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Evaluation of symptomatic drug effects in Alzheimer's disease: strategies for prediction of efficacy in humans

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In chronic diseases such as Alzheimer's disease (AD), the arsenal of biomarkers available to determine the effectiveness of symptomatic treatment is very limited. Interpretation of the results provided in literature is cumbersome and it becomes difficult to predict their standardization to a larger patient population. Indeed, cognitive assessment alone does not appear to have sufficient predictive value of drug efficacy in early clinical development of AD treatment. In recent years, research has contributed to the emergence of new tools to assess brain activity relying on innovative technologies of imaging and electrophysiology. However, the relevance of the use of these newer markers in treatment response assessment is waiting for validation. This review shows how the early clinical assessment of symptomatic drugs could benefit from the inclusion of suitable pharmacodynamic markers. This review also emphasizes the importance of re-evaluating a step-by-step strategy in drug development.

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Introduction

Process optimization in drug discovery is a laborious challenge that depends primarily on the acceptance that a paradigm shift is needed. Despite scientific progress in the development program of drugs for AD, some persisting methodological issues highlight the necessity to develop innovative strategies that assess the therapeutic potential of a drug-candidate before initiating phase II/III studies. A key problem in Alzheimer's and other neurodegenerative diseases is that cognitive tests (Alzheimer's Disease Assessment Scale-Cognitive subscale (ADASCog) and Mini Mental State Examination (MMSE) scores) currently used to assess the clinical benefit of symptomatic drugs might suffer from subjectivity and little sensitivity to subtle changes with extended evaluation time (over six months). Furthermore, there is no equivalent task for animals in particular because a verbal component is predominantly used in these tests. This fact emphasizes the need to develop new markers sensitive to pharmacological intervention of utility both in patients and in healthy volunteers (HVT) to establish the pharmacologically active range (for efficacy) before testing on larger groups of patients. Validation of these predictive markers could reduce delays and decrease the sample size required to demonstrate benefit from new therapeutic agents.

In this context, adding physiological and functional imaging markers to the current battery of neurocognitive and neurophysiological measures in the drug discovery process would seem pertinent. Indeed, the assessment of cognitive function based on task categories may be associated with brain activity which can be more directly linked to biomarkers (electrophysiology and functional imaging) currently applied to the clinical diagnosis of AD.

Nevertheless, to reliably determine the predictive value of these new tools, an extensive study should be undertaken in both AD and HVT populations to assess their sensitivity to current symptomatic drugs, specific to the cholinergic pathway or not. The complementary information provided by the intrinsic specificities of these techniques, that is high spatial resolution for functional imaging versus high temporal resolution for EEG, suggests the need for combined biomarkers rather than a single one. In addition, these non-invasive markers reflect basic mechanisms of brain functioning rather than species-dependent cognitive tasks. This promotes their use across species and may contribute to enhance the predictive value of pre-clinical studies and facilitate the translation of research evidence from animal to human. It is worth noting that great strides are being made to implement these techniques in the preclinical field to expand their use as modern translational tool in drug discovery. These new tools may compensate for the lack of translatability of the neuropsychological assessment applied to patients.

Incorporation of reliable biomarker combination early in the process of development might improve the decision-making power at the early stages particularly in terms of clinical efficacy of drug candidates (Fig. 1).

