory glutamatergic and inhibitory GABAergic activity in the large functional networks of the brain is crucial in all cognitive functions (Sengupta et al., 2013). Reduction in inhibition or increase in excitation has a key role in ictogenesis (Bonansco and Fuenzalida, 2016). Local GABAergic sprouting limits the spreading of epileptic activity to distant areas (Sutula, 2002), relatively disconnecting the epileptogenic zone from connected brain structures. Connectivity studies support the pathological findings describing increased intrahippocampal and decreased hippocampo-cortical connectivity in patients with mesio-temporal lobe epilepsy (Warren et al., 2010; Engel et al., 2013). As seizures propagate and the epileptic network extends, altered hippocampo-cortical structural connectivity could lead to less synchronized global networks, to impaired organization of rhythmic brain activities, and finally to random organization of physiological networks (Luo et al., 2012; **Figure 2**).

Growing body of evidence suggests that IEA spreads in the same pathological network as epileptic seizures, however, the underlying aberrant activity does not reach the seizure threshold (Dzhala and Staley, 2003). This hypothesis is supported by clinical observations of transient cognitive impairment (TCI) observed after IEA. TCI is characterized by a brief temporary deficit in memory encoding, attention, communication, or visuospatial abilities (Holmes and Lenck-Santini, 2006). If IEA is frequent, epileptic activity could induce long-lasting and distant changes in brain functioning (Caciagli et al., 2014). It is supported by the findings of Gelinas et al. (2016) showing that IEA shows a coupling with spindles via cortical downstates. Studies using functional MRI reinforced these suggestions, demonstrating spike-related changes in blood–oxygen-level-dependent imaging (BOLD) signal even at distant cortical sites (Federico et al., 2005). Reports applying EEG connectivity analysis revealed similar findings demonstrating that functional connectivity is increased in the epileptic network during IEA similarly to seizure-related alterations, while it is reduced between epileptic and functional networks such as default mode network (DMN) (Bettus et al., 2008; Fahoum et al., 2013). Noticeably, these changes become permanent in a long-lasting disease and remain independent from ongoing IEA (Luo et al., 2011).

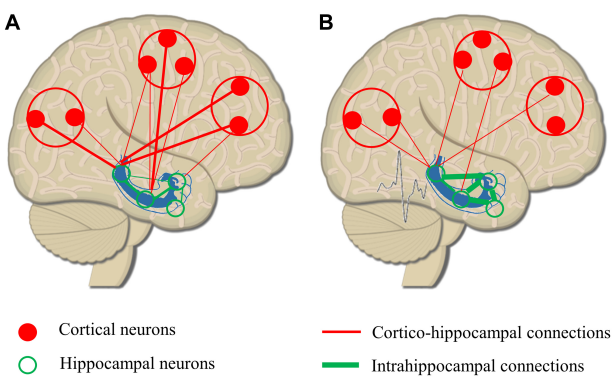

FIGURE 2 | Remodeling or hippocampo-cortical circuitry as a result of epileptic discharges. **(A)** Physiological organization of hippocampo-cortical connections with numerous, strong local connections and less and weaker distant associations. **(B)** As a result of epileptic discharges, intrahippocampal connectivity is increased, and the strength and number of long distant connections are decreased. The remodeling of network circuitry leads to a relative isolation of hippocampus from cortical areas reducing the efficacy of hippocampo-cortical coupling.

Substantial alterations of large neural networks have been shown in all conditions. Elevated intrahippocampal activity is depicted in the prodromal stages of AD (in amnestic mild cognitive impairment) (Bakker et al., 2012) correlating with cortical thinning (Putcha et al., 2011) and with disconnection to other neural networks including DMN (Pasquini et al., 2015). Similar findings showing local increase in connectivity and reduction in global connectivity have been described in various forms of dementia including DLB and FTD (Agosta et al., 2013; Dauwan et al., 2016). Hyperactivity within large-scale brain networks and decreased between-network connectivity is a core finding in pediatric ASD studies (Cerliani et al., 2015; Nomi and Uddin, 2015). Increased within-network hyperconnectivity has been described in the frontal areas of ADHD patients (Wang et al., 2009) with a loss of long distant connections. Interestingly, AD-like changes in hippocampo-cortical connectivity (increase in intrahippocampal connectivity and decrease in global connectivity) have been demonstrated in MS patients with memory impairment; however, they parallelly identified reduction in hippocampal activation (Hulst et al., 2015).

