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A Review of the Effects of Hypoxia, Sleep Deprivation and Transcranial Magnetic Stimulation on EEG Activity in Humans: Challenges for Drug Discovery for Alzheimer's Disease

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Abstract: Different kinds of challenge can alter cognitive process and electroencephalographic (EEG) rhythms in humans. This can provide an alternative paradigms to evaluate treatment effects in drug discovery. Here, we report recent findings on the effects of challenges represented by sleep deprivation (SD), transient hypoxia, and transcranial magnetic stimulation (TMS) in healthy volunteers on cognitive processes and EEG rhythms to build a knowledge platform for novel research for drug discovery in AD Alzheimer's disease (AD). Sleep pressure enhanced frontal delta rhythms (< 4 Hz) during the night, while SD increased slow rhythms in the theta range (4-7 Hz), and reduced resting state alpha rhythms (8-12 Hz) after the following day. Furthermore, SD transiently affected cognitive performance. In contrast, transient experimental hypoxia induced abnormal posterior resting state delta and alpha rhythms in healthy volunteers that resemble the abnormal EEG rhythms typically recorded in AD patients. However, the relationship between the cognitive and EEG effects of such challenges is poorly understood. TMS reversibly interfered with higher brain functions during EEG recordings, but few studies have investigated the relationship between the cognitive and EEG effects of TMS. In conclusion, SD is the most mature challenge model for testing new drugs for AD. Future investigation is needed to better understand the opportunities offered by TMS and hypoxia challenges.

Keywords: Electroencephalography (EEG), transcranial magnetic stimulation (TMS), sleep deprivation, hypoxia, alzheimer's disease, drug research, IMI PharmaCog.

1. PROCEDURES FOR INTERFERING WITH HUMAN CORTICAL ACTIVITY AND COGNITION FOR POTENTIAL USE IN DRUG DISCOVERY FOR THE TREATMENT OF ALZHEIMER'S DISEASE

It is well known that Alzheimer's disease (AD) is a progressive, neurodegenerative brain disease of the elderly characterized by memory loss and cognitive and behavioral abnormalities. Aging is the most important risk factor of AD. Memory dysfunction in senescence and early AD is related to an impairment of cholinergic basal forebrain, thalamocortical system, associative parietal-temporal areas, and the circuits linking entorhinal cortex, hippocampus, and amygdala [1]. At present, there are no effective disease modifying drugs that are able to prevent, or even slow down, the pathological process. There are two therapeutic classes licensed for the symptomatic treatment of the cognitive deficits evident in AD, namely the acetylcholinesterase inhibitors and the NMDA receptor glutamatergic antagonist, memantine. Unfortunately, these drugs have only modest effects on AD symptoms.

An important objective of modern AD research is to develop and validate procedures for an effective preliminary evaluation of new symptomatic drugs to enable early clinical proof of concept studies prior to large scale expensive phase II and III studies. This objective may be achieved by several strategies including the validation of reproducible procedures transiently interfering with cortical activity and cognitive processes in healthy volunteers, i.e. challenge models. Challenge models overcome the inherent difficulty of detecting significant improvements in cognitive performance in normal healthy subjects. Examples of these kind of challenges

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have previously been limited to pharmacological challenges such as the administration of cholinergic or glutamatergic antagonists such as scopolamine or ketamine [2-4].

The evaluation of new non-pharmacological challenge models constitutes one of the research lines of the Pharma-Cog project funded under the European Innovative Medicines Initiative (IMI). For the PharmaCog project, transient hypoxia, sleep deprivation (SD), and interfering transcranial magnetic stimulation (TMS) were selected as tentative challenge models. Pathologic hypoxia is typically caused by a reduction or change in blood supply. It induces glucose hypometabolism in the hippocampus and a number of key brain areas sub-serving cognitive functions such as mnemonic processing. Hypoxia may occur at any point in the circadian cycle. During sleep, repeated hypoxic events may be due to respiratory abnormalities with a generalized impact on cerebral oxygenation. This is especially true in rain regions with high metabolism. Furthermore, chemoreceptor sensitivity decreases, respiratory motor output and muscle contraction diminish. This alters ventilation/perfusion relationships and airflow resistance increases. These changes in pulmonary mechanisms generate alveolar hypoventilation, especially during rapid eye movement (REM) sleep, and can lead to significant nocturnal hypoxemia in patients affected by pulmonary disease such chronic obstructive pulmonary disease (COPD) [5, 6]. During the day, hypo-oxygenation of the brain may be due to several causes including chronic cerebrovascular and vasomotor deficits provoked by a poor cholinergic tone or other causes. It has been reported that these alterations may, in turn, induce amyloid-beta deposition extracellularly, and intracellular neurofibrillary cytopathology in cholinergic and other neurons [1, 7].

Sleep is characterized by stages dominated by low-frequencies (1-7 Hz) electroencephalographic (EEG) rhythms and by stages dominated by REM. REM sleep is associated with especially vivid dreams and EEG activity with a greater proportion of higher EEG frequencies (> 7Hz). A circadian and ordered sequence of non-REM and REM sleep stages is reflected in a sleep profile and is crucial for energetic homeostasis of neurons and consolidation/testing of new synapses and cognitive representations during the night [8-11]. For this reason, SD is hypothesized to have deleterious effects on a variety of cognitive and attentional abilities and may lead, eventually to cognitive disruption and death as in familial fatal insomnia [12-25].

TMS is the most effective, non-invasive and tolerated procedure for the stimulation of human cerebral cortex through the intact skull [26, 27]. It utilizes a rapidly changing magnetic field to transmit a short lasting electrical current pulse into the brain. This field can induce a synchronized activation of cortical neurons followed by a long-lasting inhibition, especially in superficial cortical layers. In a clinical setting, TMS is mainly used to examine the functional integrity of the corticospinal motor projections. However, single pulses or short bursts of TMS can perturb ongoing neuronal processing in the stimulated cortex, producing a transient and fully reversible interference with the physiological activity of the neurons involved [28-30]. This perturbation has been extensively used by cognitive neuroscientists to examine the functional relevance of the stimulated cortical area for cognitive processes and behavior [28, 31, 32].

Ideally, an effective pharmacological agent should normalize key neurophysiological mechanisms and cognitive processes impaired by the disease action. A promising candidate for the evaluation of these mechanisms is the recording of EEG activity. It is non-invasive, relatively inexpensive and cost-effective. Furthermore, it is able to probe an important emerging property of the brain, that is to say its oscillatory nature [29, 33-37]. The present review presents a contemporary analysis of the effects of hypoxia, SD, and TMS on resting state EEG rhythms in humans, as a basis for novel experiments in the framework of symptomatic drug discovery in AD.

2. QUANTITATIVE EEG

Scalp EEG recording essentially measures current changes on the scalp that directly follow those in the extracellular space, which are caused by ion flow due to excitatory and inhibitory postsynaptic potentials [29, 33-37]. EEG signals mainly reflect synchronized synaptic activity of neuron populations whose dendrites are collectively oriented radially to the cortical surface (e.g. pyramidal neurons) [38]. The temporal resolution of EEG is high and can be accurately measured in terms of fractions of milliseconds. In contrast, the spatial resolution of EEG is considered far lower than that of positron emission tomography (PET) which is approximately 1 cm and functional magnetic resonance imaging (fMRI) which has a spatial resolution of millimeters. The most popular procedures for the estimation of cortical sources of EEG activity use equivalent current dipoles as a model of neural generators. Regularized linear procedures are normally utilized for solving the inverse problem [39-41].