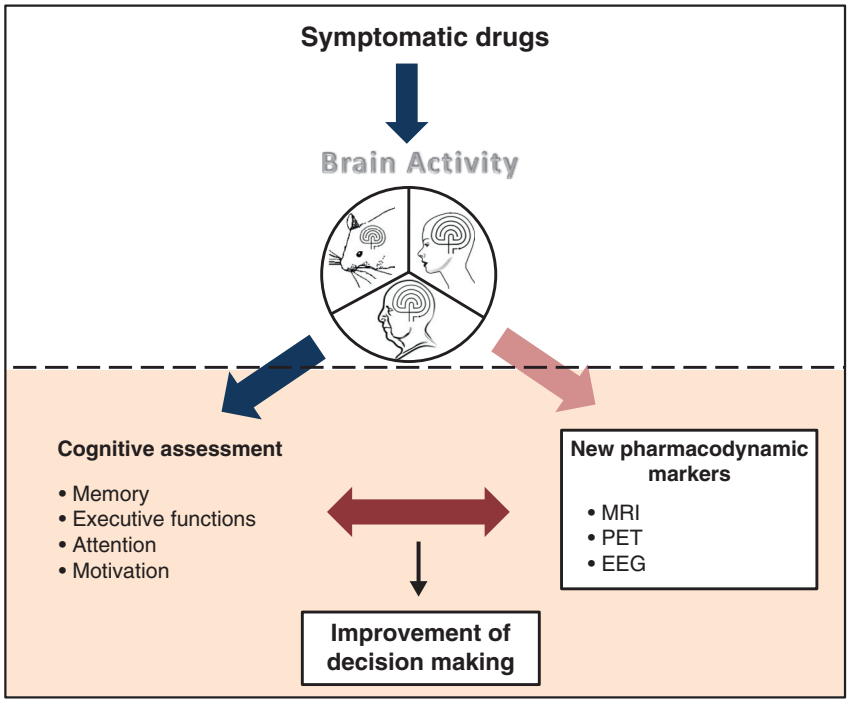

Figure 1. Integration of new pharmacodynamic markers in the symptomatic drugs evaluation design.

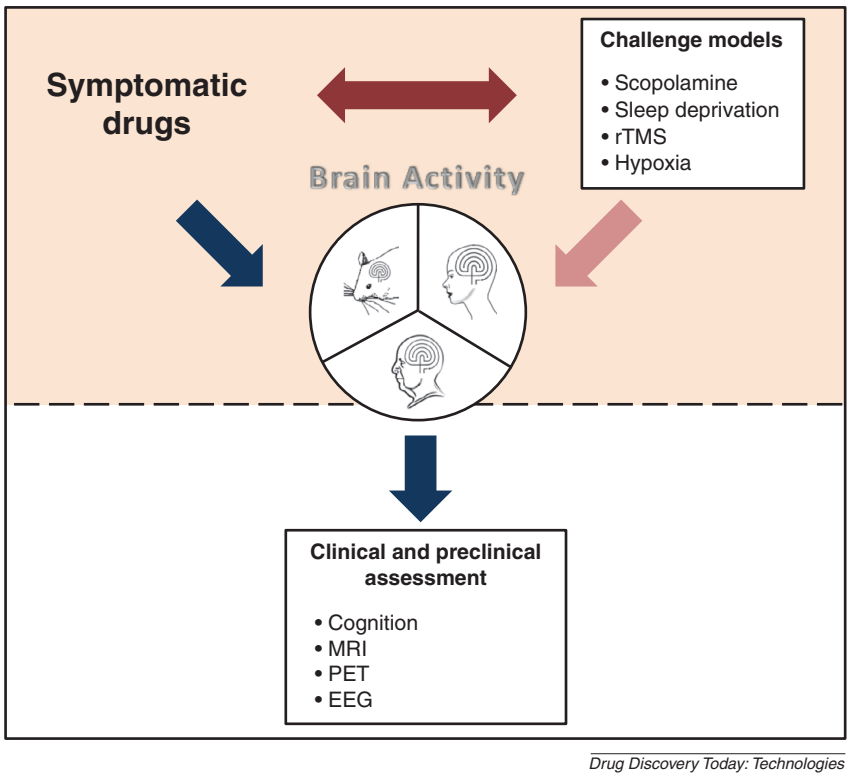

Figure 2. Use of a biomarker battery at early stage of clinical development to assess effect of symptomatic drugs alone or in combination with a challenge test.