Disruption of Sleep-Related Memory Consolidation Process

Sleep occurs in all vertebrates in regular intervals, and it is homeostatically regulated. It is well known that sleep deprivation has a harmful effect on the physical and mental health including severe changes in hormonal, homeostatic, and temperature regulation, higher occurrence of infections, and dysfunction of cardiovascular control (Shahar et al., 2001). Human sleep is distinguished into non-REM and REM sleep. The dual process hypothesis postulates that REM sleep is crucial in implicit memory formation, while non-REM sleep, especially episodes characterized by slow-wave electric activity (slow-wave sleep or SWS) is mandatory in the establishment of episodic memory (Diekelmann and Born, 2010). The widely accepted two-stage memory model differentiates brain structures into areas with short-term memory capacity having an encoding function and into regions serving as long-term storages (Walker, 2005). The memory consolidation process involves the repeated reactivation of short-term stored memory items (freshly developed synaptic connections) during offline periods (e.g., SWS) and the strengthening and adaptation of memory fragments into long-term storages (Stickgold, 2005).

The anatomical structure for the interplay is the network between hippocampus and cortical areas. In human SWS, EEG shows 0.5–4 Hz slow oscillations with dynamic

alterations of neuronal membrane depolarization (upstates) and hyperpolarization (downstates) (Csercsa et al., 2010). Dynamic changes reveal an opportunity for the reduction in weaker synaptic connections parallel with the reinforcement of stronger ones, known as synaptic downscaling (Tononi and Cirelli, 2006). Neurons during SWS show widespread synchronization in cortico-cortical, thalamo-cortical, and hippocampo-cortical networks (Dang-Vu et al., 2008). High synchrony is reinforced by animal and human neurophysiology studies showing that the top–down controlled phase-locked co-occurrence of hippocampus generated sharp-wave ripples, thalamic sleep spindles, and cortically induced slow waves (Maingret et al., 2016).

An epileptic spike is shorter but similar to sharp wave, and it associates to faster ripple oscillations than sharp wave (Bragin et al., 2002). Numerous studies hypothesized that epileptic discharges linked to fast ripples could interfere with normal memory process (Halász et al., 2019; **Figure 3**). Furthermore, they can also act as dysfunctional ("dummy") variants of sharp-wave deteriorating memory consolidation (Gelinas et al., 2016). The crucial role of sleep-associated IEA in memory formation is suggested by the following findings: IEA predominantly occurs in SWS (Bazil, 2000); it associates with longer REM latency (first occurrence of REM during the night), with reduced duration of SWS (Miller et al., 2016) and with lower number of physiological ripples (Jefferys et al., 2012) and negatively affects thalamic spindle formation (Frauscher et al., 2015).

While sleep changes might have a crucial role in the memory impairment of epileptic patients, sleep disorders are also highly prevalent in cognitive disorders. Around 40% of AD patients suffer from sleep disturbances (Tractenberg et al., 2003), namely, from nocturnal sleep disruption, increased daytime sleepiness, insomnia (Rao et al., 2008; Osorio et al., 2011), and sundowning (agitation and confusion late afternoon) (Volicer et al., 2001). About 50–83% of DLB patients suffer from REM sleep behavior disorders (Ferman et al., 2010). In MS, obstructive sleep apnea (Braley et al., 2014), restless leg syndrome (Manconi et al., 2007), and moderate or severe insomnia are frequently observed (Brass et al., 2014). Insomnia is reported in 44–83% of children with ASD (Miano and Ferri, 2010). Prominent elevation in the occurrence of restless leg syndrome, periodic limb movement in sleep, sleep-onset insomnia, nocturnal motor activity, and obstructive sleep apnea has been highlighted in numerous studies on ADHD patients (Konofal et al., 2010). Sleep microstructure seems to be highly impaired as well in cognitive disorders. Reduced REM sleep, decreased number of sleep spindles, reduction in SWS, and increase in superficial stages have been reported in ASD (Richdale and Schreck, 2009). Excessive loss of SWS is a characteristic hallmark of AD with a reduction in sleep spindles and K complexes (Petit et al., 2004). Children with ADHD show lower rate of cyclic alternating pattern and sleep spindles (Miano et al., 2006; Kirov and Brand, 2014). Thus, the role of poor sleep in cognitive impairment is not questionable in cognitive disorders.