EEG recordings can reveal temporal and spatial information about externally triggered event-related (i.e. event related potentials, ERPs) [42-44] or spontaneous on-going EEG activity [45, 46]. The measurement of ERPs requires the averaging of multiple short on-going EEG trials that are phase- and time-locked to sensory, cognitive or motor tasks. Event-related cortical activity can be quantified by measuring latencies and amplitudes of distinct ERP components. Spontaneous on-going EEG activity is usually recorded over longer time periods to assess states of vigilance or consciousness, for example sleep and resting state wakefulness with eyes-closed or -open.

Resting state, eyes-closed EEG rhythms may reflect, -at least at a group level, preclinical and clinical stages of AD. from the literature it can be concluded that (1) alpha (8-12 Hz) EEG rhythms are specifically abnormal in AD subjects when compared to normal, amnesic MCI, cerebrovascular dementia, and Parkinson disease (PD) with cognitive impairment subjects; (2) delta (2-4 Hz) and alpha rhythms are related to attention, global cognitive status, genetic factor risks for AD (i.e. ApoE4, haplotype B of CST3), biomarker (i.e. serum 'free' copper), structural neurodegenerative signs, and treatment with donepezil and ibuprofen [47-73].

3. EFFECTS OF HYPOXIA ON EEG ACTIVITY

Acute (i.e. stroke) and chronic (i.e. several cardiorespiratory disorders) periods of inadequate oxygen delivery to the brain causes cerebral hypoxia. Cerebral hypoxia can produce

abnormal EEG patterns, such as burst-suppression [74, 75], alpha coma [76-78] and brief (up to several seconds) intermittent periods of generalized suppression without associated bursts [78, 79].

Periods of chronic hypoxia predispose individuals to the development of dementias, particularly AD. It has been shown that hypoxia, even *in vitro*, can increase production of Abeta in different cell types (for a review see [80]). Evidence has been produced to indicate hypoxia alters both expression of the Abeta precursor, Amyloid precursor protein (APP), and also the expression of the secretase enzymes, which cleave Abeta from APP. Other studies implicate reduced Abeta degradation as a possible means by which hypoxia increases Abeta levels; such variability may be attributable to cell-specific responses to hypoxia [80].

An intriguing working hypothesis is that the effects of acute hypoxia on resting state EEG rhythms may represent a useful challenge model for AD research. Acute hypoxia has been implicated in causing the high levels of morbidity and mortality in patients hospitalized in Intensive Care Units (ICU) [81]. There is recent evidence that EEG measures can assist ICU clinicians in assessing cerebral hypoxia severity of patients hospitalized for acute respiratory failure and further may inform as to a patients' optimal time-point for disconnection from a respirator [82]. In patients suffering from acute cerebral ischemia, sensitive EEG parameters are frequency, amplitude and reactivity to eye opening [83]. The reduction of cerebral blood flow caused by acute cerebral ischemia has been suggested to be related to the slowing of the resting state EEG rhythms [84]. Decreased resting state alpha rhythms and increased slow-wave EEG rhythms have also been observed in patients with acute cerebral ischemia caused by a reduction in cortical blood flow [85-88].

Hypoxic hypoxia is one of the main three types of hypoxia (hypoxic, anemic, and ischemic hypoxia) during which the oxygen supply to the blood is insufficient [89-91], and seems to be promising as a challenge model for AD research. The simulation of hypoxic hypoxia can be performed in two ways, either by decreasing the oxygen concentration of the inspired gas mixtures or by decreasing the barometric pressure (hypobaric hypoxia). Hypobaric hypoxia has been recognized as a safe experimental model for the study of hypoxic conditions. It is also advantageous as it allows for the precise standardization of the hypoxic exposure [89, 90]. A slowing activity of EEG has been generally observed in many studies of hypoxic hypoxia induced by low oxygen gas mixtures [92-95] in simulated high altitude chambers [89, 90, 96, 97] and during high mountain climbing [98-100]. A quantitative evaluation of standard EEG frequency parameters during a hypobaric hypoxia experiment in healthy volunteers has been performed. The EEG change in hypobaric hypoxia was found to be similar to that observed in ischemic hypoxia [89, 101-103]. However, due to the difficulty of obtaining EEG records in the restricted experimental environment of the low pressure chambers or on mountains, only a limited number of EEG channels were examined in the early hypobaric hypoxia experiments [90, 104]. It has been reported that the spectral power density of a single channel EEG (locations P4 vs. O2) in the alpha frequency band is significantly decreased under hypobaric hypoxia induced by reduced air pressure corresponding to an altitude of 6096 m [90]. Recent progress in the processing of multichannel EEG data has allowed the quantification of the topography of the EEG rhythms under experimental conditions such as hyperbaric conditions [97, 105-107]. EEG topographical changes due to 6000 m (19,685 ft) altitude exposure have been reported by Saletu *et al*. [106] in a multichannel EEG experiment in which an increase in resting state delta and theta rhythms and a decrease of alpha rhythms were described. These studies were extended by Ozaki *et al.* [97] who estimated the topography of resting state EEG rhythms at different levels of hypobaric hypoxia induced under simulated high altitude conditions. The EEG rhythms were also analyzed after return from hypobaric to normobaric condition thus examining the recovery of the central nervous system from hypobaric hypoxia [97]. Global spectral power (16 electrodes) of the resting state alpha rhythms (10-11 Hz) was significantly decreased and, with increasing altitude, significant decrease of spectral power was observed in a wider range of the alpha frequency band [97]. In the 6000 m condition, the decrease of spectral power of the alpha rhythms in the posterior brain areas was dramatic when compared to baseline measurements. In contrast, the spectral power of the resting state theta rhythms in anterior brain areas increased significantly in the 5000 m and 6000 m conditions. After return from the 5000 m condition (without exposure to the 6000 m condition), the resting state EEG rhythms showed recovery to the level of baseline condition. However, in subjects who returned to sea level condition after exposure to the 6000 m condition, both the resting state theta and alpha rhythms were significantly suppressed. On the whole, these results suggest that the first stage of hypobaric hypoxia is characterized by selective suppression of resting state alpha EEG rhythms. Further elevation in altitudes of over 5000 m results in significant enhancement of resting state theta rhythms in the anterior brain areas and strong suppression of resting state alpha rhythms in posterior areas.