It is worth keeping in mind that these biomarkers may not be sensitive enough to detect changes in HVT, so their sensitivity in response to a challenge test may be useful. This, however, leads to a further barrier regarding the emergence of novel AD drugs. In fact, as of today, the most commonly used pharmacological challenge is with scopolamine, a non-selective muscarinic receptor antagonist. Novel cognitive enhancing therapies have been assessed with scopolamine both preclinically and clinically with varying degrees of success. The weakness of this 'pharmacological' model might be related to: (a) its targeted action on a single neurotransmitter system whereas AD is a multifocal disease in which cognitive impairment results from alteration of multiple systems, compromising its use to test drugs that are not exclusively targeting the cholinergic system; (b) the potential pharmacological interaction with therapeutic drugs could compromise the interpretation of the data.

To provide a 'non pharmacological' alternative to the commonly used scopolamine model, we suggest investigating three potential models with different approaches: sleep deprivation; hypoxia; repetitive Transcranial Magnetic Stimulation (rTMS) to induce transient cognitive impairment in HVTs and rodents. The basic requirement of these challenge models is to (i) be able to induce cognitive impairment and modify electrophysiological and imaging parameters and (ii) be sensitive to current therapy for AD with different mechanisms of action to provide a basis for new potential drugs assessment (Fig. 2).

In this review, we seek to identify the most accurate markers to assess drug effect (of commercially available AD symptomatic drugs) among neuropsycological, neurophysiological and imaging tools (PET/MRI), based on literature data. Our investigation includes clinical and pre-clinical populations: (i) healthy versus AD subjects in humans and rodents and (ii) translational physiological challenge models in humans and rodents.

Evaluation of symptomatic drug effect on biomarker battery

Currently, the field of AD drug discovery research is lacking in pharmacodynamic biomarkers capable of (i) rapidly detecting central activity (cognitive changes) in response to treatment, in healthy groups of animals and humans and (ii) predicting early therapeutic efficacy in AD clinical and pre-clinical populations.

The following paragraph summarizes the current situation.

Insights on biomarkers in the clinical field

ADAS-Cog is the most used cognitive scale to evaluate disturbances of memory, language, praxis, attention and other cognitive abilities often referred to as the core symptoms of AD. Several systematic reviews reported significant

improvement in ADAS-Cog score in mild cognitive impairment (MCI) or AD subjects treated with donepezil, reflecting beneficial effects on cognitive status. In HVT, donepezil induces a slight improvement in the retention of training on complex aviation tasks [1], verbal memory for semantically processed words [2] and might improve long term visual memory [3]. Nevertheless, some studies reported transient negative effects on episodic memory [4,5] and no improvement in the Cambridge Neuropsychological Test Automated Battery (CANTAB), a computer-based cognitive assessment system consisting of a battery of neuropsychological tests. In a recent study conducted in healthy older individuals, donepezil has been shown to impair cognitive function associated with alteration in additional neural markers including EEG and fMRI markers [6]. Moreover anticholinesterase treatment also improved attention in MCI and AD patients [7–13].

Evaluation of the effect of symptomatic drugs through the measurement of electroencephalogram variables considered as indices of cognitive processing has been extensively studied in AD patients. Data available in the literature on resting state EEG indicated that long- and short-term treatment with donepezil reduced significantly the deterioration of EEG spectral activity and correlated with cognitive improvement rate on the ADAS-cog. This improvement is mainly characterized by a reduction of the slow-wave activity (theta and delta power) in frontal and temporo-parietal areas [14–17]. In a small study, Sneddon and his colleagues demonstrated the ability of a qEEG variance combined with a delayed recognition memory task to measure accurately treatment effects on patients with AD [18]. In healthy elderly subjects, a single dose of memantine has been shown to compensate diurnal vigilance fluctuation measured by EEG recording [19]. Another EEG component, the P300 is of special interest as it is related to brain functions such as cognition and attention, which are severely impaired in patients with dementia [20]. In AD patients performing auditory and visual oddball tasks, significant changes expressed by a reduction of the P300 latency were observed already during the first month of donepezil administration [21,22] and were significantly correlated with various neuropsychological score changes [22–24].