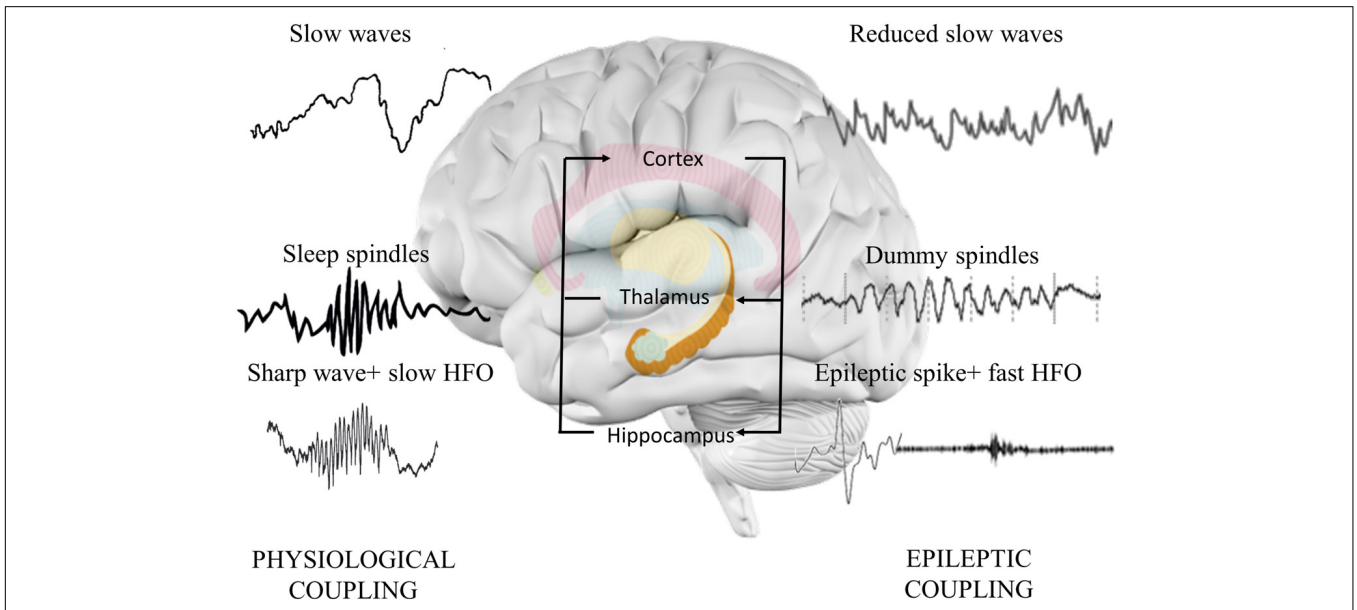

FIGURE 3 | Hippocampo-thalamo-cortical coupling in memory consolidation. In physiological memory consolidation process, synchronization of the hippocampus, thalamus, and neocortex is essential. Hippocampal sharp-wave ripples correspond to the replay of recently stored memory items in the synaptic connections of hippocampal neurons. Thalamic sleep spindles with a frequency of 12–16 Hz are essential elements of memory formation, synchronizing hippocampal activity with cortical neurons. Cortical sleep-related slow waves provide the highest synchronization state to facilitate the activation of hippocampal sharp-wave ripples and thalamic sleep spindles. Epileptic discharges correspond to the pathological transformation of sharp waves coupling with faster high frequency oscillations. The altered activity disorganizes the architecture of spindles, decreases the normal spindle activity, and induces the formation of dummy spindles with longer duration and spiky appearance. Cortical slow waves are also reduced, probably due to the spike-inducted cortical hyperpolarization (downstates). Alterations might reduce the efficacy of memory consolidation process.