Of extreme interest are the challenge hypoxia models based on carbogen inhalation with 7% CO_2. A functional MRI study with a CO_2 challenge showed impaired cerebral vasoreactivity in MCI and AD patients at the individual level [108]. Furthermore, an analogous procedure induced a reduction of cerebral blood flow velocities and increased pulsatility with a significant vasoreactivity reduction in patients with cerebrovascular dementia and AD, as indicators of impairment of cerebral microvasculature circulation in both diseases [109]. These findings support the use of resting state EEG markers with CO_2 hypoxia challenge models to better characterize patients with cognitive disorders in the clinic. In this regard, our group evaluated the effects of hypoxia on resting state EEG rhythms in amnesic MCI subjects to probe cerebral vasomotor reactivity. The preliminary results show that with respect to the baseline, hypoxia (inhalation of air with 7% of CO_2 induced an enhanced frontal oxygen percentage and a reduction of wide alpha source power in both MCI and normal elderly subjects; the higher the enhancement of the percentage of frontal oxygen, the higher the reduction of frontal alpha source power across all subjects. In this condition, the reduction of posterior alpha source power was lower in the MCI than Normal subjects. Finally, after CO_2 infusion, there was a recovery of alpha source power

which was lower in the MCI than normal elderly subjects. These preliminary results suggest that the decline of resting alpha rhythms in amnesic MCI subjects might be associated with an impairment of vasomotor reactivity and neurovascular coupling, which is especially reflected in an impaired recovery period.

Few studies have shown that hypobaric hypoxia induces a variation of ERP peaks and slow contingent negative variation (CNV) developing before a warned imperative stimulus during discrimination tasks [110, 111]. During hypobaric hypoxia at 6000 m (i.e. hypobaric hypoxic conditions at altitudes of 0-6000 m were simulated in an experimental decompression chamber), CNV decreased significantly compared with that at 0 m. Early CNV following the presentation of warning stimuli also showed a significant decrease, but there was no significant change in the late CNV immediately preceding the imperative stimuli [110]. Hypobaric hypoxia at 4572 m also induced a remarkable modulation in amplitude of CNV in relation to changes in reaction time [110].

(Table **1**) provides an overview of the main bibliographic evidence on the effects of experimental hypoxia on the resting state eyes closed EEG rhythms recorded in healthy volunteers.

4. EFFECTS OF SLEEP DEPRIVATION ON EEG ACTIVITY

Sleep deprivation (SD) or restriction is a common phenomenon in our society [112-114]. Laboratory studies have reported increased daytime sleepiness and prolonged sleeping times during the weekends [113, 115-117] even when the normal sleep duration during weekdays is 7–8 h. These results are supported by a study that examined 1010 adults in the United States and reported average sleep durations of 6.9 h during weekdays compared to 7.5 h during weekends. Fifteen percent of the respondents slept even less than 6 h during weekdays [118]. These moderate sleep restrictions may lead to impairments in psychomotor vigilance tests [17, 119, 120]. Although this issue is beyond the limit of the present review, it is worth mentioning that only the first 5–6 h of sleep are important and that people can adapt to shortened sleep times without experiencing major daytime fatigue or other deficits [121].

SD impairs performance on a number of verbal and non-verbal memory tasks [8-10] and produces deleterious effects on a variety of cognitive and attentional abilities [12, 13, 16-25]. The broad cognitive impairment induced by SD has lead to further study of the neurophysiological basis of these deficits as assessed by EEG activity.

Total SD enhanced the frontal predominance of delta rhythms (2-4 Hz) only in the left hemisphere [122, 123]. These effects may be related to functional asymmetry between the dominant and non-dominant hemisphere, and provide further evidence for the existence of local aspects of sleep regulation [122, 123]. Furthermore, both delta and theta rhythms (4-7 Hz) increased during total SD [124-126]; theta rhythms were found to correlate to subjective sleepiness [124] and subjective fatigue [125]. A high correlation between theta rhythms and subjective sleepiness was observed during a mental task during nighttime train driving [127]. The alpha rhythms (8-12 Hz) during total SD showed different responses between resting state eyes-open and -closed conditions. Under eye-open conditions, alpha rhythms increased with time throughout SD [125, 127-129]. In contrast, resting state eyes-closed alpha rhythms decreased with the increased sleepiness level following nocturnal SD [128]. Extended SD for 40 hours induced an increase in the modulation of theta and alpha rhythms during mental tasks [128]. During total SD, exposure to the bright light (more than 2500 lux) under a rest condition was enough to delay the increase in theta and alpha rhythms and subjective sleepiness [130, 131].

Repeated partial SD has been reported to induce long-lasting changes in the delta, theta, and alpha rhythms during the REM sleep periods of 3 consecutive recovery nights [132, 133]. The authors attributed these EEG changes to the accumulating and dissipating REM sleep deficit; hypothesizing that they may reflect the intensity dimension of a REM sleep-dependent process [133].

Several studies have also shown that the selective EEG slow-wave sleep (SWS) deprivation affects the EEG rhythms [126, 134-137]. During the SWS deprivation nighttime epochs, large decreases of EEG power were found at frontopolar, central and parietal derivations encompassing delta, theta and alpha rhythms, while only slow delta rhythms (0.5-2 Hz) were affected at the frontal derivation [136]. Recovery sleep was characterized by a generalized increase of power during non-REM sleep encompassing delta, theta and alpha rhythms, with a clear anteroposterior gradient. The coherent behaviour of different EEG bands with traditionally different electrophysiological interpretations during non-REM sleep suggests that a re-examination of the functional role of EEG rhythms during sleep is needed. The resistance to selective EEG SWS deprivation of the frontal area, together with its larger increase of EEG power during recovery, may be interpreted as a sign of a greater sleep need of frontal cortical areas compared to other cortical regions. This would confirm that some aspects of the regulatory processes of human sleep are local in nature and endowed with use-dependent characteristics [136]. The EEG SWS deprivation was slightly more effective in the right than the left hemisphere, but the left hemisphere showed a markedly larger increase of EEG power in the 1–25 Hz range during recovery-night non-REM sleep, combined with a larger increase of EEG power during both deprivation-night and recovery-night REM sleep [137]. These results support the greater need for sleep restorative processes of the left hemisphere, which may suggest that local sleep regulation processes may also act during REM sleep [137]. In addition, EEG SWS deprivation advances the shift to an anterior-to-posterior directionality of functional cortical coupling, possibly as a consequence of heightened sleep pressure [138]. These findings support the notion that a spread of synchronizing signals from associative prefrontal to posterior areas plays a role in wake-sleep transition [138].

Finally, a typical example of hypoxia is obstructive sleep apnea (OSA), a sleep-related breathing disorder characterized by repetitive episodes of apnoea–hypopnoea due to partial or complete upper airway obstruction during sleep. These episodes are usually accompanied by blood gas abnormalities (hypoxemia and hypercapnia) and sleep fragmentation.

Table 1. Overview of the main bibliographic evidence on the effects of experimental hypoxia on the resting state electroencephalographic (EEG) rhythms in healthy volunteers.