With regards to the MR technique, various studies have undertaken anatomical analysis of hippocampal volume. They indicated a decrease in hippocampal volume loss/in the rate of hippocampal atrophy following treatment with donepezil or memantine that closely relates to neuropathologic and clinical data [25–27]. Functional MRI is uniquely suited for evaluation of cognitive-enhancing agents. In 2 cognitive paradigms of visual memory, donepezil has been reported to produce activation in the ventrolateral prefrontal cortex (PFC) and in the fusiform gyrus in patients with MCI or AD [28,29]. Another study demonstrated that donepezil reversed the deficit of activation in fronto parietal region during a working memory task in MCI patients [30]. Finally, a reduced activation in the PFC and anterior cingulate cortex was observed after memantine administration during an auditory attention control task [31]. Using [^{18}F]fluorodeoxyglucose (FDG-PET) imaging, a study under resting conditions demonstrated that treatment with donepezil in AD patients may slow the decline in functional brain activity in the right parietal lobe, left temporal lobe and right and left frontal lobes [32]. In addition, Teipel *et al.* showed that the metabolic changes induced by donepezil, during a passive audio-visual stimulation, were limited to the right hippocampus and the left PFC and independent of the effects on cognitive performance [33]. Finally, in patients treated with memantine for 52 weeks, glucose metabolism in all brain areas was preserved [27].

These insights support the potential suitability of electrophysiological and imaging measurements to monitor the effects of symptomatic AD drugs in a context of disturbed (in AD patients) or non-disturbed (in HVT) subjects.

Insights on biomarkers in the pre-clinical field

To bridge the gap between pre-clinical and clinical research studies, great progress has been made in the development of a wide range of pathological models of AD and improvement of functional analysis techniques very similar to those performed in the clinical setting (micro PET, wireless microchip technologies for EEG).

The disease models attempt to reproduce most of the neuropathology including neurophysiological and behavioral hallmarks of AD pathology [34,35]. Since the prominent feature of AD is the loss of neurons and synapses in the hippocampal formation and related areas, neurobehavioral phenotyping of mouse models of AD have focused on the evaluation of hippocampal-related cognitive areas including spatial-, contextual-, working-and recognition memory. These cognitive functions have been extensively explored through a classical battery of experimental tasks that contributed to the characterization of cognitive profile associated with each transgenic line [35]. Despite their limitations, the comprehensive study of these transgenic mouse models provided a good opportunity to evaluate their translational and predictive value of drug efficacy. The next section summarizes the data on the sensitivity of cognitive tests and of new technological biomarkers that aim to measure the response to AD drugs in genetically modified and wild type mice.

In a substantial work, Van Dam and colleagues attempted to validate the APP23 model [36] by studying the reversibility of spatial learning and memory impairment in response to therapeutically relevant doses of three acetylcholinesterase (AChE) inhibitors and memantine. They reported that the lowest dose of Donepezil, rivastigmine and to a lesser extent galantamine, elicited beneficial effects on learning and

memory performance measured in the Morris Water Maze (MWM) task [37]. As for memantine, modest effects have been noted with only partial improvement of spatial learning abilities [37]. However, in a subsequent report, the authors demonstrated that longer administration of memantine (8 weeks) improved the effect [38]. In the Tg2576 mouse model of [39] AD, Dong and colleagues also demonstrated beneficial effects of donepezil on spatial learning/memory with regards to improvement of acquisition in the water T-maze and in contextual memory in a conditioned fear test [40]. Furthermore, administration of memantine through drinking water for 3 weeks to APP/PS1 transgenic mice [41] improved acquisition but no retention abilities in the water maze task [42]. Romberg and colleagues demonstrated that Donepezil rescues the attention deficit by improving response accuracy on the 5-CSRTT adversely affected in the 3xTgAD mice model [43,44].