While SWS is reduced in all cognitive disorders, SEA seemingly still accumulates in deep sleep similarly to epileptic patients. Approximately 90–100% of epileptic discharges are detected in SWS in studies examining patients with AD (Vossel et al., 2016) or with ASD (Chez et al., 2006). Furthermore, the occurrence of epileptiform discharges on nocturnal EEG is positively related to higher attention deficit and higher impulsivity in ADHD patients (Danhofer et al., 2018). Since epileptic activity compromises the organization of sleep structure and disturbs the sleep-related memory consolidation processes, it is intriguing to state that SEA might accelerate the disorganization of sleep structure and contribute to the decline of memory functions.

THERAPEUTIC ASPECTS

Current Findings and Recommendations

The primary application of AEDs is to effectively reduce or eradicate epileptic seizures with an optimal side effect profile. Approximately 30 types of AEDs are available on the market with first, second, and third lines of indications regarding the type of seizures, the age, physical condition, and the current drug use of the patient. While we do not understand completely the mechanism of all AEDs, their efficacy is measured as the extent of decrease in the number of seizures. While we have tremendous experience and recently updated guidelines for controlling seizures in epilepsy patients, we have relatively limited data on the AED selection in cognitive disorders. In AD, studies are available on levetiracetam (LEV), lamotrigine (LTG), gabapentin, carbamazepine, valproic acid, phenytoin, and phenobarbital (Horváth et al., 2016; Vossel et al., 2017). Only LEV and LTG reached excellent efficacy (60–70% reduction in the number of seizures in a 1-year follow-up) and tolerability without cognitive side effects (Belcastro et al., 2007; Cumbo and Ligori, 2010; Lippa et al., 2010). Notably, treatment with LEV resulted in marginally increased cognitive scores (MMSE and ADAS-Cog), and application of LTG was associated with significantly improved mood (Cumbo and Ligori, 2010), but the study was not placebo controlled. Studies on AED application in other NCDs for controlling seizures are absent (Horváth et al., 2018a). According to the current guidelines, the management of seizures does not differ in other cognitive disorders compared to epilepsy patients (Myers and Johnson, 2007; Kelley and Rodriguez, 2009; Felt et al., 2014).

While growing body of evidence supports the central role of epileptic discharges in cognitive deterioration, studies on affecting SEA are limited in cognitive disorders. In NCDs, LEV treatment for 2 weeks significantly improved performance in pattern separation, but no other cognitive scores of non-epileptic MCI patients in line with the normalization of hippocampal and entorhinal cortical activity measured with functional MRI (Bakker et al., 2015). In the study of Musaeus et al. (2017) using single-dose LEV, while antiepileptic therapy marginally increased the power of beta band in AD patients, positive cognitive effect was not detected. Unfortunately, studies on other forms of NCDs have not been conducted. Furthermore, most MCI and AD studies are not double-blind observations and did not use SEA as a selection criterion or a marker of therapeutic response. Ongoing clinical trials (e.g., ILiAd, NCT03489044; LAPSE, NCT04004702; LEV-AD, NCT02002819 studies) on LEV already assess SEA for the identification of target groups, but results have not been published yet. In ASD patients without epileptic seizures, seven placebo-controlled, randomized studies on the use of AED are available. These studies analyze the utility of valproic acid, topiramate, LTG, and LEV. Based on the findings of a meta-analysis, AED did not have a significant effect on behavioral symptoms, however, studies have not differentiated subgroups of patients with SEA and were not EEG controlled (Hirota et al., 2014). In ADHD, only independent, single reports are available on the use of AED in non-epileptic patients. A study using valproic acid reported reduction in frontal SEA in 62% of ADHD patients, and the decrease was correlated with improvements in ADHD rating scale (Kanemura et al., 2013). In the study of Öncü et al. (2014), LTG improved mood scores in 78% of ADHD patients with comorbid bipolar disorder or depression, however, EEG was not applied. While AED are frequently prescribed in MS for neuropathic pain, studies on SEA or on cognitive impact were not conducted (Solaro et al., 2005).