EEG Marker	Group	Condition	Main Results	Reference
Spectral power density	N=24	Hypoxic/ Ischemic hypoxia (standardized hyperventilation)	Amplitude of delta power increased and that of alpha and beta power decreased in relation to a reduced blood flow velocity (40%).	Kraaier *et al.*, 1988
Spectral power density	N=36	Hypoxic and hypobaric hypoxia	Amplitude of delta power increased and that of alpha decreased.	Kraaier *et al.*, 1988
LORETA	N=10	Ischemic hypoxia	Compared to healthy subjects, both AD and congestive heart failure (CHF) patients presented higher delta and lower alpha temporal sources	Vecchio *et al.*, 2011
Spectral power density, coherence, phase shift		Hypoxic hypoxia (gas mixture with 8 % of oxygen)	No result on spectral power density. Delta and alpha coherence decreases; beta coherence increases; phase-shift increases in delta-and theta- and decreases in beta.	Burykh, 2005
Spectral power density	N=14	Hypoxic hypoxia (low oxygen gas mixtures)	Low oxygen gas mixtures induced a slowing of the EEG activity.	Schellart and Reits, 2001
Spectral power density	N=18	Hypobaric hypoxia	Alpha decreased in the posterior brain areas and theta increased in anterior brain areas.	Ozaki *et al.*, 1995
Spectral power density, visual evoked responses (VER)	N=7	Hypoxic hypoxia	In three subjects, EEG frequency was increased, amplitude decreased, and/or spiking became evident. In four subjects, VER amplitude was reduced.	Forster *et al.*, 1975
Spectral power density	N=10	Ischemic hypoxia.	Spectral analysis of the EEG recorded under hypoxia demonstrated neurophysiological alterations indicative of a deterioration in vigilance.	Saletu *et al.*, 1984
Spectral power density	N=16	Hypoxic hypoxia	Delta/theta increased in parietal, temporal and central regions while there was a widespread decrease of alpha activity.	Saletu *et al.*, 1990
Spectral power density	N=10	Hypoxic hypoxia.	Power of theta and alpha bands increased during hypoxia.	Papadelis *et al.*, 2007
Contingent negative variation (CNV)		Hypobaric hypoxia	The complete CNV decreased significantly; early CNV showed a significant decrease; there was no significant change in late CNV.	Takagi and Watanabe, 1999
Event related potentials (ERPs)	N=10	Hypobaric hypoxia	Attenuation of P1 and enhancement of N1-P3 amplitudes; delay of P2 latency for targets and nontargets; delay of P3 latency for nontargets.	Tsarouchas *et al.*, 2008

OSA syndrome is characterized by hypoxia during sleep and/or sleep fragmentation. The number and duration of apnoea–hypopnoea events define the extent of hypoxia during sleep in OSA patients. OSA is common in patients suffering from AD with prevalence rates reported to be greater than 40% in those who are institutionalized [139]. OSA affects cognitive function and EEG activity. Untreated OSA itself can have deleterious effects on cognition and daytime functioning [140-142], and may exacerbate the primary cognitive and functional deficits associated with AD [143-145]. The most effective treatment of OSA is continuous positive airway pressure (CPAP). In a 6-week randomized placebo controlled clinical trial of CPAP in patients with mild-moderate AD and OSA, CPAP improved OSA, objective sleep parameters, and daytime sleepiness and resulted in modest improvements in measures of cognitive functioning [146-149]. Finally, previous EEG studies have shown that compared to healthy subjects, OSA patients reveal an increased resting-state delta and theta power as well as an increased ratio of delta/theta to alpha/beta power during rapid eye-movement sleep and wakefulness [150-152].

(Table **2**) provides an overview of the main bibliographic evidence on the effects of SD on the on-going EEG rhythms measured in healthy volunteers.

5. EFFECTS OF TMS ON EEG ACTIVITY

5.1. TMS As a Tool

Since its introduction in 1985 [26], the scientific applications of TMS have rapidly expanded. TMS has become a

Table 2. Overview of the main bibliographic evidence on the effects of sleep deprivation (SD) on the on-going EEG rhythms in healthy volunteers. Of note, the heterogeneity of the EEG markers (i.e. spectral power, LORETA, spectral coherence in different frequency bands as well as phase shift, visual evoked responses, contingent negative variation, event related potentials) reported in the cited studies did not make possible to perform the meta-analyses.

EEG Marker	Group	Condition	Main Results	Reference
Spectral power density	N=8	Total sleep deprivation	Sleep deprivation enhanced the anterior predominance of delta activity in the left hemisphere but not in the right one.	Achermann at al., 2001
Spectral power density	N=8	Total sleep deprivation	Sleep deprivation enhanced the frontal predominance of delta rhythms in the left hemisphere.	Kattler *et al.*, 1994
Spectral power density	N=14	Total sleep deprivation	Sleep deprivation enhanced theta rhythms; these rhythms were found to correlate to subjective sleepiness.	Dumont *et al.*, 1999
Spectral power density	N=9	Total sleep deprivation	Sleep deprivation enhanced theta/ alpha power density and was correlated to subjective fatigue.	Cajochen *et al.*, 1995
Spectral power density	N=11	Total sleep deprivation	Spectral power density in the theta, delta and alpha bands increased during the nighttime train driving. A very high correlations was found between sleepiness and alpha and theta.	Torsvall and Åkerstedt, 1987
Spectral power density	N=9	Total sleep deprivation	Sleep deprivation enhanced theta/ alpha rhythms during a visual vigilance task.	Corsi-Cabrera *et al.*, 1996
Spectral power density	N=8	Total sleep deprivation	Alpha rhythms decreased with the increased sleepiness level following nocturnal sleep deprivation.	Daurat *et al.*, 1996
Spectral power density		Partial sleep deprivation.	Repeated partial sleep deprivation induced long-lasting changes in the delta, theta rhythms during the REM sleep periods.	Brunner *et al.*, 1990
Spectral power density	N=9	Partial sleep deprivation.	Repeated partial sleep deprivation induced long-lasting changes in the delta, theta, and alpha rhythms during the REM sleep periods of 3 consecutive recovery nights	Brunner *et al.*, 1993
EEG slow-wave sleep (SWS)	N=10	Total sleep deprivation	An almost complete selective SWS suppression during both deprivation nights was achieved. A significant increase of S4 and SWS in the REC as compared to the BSL-A paralleled a significant shortening of S3 and S4 latencies. S2 percentage significantly increased during both DEP nights with respect to the other experimental nights. There was no significant difference among nights with regard to total sleep time, percentage of REM sleep, stage 1, movement time, number of awakenings and number of movement arousals	Ferrara *et al.*, 1999
EEG slow-wave sleep (SWS)	N=10	Total sleep deprivation	There was an almost complete selective SWS suppression during the deprivation nights, and a significant SWS rebound during the recovery sleep.	Ferrara *et al.*, 2000
EEG slow-wave sleep (SWS)	N=10	Total sleep deprivation	During selective SWS deprivation nights, there was a significant reductions of sleep EEG power in the delta–theta–alpha frequency range at frontopolar, central and parietal derivations.	Ferrara *et al.*, 2002

(Table 2) contd….