With respect to investigations of electrophysiological and imaging markers in experimental AD models, there is limited data. An interesting paper reviewed the findings obtained to date from studies using PET imaging and EEG to monitor pathological changes in rodent AD models. They compared FDG-PET phenotypes in 3 cohorts of transgenic strains and indicated that metabolic activity was reliably compromised in PSAPP and PLB1triple transgenic mice [45,46] but not in APP/PS1 mice [41] which carry a different mutation in the PS1 transgene [47]. In addition, with FDG-based autoradiography, metabolic changes have also been reported in the 3xTg mouse [48]. However the AD-relevant brain region specificity is discussed. Furthermore, a comprehensive analysis of sleep patterns, vigilance staging and qEEG conducted in APP/PS1-overexpressing mice highlighted a decreased in low and an increased in high frequency spectral EEG power [49]. Similarly, AD-like shifts in spectral power recorded in the parietal cortex area, have been reported in the triple transgenic mice (PLB1 triple), in agreement with FDG hypometabolism maps [50]. Moreover the authors suggested that these changes might precede the cognitive decline onset [44]. Although the method to evoke a P300 in rodent is now well established, data are missing in the field of transgenic animals of AD.

Thus, many of these technological tools are so recent that their sensitivity to therapeutic intervention has not yet been fully investigated. Only Scholtzova and colleagues followed up the therapeutic response to memantine in APP/PS1 transgenic mice regarding amyloid deposition by micromagnetoc MRI. Despite modest changes between control and treated groups this tool was able to detect reduction in amyloid burden, which correlates with cognitive improvement [51].

Fortunately, the good correlation between the preclinical preliminary results on transgenic strains and human outcomes encourages the analysis of the modulation of these markers in a therapeutic context. Nevertheless, it is interesting to underline that no change in acquisition or retention performance was observed in wild type (WT) mice among treated groups [37], contrasting with some results obtained in HVT. However, Spowart-Manning and co-workers reported that the T-maze continuous alternation task assessing the spatial exploratory performances developed by Gerlai [52,53] was sensitive enough to respond to donepezil administration by improving the spontaneous alternation rate in control mice [54]. Overall, studies on healthy animals cannot be discussed since the differences of performance between strains are too heterogeneous and the majority of tasks are sensitive to genetic differences. The sensitivity of a particular experiment depends at least partially, upon the value of the control group.

This literature overview highlights the heterogeneity of the cognitive assessment performed in the preclinical studies with various different stimuli and some methodological issue (confounding factors). These data cannot yet conclude which biomarker array is most pertinent.

Evaluation of the effect of a cognitive challenge on the biomarker battery - combined effect with symptomatic drugs

Due to the inherent difficulty of detecting significant improvements in cognitive performance in normal healthy subjects, pre-clinical and clinical scientists have developed a number of experimental paradigms to artificially induce cognitive impairments akin to those observed in AD. The identification and validation of HVT challenge models suitable for use in early clinical development might support 'hint of efficacy' studies that can be back translated to preclinical studies. The complexity of AD prevents recapitulating both the pathophysiology and symptoms in one animal model. Currently, each animal model only partially reflects the underlying pathology, for example, the amyloid aggregates. Conversely, pharmacodynamic models are more straightforward than disease models and should exhibit one or more transient cognitive impairments (also typical of AD) that are sensitive to the pharmacological active range of the compound in question. Validation of such models will rely on (a) the characterization of the cognitive and neurofunctional deficits induced, (b) their sensitivity to current symptomatic treatment and (c) their bidirectional translatability. The nature of these challenge models is very important. We propose to study three distinct non-pharmacological models that develop a transient cognitive impairment in HVT or rodents following sleep deprivation, hypoxia and rTMS challenges. Thus, in the next section, we will review studies that have examined the effects of provocation challenges and how these dynamically alter cognitive, electrophysiological and neuroimaging markers and how these markers respond to symptomatic treatment. To highlight the translational properties of each challenge model, clinical and preclinical findings will be discussed in parallel.

Beyond identifying the most appropriate model, the second objective, in this context of challenge models, is to select the most relevant biomarkers for the assessment of treatment efficacy.