Age-related changes in pharmacodynamics and pharmacokinetics make AED studies by the elderly complicated. In the selection of AEDs, safety issues and contraindications have to be carefully considered in these patients. In previous studies, the use of AEDs has been associated with elevated risk for fall (Seppala et al., 2018), stroke (Sarycheva et al., 2018), fractures (Shen et al., 2014), pneumonia (Taipale et al., 2019), and adverse drug–drug interactions (Anderson, 2004). Application of traditional AEDs (e.g., phenytoin, valproic acid) was associated with unplanned hospital admissions and impaired motor functions (Lin et al., 2017). Use of carbamazepine and oxcarbamazepine was attached to adverse cardiac events, hyponatremia, and sedation (Spina and Perucca, 2002). Thus, contraindications have to be considered individually.

For the understanding of the potential role of AED in the therapy of cognitive impairment, double-blind, placebo-controlled studies are needed in various cognitive disorders. The detection of SEA with EEG might have a crucial role in the accurate identification of target groups of patients, and it might serve as a fundamental outcome and therapeutic response measure. However, it should be noted that AEDs are primarily applied for seizure control. Thus, their effect on inhibiting epileptiform discharges (including IEA or SEA) is limited or unknown. Furthermore, the identification of novel targets and development of new drugs are crucial for the proper therapy of hyperexcitability in cognitive disorders.

Potential Novel Directions

As we have described, many AEDs have been introduced to the market over the recent decades and even more are in development. Fundamentally, all AEDs have been designed or optimized to restore an abnormal balance between excitatory and inhibitory neurotransmission, which is a hallmark of epilepsy. Most AEDs, especially those from the first generation, lack selectivity and act on essential mediators of neuronal

excitability such as ion channels, glutamate, or $GABA_A$ receptors. These drugs exert widespread effects on neuronal networks and cause a range of undesired side effects such as sedation and cognitive deficits (Ortinski and Meador, 2004). Therefore, newer antiepileptics that have more selective targets modulating excitability in discrete neuronal circuits are better positioned for potential treatment of SEA or IEA associated with various neuropsychiatric disorders.

One such drug with a unique modulatory effect on neuronal excitability exerted by binding to the synaptic vesicle protein 2A (SV2A) is LEV (Löscher et al., 2016). Compared to more conventional AEDs, which typically act on postsynaptic receptors or ion channels, levetiracetam tends to be better tolerated by patients and does not induce strong sedative effect (Cramer et al., 2003; Abou-Khalil, 2008). Interestingly, levetiracetam has been initially developed as a cognitive enhancer after chemical modification of its predecessor, piracetam. Several lines of evidence indicate that low doses of levetiracetam improve cognitive performance in both animal models and clinical setting. These therapeutic activities of the drug are attributed to modulation of hippocampal hyperactivity (Haberman et al., 2017). Interestingly, levetiracetam is one of the few AEDs that display clear-cut effect on IEA not only in patients with adult (Stodieck et al., 2001) and childhood epilepsies (Larsson et al., 2010), but also in children with ADHD (Bakke et al., 2011) resulting in improvement in clinical symptoms (e.g., restless leg) (Gagliano et al., 2011). Further, preclinical evidence indicates that levetiracetam improves cognitive performance in models of AD and schizophrenia (Sanchez et al., 2012; Koh et al., 2018). There may be a connection with the mechanisms of action of levetiracetam since several studies using SV2A PET tracers show reduction in SV2A expression associated with several neuropsychiatric and neurodegenerative diseases that are associated with cognitive deficits (Heurling et al., 2019). This was most clearly demonstrated in patients with AD (Chen et al., 2018). It is believed that SV2A is a marker of synaptopathy reflecting pathological changes in synaptic circuits (e.g., hippocampus) associated with cognitive performance. These observations have led to a number of clinical trials exploring the potential of levetiracetam as treatment for cognitive deficits associated with increased cortical activity or with SEA in AD (Bakker et al., 2015; Vossel et al., 2016), and numerous ongoing double-blind trials are going to conclude soon as well.