EEG Marker	Group	Condition	Main Results	Reference
EEG slow-wave sleep (SWS)	N=10	Total sleep deprivation	During the SWS deprivation, there was a markedly larger increase of EEG power during recovery-night non-REM sleep, and a larger increase of EEG power during both deprivation-night and recovery-night REM sleep.	Ferrara *et al.*, 2002
Directed transfer function (DTF); slow-wave sleep (SWS)	N=10	Total sleep deprivation	SWS deprivation advanced the shift to an anterior-to-posterior directionality of functional cortical coupling.	De Gennaro *et al.*, 2005
Spectral power density	N=9	Total sleep deprivation	Performance recovery was predicted by increased delta power and decreased sigma power in recovery sleep compared to normal sleep.	Mander *et al.*, 2010

valuable tool to probe the excitability of intracortical circuits in the motor, sensory and visual cortices [153]. As described above, single pulses or short bursts of TMS can effectively perturb ongoing neuronal processing in the stimulated cortex even beyond the time of stimulation [154, 155]. These neuromodulatory effects of TMS have been exploited in many *in vivo* studies on cortical plasticity in humans [156-160], and may be of some use in patients with neurological and psychiatric diseases to maintain or restore brain function [161]. Furthermore, rTMS has added a new dimension to human brain mapping and has provided unique opportunities to probe causality at a systems level of sensory, cognitive and motor brain networks [162]. For instance, perturbative effects of rTMS can be used to make causal inferences regarding the functional contribution of the stimulated cortex to a specific brain function [28, 31, 160, 163]. Of note, the effects of rTMS are not limited to the stimulated cortex but focal rTMS gives rise to functional changes in interconnected cortical areas [160]. Studies combining TMS with functional neuroimaging techniques in humans revealed that these effects occur both in the main cortical stimulated region as well as, due to trans-synaptic effects, in other distant areas [164]. TMS effects can be either facilitatory or disruptive depending on the time point of stimulation [165]. Several studies have found behavioral facilitations when single-pulse TMS is applied shortly before the onset of a cognitive process [156, 166]. In contrast, the better-known "virtual lesions" or disruptions in perception and behavior are generally obtained when TMS is applied during the perceptual/cognitive process [167, 168].

TMS has been shown to impact episodic memory, which is the most vulnerable cognitive domain in AD and amnesic MCI. Although mnemonic processes are crucially related to the integrity of medial temporal lobe structures, other brain areas including the dorsolateral prefrontal cortex also have a relevant role both in encoding and retrieval mechanisms of long-medium term episodic memory as revealed by fMRI and PET [169-172]. Noteworthy, several studies have pointed to a prevalence of activity in the left prefrontal cortex during the encoding of verbal and spatial materials, while activity was enhanced in the right prefrontal cortex during the retrieval of that information content [173-175]. It has been shown that rTMS applied over the left dorsolateral prefrontal cortex results in distal changes of neural activity, relative to the site of stimulation, and that these changes depend on the patterns of brain network activity during resting-state [176]. Furthermore, rTMS of dorsolateral prefrontal cortex, but not posterior parietal cortex, can transiently interfere with the encoding and consecutive retrieval of visuospatial episodes [163, 177]. In separate experiments, the same visuospatial episodes have induced increased fronto-parietal EEG gamma (around 40 Hz) power and functional coupling [47, 178].

5.2. TMS Interferes with the Generation of EEG Activity

To better understand the effects of TMS on cortical activity several studies have recorded EEG and TMS simultaneously, i.e. the TMS-EEG approach, which allows several lines of empirical observations. First, EEG activity can be compared before and after TMS over a cortical region to understand how spontaneous EEG activity and causal modulation of that activity affect sensory and cognitive processes. For example TMS over occipital cortex evoked phosphenes as a function of alpha activity before TMS [179]. Second, the comparison of EEG activity before and after TMS has revealed changes in the EEG power spectrum. A single TMS pulse has been reported to transiently synchronized activity in the beta (14-30 Hz) range [180]. Furthermore, rTMS trains of 1 Hz [181] and 5 Hz rTMS [182] were associated with concurrent changes in cortical alpha (about 8-12 Hz) and beta activity. Third, the TMS-EEG approach allows the study of functional connectivity between cortical areas probing the effect of TMS over one cortical site on the ERPs evoked in another area. A single TMS-pulse evokes in the EEG a cortical potential which strongly differs in polarity and amplitude of its peak components. This is dependent on several factors including position and orientation of the TMS coil, stimulation intensity, and electrode position. However, supra-threshold stimulation (biphasic pulse configuration) of the cortical motor hand area reliably evokes a response at the vertex with the following components: N10, P14, N15/18, P30, N40/45, P55/60, N100, P180/190 and N280 [180, 183]. As an alternative to peak analysis, especially for hd-EEG recordings, the calculation of global mean field power (GMFP) has been introduced as a reference-free measure of

local EEG variability [184]. As the number of neurons recruited by a single TMS-pulse is directly related to their excitability, GMFP amplitude change has been proposed as a measure of cortical excitability, which is sensitive to TMS-induced changes in cortical plasticity [185-187]. Along this line, temporospatial propagation of TMS-evoked cortical activity can be traced to gain insight into the temporospatial dynamics of corticocortical connectivity patterns activated by TMS [188, 189]. During a typical TMS-EEG session, several measurements can be obtained: (i) Strength of its immediate response in the cortical target area of interest [190], (ii) Temporospatial dynamics of the ensuing spread of activation [191, 192], (iii) Corticocortical conduction times [193, 194], and (iv) Complex dynamics such as phase locking or power modulation of EEG rhythms [180-182, 195].

Fourth, the TMS-EEG approach provides an important opportunity to associate causal changes in EEG activity with performance in cognitive tasks. These data complement previous fMRI studies showing that dorsal brain networks, including right intra parietal sulcus (IPS), subserve the top-down control of parieto-occipital activity underlying visuospatial attention processes [196, 197]. Consistent with this, recent evidence of combined TMS-functional MRI approach demonstrated that attention-related activation of visual cortex was affected when TMS is applied over right IPS [198, 199]. Furthermore, right IPS-rTMS interference on presentation of a visual cue stimulus abolished typical preponderant pre-target activation of parieto-occipital areas (i.e. power reduction of alpha rhythms at about 8-12 Hz), especially in the hemisphere contralateral to the hemifield where the visual target was attended [200]. Moreover, such right side IPS-rTMS interference impaired target identification even when the stimuli were presented at unattended locations. This was despite the finding that the amplitude of earlier components (i.e. P1/N1) of parieto-occipital visual evoked potentials was unaffected [200, 201]. To explain this effect, it can be hypothesized that the IPS-rTMS interference on presentation of the visual cue stimulus affected later cortical activity and attention processes, possibly reflected by subsequent ERP components. In this line, the IPS-rTMS interference on presentation of the visual cue stimulus affected late positive event-related potentials following the invalid (rare) target stimuli spatially incongruent to the cue stimuli (i.e. P3) [202]. Furthermore, the application of rTMS during a visual search task disrupted an ERP component following P1/ N1, i.e. N2pc, which is considered a marker for a shift of attention during visual scanning [203].

It is important to note that it has been shown the frontal areas are important in controlling visual processing in posterior visual brain areas during the orienting of spatial attention [204]. Furthermore, the dorsal medial frontal cortex influences lateralized ERPs in primary motor cortices during conflict resolution in an action selection task [205].

(Table **3**) provides an overview of the main bibliographic evidence on the effects of TMS on the EEG activity in healthy volunteers.

6. INNOVATIVE CHALLENGE MODELS FOR DRUG DISCOVERY IN AD

The data reviewed above show that SD, transient hypoxia, and rTMS can alter cognitive processes and EEG rhythms in humans. The PharmaCog project aims to identify the most sensitive of these models to induce transient cognitive deficits when applied in healthy volunteers and to reverse them by symptomatic drugs against AD such as Achetylcholinesterase inhibitors (e.g. donepezil) or NMDA receptor antagonists (e.g. memantine).