Sleep deprivation challenge

Sleep deprivation (SD) has been largely investigated in clinical trials due to its association with deficits in the memory process. Many behavioral adverse effects of SD including the degradation of a wide range of cognitive functions have been fully described (for review [55]). On the basis of studies that have focussed on total sleep deprivation (TSD) protocols, there is extensive literature reporting the deterioration of executive functioning including decision making, flexible thoughts, semantic processing and inhibition as well as working memory [56–59]. Episodic memory was also impaired in verbal and visual domains and simple and complex attention processes have been shown to be disrupted [60–62]. Interestingly, the duration of TSD is not correlated with the magnitude of cognitive impairments in that under prolonged TSD performance remains stable [63–65]. In addition, cognitive deficits are inversely correlated with cerebral activity as measured by functional imaging investigations. In a PET study, Thomas and colleagues noted a decreased glucose metabolism in fronto-parietal and thalamic areas [66]. Other authors demonstrated a hypometabolism in the frontal regions that correlated with disturbances in working memory performances [67–69]. Furthermore, metabolic decreases in posterior parietal cortices have been associated with deficits of verbal working memory [70]. More recently, the modulation of blood-oxygen-level-dependent (BOLD) fMRI signal intensity has been assessed in sleep-deprived subjects performing a divided attention task and the vulnerability of pre-frontal cortex to sleep deprivation previously observed has been confirmed [71,72]. However some inconsistent findings may be noticed between clinical trials. These differences may be partly explained by the inter-individual heterogeneity in the subjects' sensitivity to SD. Studies of SD on EEG power spectra have revealed an increase in low-frequency EEG power (delta and theta frequencies) after TSD [73–75]. Similarly, significant reduction of alpha power in rapid eye movement (REM) sleep has been detected after total and partial SD [73,76]. Topographical analysis combining EEG to PET has been suggested to access the spatio-temporal changes of EEG signal in pharmacological studies [75]. Similarly, there is evidence of a negative impact of sleep deprivation on the auditory event related potential (ERP). SD has been shown to induce a delay of P300 latency and a reduction of its amplitude [77–79]. Other ERP components seem sensitive to SD to a lesser extent [77,79].

On the basis of this core feature of sleep deprivation some authors have investigated whether such read-outs are reversed in response to treatment with symptomatic drugs. In two consecutive studies, Chuah and colleagues have explored the effects of donepezil on cognitive and neuroimaging deficits that developed in a cohort of total sleep-deprived subjects. The first study conducted on 28 HVT showed that donepezil administered for 17 days enhanced both visual short-term memory and visual attention in sleep-deprived HVT. This behavioral benefit, observed in individuals vulnerable to the effects of sleep deprivation, primarily correlates with an increase in neural activation in the parieto-occipital regions that mediate attention and visual sensory processing [80]. The second study performed on the same subjects showed that donepezil could improve performance in an episodic memory task. They also demonstrated a significant correlation between left inferior prefrontal activation at encoding, and performances in recognition memory that may relate specifically to episodic memory [81]. This relationship between fMRI pattern and behavioral drug effects emphasize the value of combining these two measures for future drug candidate assessment. Furthermore, in the context of sleep deprivation-induced episodic memory deficit, donepezil enhanced activation of cerebral regions involved in attention and memory encoding processing during a semantic judgement task [81]. A recent work failed to find any donepezil-induced improvement of memory or non-memory cognitive tasks impaired by sleep deprivation [82].