A promising potential therapeutic approach can be attributed to subunit selective modulators of $GABA_A$ receptors, which have discrete localization in the brain areas associated with SEA or IEA. The key advantage of such compounds is their improved safety and tolerability versus conventional, non-selective drugs such as benzodiazepines. In this context, selective positive allosteric modulators of alpha-5 subunit containing $GABA_A$ receptors might have a potential to reduce the occurrence of epileptiform discharges (Biagini et al., 2010), and studies have shown promising effects on cognitive and memory performance in animal models (Koh et al., 2013).

Abnormality in glutamate uptake is another important mechanism that is shared by several neuropsychiatric diseases that are associated with SEA and deficits in cognition (O'Donovan et al., 2017). Recent work indicates that neuronal hyperexcitability observed in the limbic regions in patients with Alzheimer's disease may be initiated by suppression of glutamate reuptake and can trigger a vicious cycle of neurodegeneration driven by β-amyloid (Zott et al., 2019). Therefore, restoration of glutamate uptake by drugs increasing the expression or function of excitatory amino acid transporter 2 (EAAT2) could find a novel therapeutic indication for treatment of SEA or IEA associated with various cognitive disorders (Fontana, 2015).

DISCUSSION

Cognitive disorders including NCDs, ASD, ADHD, and MS have a high overall prevalence affecting approximately 40–50% of the population. A common hallmark of these variable conditions is the higher occurrence of epileptic seizures during the course of the disease suggesting that hyperexcitation might play a role in the pathomechanism of cognitive impairment (Tuchman and Rapin, 2002; Austin and Caplan, 2007; Horváth et al., 2016). Prevalence and impact of IEA and SEA are less investigated in cognitive disorders in comparison to epileptic seizures. However, the study of these phenomena might represent an important future direction, since modern epileptology recognized that isolated but frequent epileptic activity could compromise the cognitive function of epilepsy patients more than epileptic seizures (Berg, 2011).

Proper definition and/or distinction of IEA and SEA are also missing, making it difficult to compare the results of various prevalence studies. While the interictal terminology postulates the presence of ictus (seizure), IEA is frequently used to describe epileptiform activity without overt clinical seizures. However, traditional epileptology recognizes epileptiform discharges without detectable clinical or electrographic seizures as benign EEG variants (Santoshkumar et al., 2009). From the epileptological viewpoint, benign means that the detected activity does not associate to clinically diagnosed epilepsy or any other neurological or psychiatric disorder. However, in our opinion, independency from seizures does not necessarily equal to clinically benign behavior. A possible explanation is that epileptiform discharges and epileptic seizures are consequences and markers of increased cortical excitability, however, they represent the different ends of the spectrum (Badawy et al., 2009a). If network excitability exceeds a certain threshold, the affected patient develops epileptic seizures and frequent interictal epileptic discharges, leading to the diagnosis of epilepsy (Dzhala and Staley, 2003). If it does not reach the threshold, SEA is detectable and indicates increased excitability as a general marker (Badawy et al., 2009b). Since more and more neuropsychological and neuroimaging studies suggest that SEA correlates with cognitive deterioration (Vossel et al., 2016), we propose to reconsider the use of "benign" term for epileptiform EEG graphoelements without detailed neuropsychological investigation. In our review, we systematically separated the two terms, IEA and SEA, and propose the exclusive use of SEA for epileptiform events in the absence of proved epileptic seizure. However, the distinction resulted in an important

conclusion: SEA shows the similar characteristic as IEA regarding the temporal, spatial characteristic, and the impact on cognitive functions.