In the PharmaCog project, the utility of SD as a suitable challenge in human healthy volunteers will be tested as follows. Healthy volunteers will be instrumented for EEG recordings. EEG activity will be recorded during normal sleep for one night as a baseline control condition and, then, during sleep deprivation experiment in the subsequent night. During the daytime following both the control and the SD night, EEG recordings will be performed in resting state eyes-open and -closed conditions as well as during a standard auditory oddball P300 paradigm. It is hypothesised that the effects of SD on cognition and EEG markers may be attenuated by chronic administration (i.e. 14 days) of donepezil and memantine in a double blind and placebo controlled trial.

With respect to hypoxia, the PharmaCog project plans the following challenge in human healthy volunteers: various gas mixtures will be delivered through a mask to healthy volunteers in a preliminary experiment. Gas mixtures will be realized extemporaneously using a servo ventilator (Siemens SV900), while vital signs, pulse oxymetry, O2, CO2, and N2O will be controlled. The psychometric evaluation will comprise a battery of vigilance and cognition tasks whilst the EEG measurements will include resting state, eyes-open and -closed conditions, as well as a standard auditory oddball P300 paradigm. This step will allow the selection of the most appropriate procedure to be used in the subsequent pharmacological studies. Cognitive and EEG assessments will be taken before (baseline), during and after hypoxia challenge. Once a robust challenge has been established, cognitive performance, mood, vigilance and EEG markers will be evaluated in double blind and placebo controlled trials of the reference drugs (donepezil and memantine).

Finally, rTMS will be evaluated as a challenge model in human healthy volunteers as follow: rTMS will be used to induce transient episodic memory impairments by stimulating the dorsolateral prefrontal cortex (DLPFC) during a cognitive task in healthy volunteers. The cognitive test is a visual episodic encoding memory task, in which 16 complex colored magazine pictures (representing indoor and outdoor scenes) are pesented. Real and sham rTMS high frequency trains (20Hz, 500 msec) with relatively low intensity (10% of subthreshold) will be delivered to the DLPFC during the performance of the memory task. In several studies, it has been demonstrated that administration of rTMS robustly impairs this kind of episodic memory (as observed by a higher proportion of incorrect vs correct responses and/or the reaction time latencies during the recognition memory test). it has been reported that, left DLPFC stimulation impairs memory encoding, whereas right DLPFC impairs memory retrieval in young healthy individuals [206]. In the elderly healthy subjects, both kinds of stimulation impair episodic memory processes consistently [207]. EEG measurements (resting state, eyes open and eyes closed conditions, standard auditory oddball P300 paradigm) will be recorded at baseline

Table 3. Overview of the main bibliographic evidence on the effects of transcranial magnetic stimulation (TMS) on the EEG activity in healthy volunteers.

EEG Marker	Group	Condition	Main Results	Reference
Temporal spectral evolution (TSE) and ERD/ERS	N=15	TMS to induce illusory visual percepts (phosphenes) in blindfolded participants	TMS over occipital cortex evoked phosphenes as a function of alpha activity before TMS	Romei eta al., 2008
Spectral power density and ERP	N=7	TMS was applied over the left primary motor cortex (M1)	A single TMS pulse induced a brief period of synchronized activity in the beta range in the vicinity of the stimulation site. TMS pulses evoked a waveform consisting of a positive peak (P30), followed by two	Paus *et al.*, 2001
ERD/ERS	N=6	Low frequency (1 Hz) TMS on left primary motor cortex (MI)	TMS induced a synchronization of the alpha, that increased with the duration of the stimulation, and this increase was inversely correlated with motor-evoked potentials (MEPs) amplitude	Brignani *et al.*, 2008
ERPow and ERCoh	N=11	High-frequency (5-Hz) rTMS on left primary motor cortex (MI)	rTMS induced an ERPow increase, for the alpha, during the trains of stimulation, mainly in frontal and central regions ipsilateral to stimulation, and a similar synchronization of cortical oscillations for both rTMS intensities, for the beta. Moreover, rTMS induced a specific ERCoh decrease over the posterior regions during the trains of stimulation for alpha and beta	Fuggetta *et al.*, 2008
Global mean field power (GMFP) and SWA	N=10	High-frequency (5-Hz) TMS on motor cortex	TMS induced a localized potentiation of TMS-evoked cortical EEG responses. The change in amplitude of GMFP between 100 and 140 ms was the best predictor of the local increase of sleep slow wave activity (SWA), in the sleep episode following TMS.	Huber *et al.*, 2007
GMFP and SWA	N=19	High-frequency (5-Hz) TMS on contralateral cortical hand area (PAS protocol)	TMS produced large deflections in scalp voltage. Changes in TMS-evoked cortical EEG response and change in sleep slow wave activity (SWA) were localized to similar cortical regions and were positively correlated. These results suggest that changes in cortical excitability in opposite directions lead to corresponding changes in local sleep regulation, as reflected by SWA, providing evidence for a tight relationship between cortical plasticity and sleep intensity. GMFP contained distinct peaks, which had similar latencies when compared between the pre and post TMS test phases	Huber *et al.*, 2008
GMFP	N=7	High-frequency (5-Hz) rTMS on hand area of motor cortex	rTMS produced EEG responses significantly potentiated (expecially at EEG electrodes located bilaterally over premotor cortex).GMFP contained distinct peaks with similar latencies when compared between the pre and post rTMS test phases. The strongest activation was initially produced in sensorimotor cortex, and during later peaks was generated in either sensorimotor cortex or premotor cortex	Esser *et al.*, 2006

(Table 3) contd….

EEG Marker	Group	Condition	Main Results	Reference
SWA	N=15	Low frequency (<1 Hz) TMS	TMS enhanced sleep SWA, locally and globally.TMS triggering of slow waves reveals intrinsic bistability in thalamocortical networks during non-rapid eye movement sleep. Moreover, evoked slow waves lead to a deepening of sleep and to an increase in EEG slow-wave activity	Massimini *et al.*, 2007
GMFP	N=7	TMS on left and right primary motor cortex	TMS produced a GMFP featured by five deflections peaking at 17, 30, 54,104, and 187 ms (average values)	Komssi *et al.*, 2007
ERP	N=4	Low frequency (<1 Hz) TMS on visual and motor cortex	TMS produced the strongest EEG activity at the site of the strongest induced current flow. In all subjects and for both motor and visual cortex stimulation, neuronal activation during the first 5 ms post-stimulus produced a current flow in the anterior–posterior direction; at 6–7 ms, the direction of the current was reversed	Ilmoniemi *et al.*, 1997
ERP	N=6	TMS	During quiet wakefulness, an initial response at the stimulation site was followed by a sequence of waves that moved to connected cortical areas several centimeters away. During non-REW sleep, the initial response was stronger but was rapidly extinguished and did not propagate beyond the stimulation site	Massimini *et al.*, 2005
MEP	N=10+14	Low (<1 Hz) and high-frequency (5-Hz) TMS	High-frequency TMS (n=10) produced an immediate decrease in MEPs, 1 min after the exercise, followed by a gradual increase to pre-exercise values. Low-frequency TMS (n=14; same task) produced a slight no significant decrease in MEPs, up to 15 min post-exercise	Bonato *et al.*, 2002
Spectral power density and cortical current-density distributions	N=6	TMS on left sensorimotor cortex	The activation had spread from below the coil center to the surrounding frontal and parietal cortical areas, having a very lateral maximum.The current-density distributions suggested activation of premotor and posterior parietal cortex, and the temporo-parietal junction.	Komssi *et al.*, 2002
ERPow and ERCoh	N=10	TMS on left primary motor cortex	TMS modulated cortical oscillations within the first half second for both alpha and beta, and enhanced the electrode connectivity of both hemispheres. ERPow reflected regional oscillatory activity of neural assemblies, while ERCoh reflected the inter-regional functional coupling of oscillatory neural activity.	Fuggetta *et al.*, 2005
ERD/ERS	N=33	High-frequency (20-Hz) rTMS	Right IPS-rTMS interference on presentation of a visual cue stimulus abolished typical preponderant pre-target activation of parieto-occipital areas (i.e. power reduction of alpha rhythms), especially in the hemisphere contralateral to the hemifield where the visual target was attended	Capotosto *et al.*, 2009