Many analogies have been proven in pre-clinical studies challenging TSD (REM sleep deprivation has not been taken into account). In terms of cognition, long-term and short-term SD has been shown to alter hippocampal-dependent spatial learning and memory in the MWM task [83,84]. Repetition of brief epochs of SD over a long period of time [85] or protocols of sleep fragmentation [86–88] lead to similar effects. Nevertheless, in light of these studies, the consolidation process of spatial information seems to be most vulnerable to sleep deprivation. Furthermore, in an alternation paradigm measuring working memory ability, where SD was given after training, disturbed the alternation rate [89,90]. The fear conditioning paradigm has also been used to assess declarative-like memory in sleep-deprived animals. Findings revealed that SD negatively affects the acquisition and consolidation phase of contextual fear conditioning related to the hippocampus [91–94] whereas performance of cued fear conditioning related to the amygdala is spared [91]. Using the Novel Object Recognition (NOR) task, considered to measure episodic memory as that in human [95], researchers indicated that SD administered after the acquisition phase severely impairs object recognition during the retention test [96,97]. Finally, attention processes have also been explored in the SD context in rodents. SD was shown to impair animals' speed and accuracy in the five choice reaction time task however no deficit in the executive control of the task was observed [98]. These findings are in agreement with another study using a sleep fragmentation protocol [99].

In terms of electrophysiology, studies in rats and mice have shown an overall increase in slow wave activity after SD [100–102]. ERP parameters have not been explored in the SD preclinical challenge. Effect of SD on modulation of neural activity measured by functional imaging in rodents has yet to be reported. Similarly, the reversal effect of symptomatic drugs on modification of markers induced by sleep deprivation has not been probed.

rTMS challenge

Transcranial magnetic stimulation is a painless method based on the induction of a small electrical current in the brain by a magnetic field applied to a small area of the skull. This technique has been described as a non-invasive transient way to interfere with cognitive functions by the stimulation of specific areas of interest [103,104]. Despite the incontestable interest of this method as a 'virtual lesion technique' to study cognitive functions, its application remains limited due to its limited depth of penetration [105]. At present, deep areas of the brain located many centimeters below the scalp such as hippocampus, amygdala or mammillary bodies cannot be directly reached. However, combination of TMS with fMRI seems to demonstrate that the activity of these areas can be actually modulated by TMS, presumably due to transynaptic effects. Thus, research is mainly focused on stimulation of frontoparietal networks involved in working memory as well as the encoding and retrieval of novel items. In particular several studies targeted the role of the PFC in episodic memory. These works, using different experimental tasks requiring wording, demonstrated that recall performances were impaired following rTMS over the left dorsolateral PFC during the encoding process [106–111]. A similar effect was observed, for some of these works, following rTMS over the right dorsolateral PFC during retrieval suggesting that there is a left encoding, right recognition PFC asymetry for memory processes [108–111]. These findings were in agreement with a recent study demonstrating that paired-pulse TMS applied over the left or right dorsolateral PFC interfered with episodic encoding and retrieval, irrespective of the type of material used (verbal versus non-verbal) [112]. In addition, TMS over the left antero-ventral inferior PFC region has been shown to disrupt episodic encoding of non-verbal material [113]. Regarding working memory, many investigations highlighted the negative impact of rTMS in various brain areas on this cognitive function (for review [114]). Mottaghy and colleagues reported disruption in working memory for faces (face recognition delayed response task) when rTMS was applied to ventral PFC and dorsolateral PFC while spatial working memory was affected following rTMS over the dorsolateral PFC and dorsomedial PFC [115]. Impairment of visual working memory after left dorsolateral PFC stimulation has also been described [116–118]. Moreover Wagner and colleagues provided evidence for delayed rTMS effects on divided attention task performances since visual reaction time is slowed over 30 min after rTMS session at the dorsolateral PFC [119]. Other studies report that applying rTMS to the superior parietal lobule produces a disruption of cognitive behavior [120–122]. Kessel and colleagues also indicated that the reaction times measured in a spatial working memory task were slower during right-parietal rTMS than during left-parietal rTMS [123]. However discrepant findings were observed for the effect of rTMS on attentional processes [124]. Finally, cerebellar TMS is able to influence memory abilities including working memory [125,126]. Multimodal approaches have also been conducted by combining TMS with neuroimaging or electrophysiological methods to assess spatial distribution of TMS-evoked activity and brain connectivity [127]. These investigations suggested an activation of brain structures distant from the TMS-stimulated area [128–135]. Other studies assessed the brain–behavior relationship, that is the modulation of brain function during a cognitive task, by combination of TMS with PET during cognitive performances. Mottaghy and co-workers emphasized the concomitant alteration of working memory task performance with a change in the activation pattern as revealed by PET, in the condition with rTMS of the left or right dorsolateral PFC [136]. The same team demonstrated a positive correlation between the regional cerebral blood flow changes in the superior frontal gyrus functionally connected to the stimulation site and performances in the working memory task [137] reviewed in [114].