IEA and SEA both accumulate in sleep in AD patients (Vossel et al., 2016; Horváth et al., 2017a), in ASD patients (Chez et al., 2006), and in ADHD (Silvestri et al., 2007). IEA and SEA both recorded mainly over the frontotemporal areas in AD (Rao et al., 2009; Vossel et al., 2013, 2016; Cretin et al., 2016; Sarkis et al., 2016; Horváth et al., 2018b), in ADHD (Lee et al., 2016), and in ASD (Chez et al., 2006). Frequent occurrence of interictal discharges associate to decreased therapeutic response, poorer postsurgical outcome, and augmented cognitive decline in epilepsy patients (Drane et al., 2016). Higher frequency of IEA in SWS defines more prominent impairment in language function of epileptic patients with ESES (Scheltens-de Boer, 2009). Therapeutic reduction in IEA improved the behavioral problems of children with focal epilepsy (Pressler et al., 2005). SEA is attached to two times faster progression of AD (Vossel et al., 2016), higher prevalence of regression in ASD (Giannotti et al., 2008; Stefanatos, 2008), elevated number of active lesions in MS (Lebrun, 2006), and higher incidence of future seizures in ADHD (Lee et al., 2016). Reduction in SEA by AED led to 60% improvement in behavior scores in ADHD patients (Bakke et al., 2011), improved cognitive scores in patients with mild cognitive impairment (Bakker et al., 2015), and significant positive changes in cognitive scores of ASD patients (Hollander et al., 2001).

Based on literature overview, there are various ways how SEA could have a detrimental effect on cognition. The common link is the glutamatergic system that is compromised in all cognitive disorders (Gonsette, 2008; Esposito et al., 2013). Increased excitatory activity results in related excitotoxicity leading to neurodegeneration of various neural structures in different cognitive disorders (Mehta et al., 2013). In the neurodegenerative process, epileptic discharges accelerate the accumulation of toxic proteins (e.g., tau, amyloid) facilitating neural loss (Liang et al., 2009; Dolev et al., 2013). These processes transform into a vicious circle since misfolded proteins also induce excessive release of glutamate (Ittner and Götz, 2011). The described changes lead to a local hyperexcited neural network and activation of compensatory remodeling mechanisms (Sutula, 2002). Remodeling can lead to the disconnection of the affected structures to other functional networks (Engel et al., 2013). It is supported by numerous studies demonstrating the loss of distant connections in cognitive disorders (Liu et al., 2014). Extending general excitability might have a crucial role in the spreading of pathological proteins in the functional neural networks of patients with NCDs (Pievani et al., 2011; Hoenig et al., 2018). When large functional circuits are involved in the pathological process, their function shows abnormalities, and it is demonstrated by the alteration of sleep structure (Palop and Mucke, 2010). Increasing number of epileptic discharges leads to reduction in slow-wave sleep, overproduction of dysfunctional, dummy sleep spindles, and finally loss of sleep function in the memory encoding and consolidation process (Malow, 2007; Halász et al., 2019). Since epileptic discharges are crucial in the pathological process, modification might have a novel therapeutic potential in cognitive disorders (Bakker et al., 2015).

Rapidly emerging observations and data linking SEA or IEA with a wide range of neuropsychiatric and cognitive disorders open several previously unexplored therapeutic opportunities that could be focused on targeting neuronal hyperexcitability. In this context, an obvious solution would be application of existing AEDs, which should be able to normalize such abnormal neuronal activity. However, despite some promising results with selected AEDs, this class of drugs is generally associated with poor tolerability, narrow therapeutic window, and worsened cognitive abilities. Therefore, a more selective and perhaps milder modulation of neuronal excitability in discrete brain regions in stratified subpopulation of patients with documented SEA or IEA could lead to significant therapeutic benefits and become a novel class of therapy for cognitive disorders.

AUTHOR CONTRIBUTIONS

AH and EC conceived the topic and wrote the manuscript. SL, RK, and AK contributed to writing and reviewing and editing the manuscript. All authors contributed to the article and approved the submitted version.

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