(Table 3) contd….

EEG Marker	Group	Condition	Main Results	Reference
ERD/ERS	N=15	High-frequency (20-Hz) rTMS	Right and left IPS-rTMS interference disrupted the normally lateralized anticipatory modulation of occipital visual cortex, with stronger alpha desynchronization contralaterally to the attended visual field. In contrast, only interference with right IPS induced a paradoxical pretarget synchronization of alpha rhythms and bilateral deficits of target identification.	Capotosto *et al.*, 2010
ERD/ERS	N=24	High-frequency (20-Hz) rTMS	Right IPS-rTMS impaired target detection, especially for stimuli presented at unattended locations; it also caused a modulation of the amplitude of parieto-occipital positive ERPs peaking at about 480 msec (P3) post-target. The P3 significantly decreased for unattended targets and significantly increased for attended targets after right IPS-rTMS as compared with sham stimulation. Similar effects were obtained for left IPS stimulation albeit in a smaller group of subjects.	Capotosto *et al.*, 2012
ERP	N=7	TMS on vertex (Cz) and right posterior parietal cortex (rPPC)	Single-pulse TMS over rPPC delayed response times to targets during conjunction search, and this behavioral effect had a direct ERP correlate. The early phase of the N2pc component was eliminated over the right hemisphere when TMS was applied there but was present when TMS was delivered to a control site (vertex)	Fuggetta *et al.*, 2006
ERP	N=18	High-frequency (10-Hz) rTMS on the right frontal eye field (FEF)	TMS affected the neural activity evoked by visual stimuli, as well as the ongoing neural activity recorded during earlier anticipation of the visual stimuli	Taylor *et al.*, 2007
ERP	N=16	High-frequency (10-Hz) rTMS on left dorsal medial frontal cortex (dMFC)	dMFC influences lateralized ERPs in primary motor cortices during conflict resolution in an action selection task	Taylor *et al.*, 2007

and during and after the rTMS challenge. Once a robust protocol has been established, cognitive performance, mood and vigilance will be evaluated in a double blind, placebo controlled trials using the reference drugs (donepezil and memantine).

The ideal healthy volunteer model will be a) reversible with clinically active treatments, b) applicable for a broad range of therapeutic targets, and c) predictive of clinical outcome. The PharmaCog project aims to develop and validate a healthy volunteer model which can predict the beneficial effects of new treatments in a relatively short time frame and in controllable conditions, providing the opportunity to identify the most promising new therapeutic agents.

7. CONCLUDING REMARKS

The use of cognitive 'challenge' models such as SD, transient hypoxia, and TMS in healthy volunteers, as well as the validation of non-invasive, translational, and low-cost neural correlates of these challenges such as resting state EEG rhythms constitute a promising approach to evaluate effects of pharmacological treatment on biomarkers as instrumental secondary end points in drug discovery. Indeed, it has previously been shown that the resting state EEG rhythms in amnesic MCI and AD subjects are characterized by an increase in delta rhythms concomitant with a decrease of alpha rhythms in occipital, parietal, and temporal areas. Furthermore, these features were related to global cognition and some clear signs of neurodegeneration. In this article, we have reported relevant literature on the effects of hypoxia, SD, and TMS on these EEG rhythms and cognitive performance in humans, in order to form a knowledge platform for novel research for drug discovery in AD (see Tables **1**, **2**, and **3** for a summary and bibliographic references). It has been shown that sleep pressure enhanced frontal delta rhythms (< 4 Hz) during the night, while SD increased slow rhythms in the theta range (4-7 Hz), and reduced resting state alpha rhythms (8-12 Hz) during the day after. Furthermore, SD transiently affected cognitive performance. In contrast, transient experimental hypoxia induced abnormal posterior resting state delta and alpha rhythms in healthy volunteers that resemble the abnormal EEG rhythms typically recorded

in AD patients (this suggests that EEG abnormalities observed in amnesic MCI and AD subjects might be associated with an impairment of vasomotor reactivity and neurovascular coupling that could be, at least in part, due to chronic and/or acute hypoxia effects). However, the relationship between the cognitive and EEG effects of such challenge is poorly understood. In the same line, the EEG literature on TMS reports that the combined TMS-EEG approach has the potential to investigate neurophysiologic mechanisms at the basis of functional cortical connectivity and cognitive functions in humans. Among them, causal interference with TMS over fronto-parietal attention networks induced changes in posterior alpha rhythms and attention. However, few TMS-EEG studies investigated the effects of this challenge on episodic memory or executive functions (i.e. the cognitive benchmark of AD) and EEG rhythms. On the whole, the findings reviewed above encourage the use of the SD challenge model and the resting state EEG rhythms in healthy volunteers to assess the potential of novel symptomatic drugs to provide early clinical proof of concept in AD drug discovery. Specifically, the most interesting EEG markers for translational purposes may be delta (<4 Hz) and low-frequency (8-10 Hz) rhythms recorded in the posterior scalp regions. Furthermore, the findings reviewed above encourage future investigations to better understand the opportunities offered by TMS and hypoxia challenges.

Keeping in mind the above data and considerations, we propose a speculative physiological model predicting the effects of hypoxia, SD, and TMS challenges on resting state EEG rhythms. In the resting state (eyes closed) condition, normal brain typically shows dominant alpha rhythms (8–12 Hz) in the posterior cortical regions, which would denote the back-ground, spontaneous synchronization around 10 Hz of neural networks regulating the fluctuation of subject's global arousal and consciousness states. These networks would span neural populations of cerebral cortex, thalamus, basal forebrain and brainstem, including glutamatergic, cholinergic, dopaminergic and serotoninergic parts of the reticular ascending systems. We posit that AD neurodegenerative processes affect the interactions among these neural populations, thus inducing an amplitude increase of widespread delta (2-4 Hz) and theta (4-8 Hz) rhythms and an amplitude decrease of the dominant alpha rhythms. This "slowing" of the EEG rhythms would mainly reflect a sort of thalamo-cortical "disconnection mode" (Fig. **1**), and would be functionally mimicked by the transient and reversible effects of hypoxia, SD, and TMS challenges on the resting state EEG rhythms recordable in healthy subjects (Fig. **2**). A further intriguing prediction is that some classes of novel symptoms drugs for AD might impinge upon the neuromodulatory systems regulating EEG rhythms and subjects' global cortical arousal in the resting state condition.

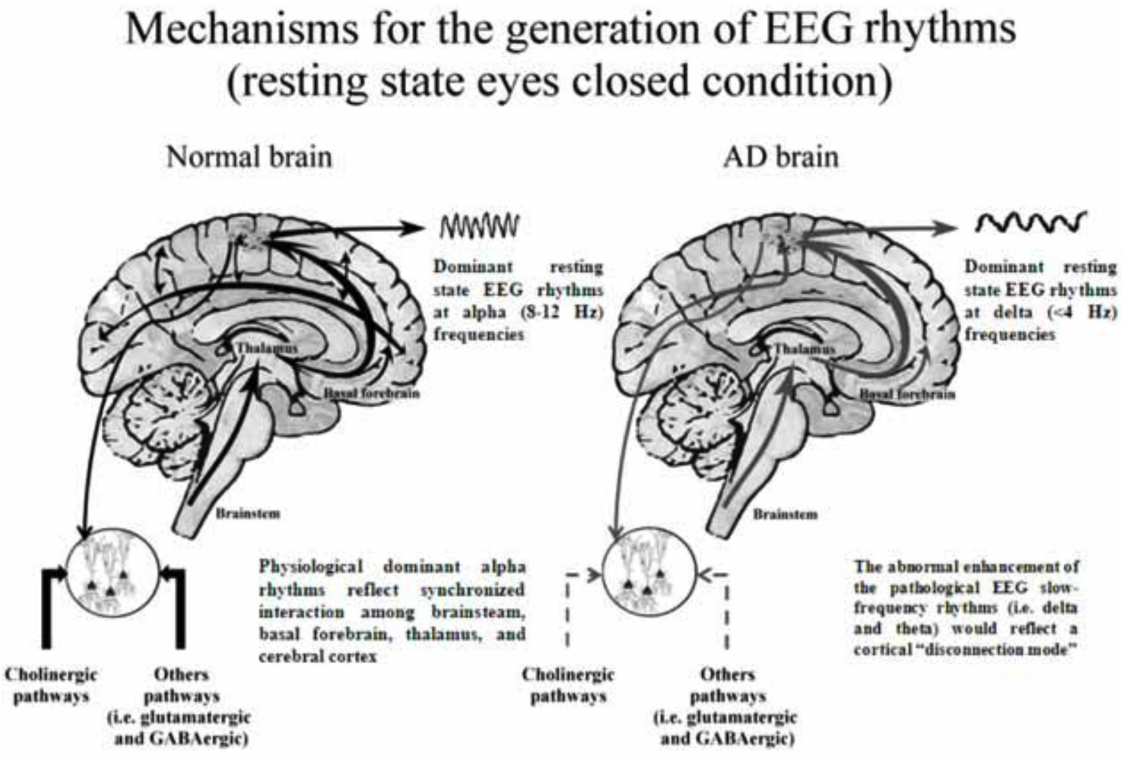

Fig. (1). Tentative physiological model of generation of resting state eyes-closed electroencephalographic (EEG) rhythms in the brain of healthy subjects and Alzheimer's disease (AD) patients. In the normal brain, dominant EEG rhythms are observed at alpha frequencies (8-12 Hz), which would denote the back-ground, spontaneous synchronization around 10 Hz of neural networks regulating the fluctuation of subject's global arousal and consciousness states. These networks would span neural populations of cerebral cortex, thalamus, basal forebrain and brainstem, including glutamatergic, cholinergic, dopaminergic and serotoninergic parts of the reticular ascending systems. In the brain of AD patients, the amplitude of these rhythms is reduced (i.e. desynchronization) together with an amplitude increase of the pathological EEG slow frequencies spanning delta (<4 Hz) and theta (4-7 Hz) rhythms. This "slowing" of the EEG rhythms would mainly reflect a sort of thalamo-cortical "disconnection mode".

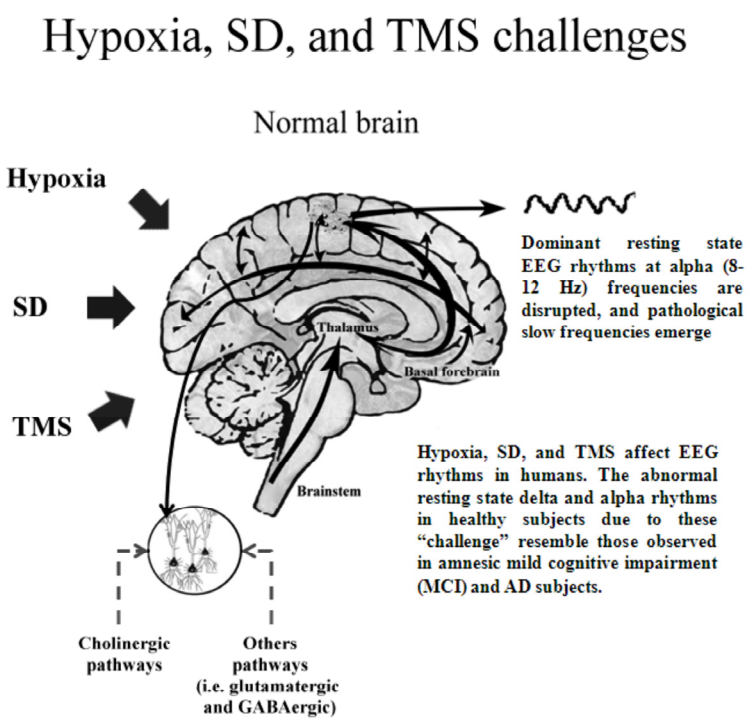

Fig. (2). Tentative physiological model of generation of resting state eyes-closed EEG rhythms in the brain of healthy subjects immediately after the exposition to transient hypoxia, sleep deprivation (SD), and transcranial magnetic stimulation (TMS) challenges. We posit that these challenges induce a transient and reversible "slowing" of the EEG rhythms that mimics that observed in AD patients when they experience a resting state eyes-closed condition.

As a final concluding remark, an important question is as to whether these challenges and EEG markers can be used to make decisions regarding the therapeutic potential of novel symptomatic drugs in AD. Unfortunately, no definitive conclusion can be drawn based on the data available. However it can be speculated that these challenge models and spectral EEG markers may be invaluable at the earlier stages of the drug development, i.e. preclinical through to early PI/PII clinical trials. Indeed, quite similar EEG experiments and the same kind of spectral data analysis can be performed in animal models of AD, in healthy young volunteers, and in elderly individuals with prodromal or manifest AD. Furthermore, the procedures for SD, transient experimental hypoxia, TMS, and recording of the resting state EEG rhythms in humans can be easily back-translated to the experiments to be carried out in wild type and transgenic animal models of AD. Furthermore, the same FFT procedures can be used for the estimation of the spectral power density in the preclinical and clinical EEG data. A number of such translational studies using the mentioned challenges and EEG markers are planned or being performed within the framework of the European IMI project "PharmaCog".

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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