The emergence of a new method to avoid the artefact problem caused by the magnetic pulse proposed by Thut and colleagues have provided a means for studying the impact of TMS on the functional electrophysiological signals associated with (cognitive) task performance, such as ERPs [138]. An equivalent procedure has also been developed in animals [139]. Effect of rTMS on auditory oddball task has been investigated after stimulation over dorsolateral PFC. Using an auditory oddball task, the authors demonstrated a widespread significantly reduced alphe desynchronisation post-TMS while the P300 component (amplitudes or latencies) was not affected [140]. In contrast, stimulation of the supramarginal gyrus by TMS applied at 200ms after the oddball sounds presentation delayed the peak response of P300 [141]. Moreover, EEG power spectrum has also been examined immediately following rTMS. Studies reported a temporal increase in frequency and amplitude of EEG within the first 2 min after high frequency rTMS over left frontal cortex [142]. Schutter and colleagues also indicated that medial cerebellar rTMS affected the high frequency band (gamma band) of EEG spectrum [143].

The effect of acetylcholinesterase inhibitors or memantine on neurophysiological parameters, fMRI or cerebral glucose metabolism has not been yet studied in the context of rTMS.

Compared to the large number of studies in humans, there a few studies describing the effects of rTMS on memory and learning in animal models have been little studied. This could be related to the difficulty of precisely stimulating a specific brain area with this technique in animals. Moreover, many stimulation parameters such as frequency, intensity, duration and number of pulse can modulate the behavioral response. An interesting study has investigated the impact of the modulation of stimulation frequency (chronic versus acute) on memory performance in rats [144]. The findings indicated that chronic low frequency stimulation could impair the retrieval of both short- and long-term spatial reference memory sparing the acquisition process; while after acute rTMS only the long-term reference memory was impaired. In another study Ahmed and Wieraszko highlighted the importance of the magnetic field properties applied. They demonstrated a temporary deterioration of performances in the NOR test performed immediately after stimulation at lower frequencies (1 and 8 Hz) while performance was improved after stimulation at higher frequency (15 Hz). However, when the test is performed 1 h or 3 days after high frequency rTMS, performances in NOR test are also impaired [145]. Other researchers concluded that 50 brief pulse transcranial magnetic stimulations may cause a disruption of retrograde memory for conditioned taste aversions [146]. Regarding cortical electrical activity, rats subjected to low frequency rTMS display a reduction in amplitude of the power spectra in the high frequency bands (beta and gamma) [147] consistent with clinical findings [142,143].

Hypoxia challenge

The interest in hypoxia as a potential inducer of cognitive disorder results from studies conducted in the context of aviation and mountain ascent. Nevertheless the role of hypoxia on cognitive domains remains relatively unexplored. Overall, studies in the literature indicated that cognitive impairment caused by hypoxia is modulated by the severity of hypoxic level and the population considered (experienced versus non experienced participants). To accurately compare clinical and preclinical data, we focused our interest on behavioral and neurophysiological effects of hypoxia on non-experienced subjects (naïve from hypoxic environment). Cognitive functions including executive functioning, working memory and attention have been reported to be affected by hypoxia. Unfortunately, results seem somewhat contradictory due to a lack of methodological consistency among studies. Nevertheless a core of findings seems to demonstrate alteration of performance in serial recognition of words, the binary choice task and auditory and visual reaction time [148]. Noble and co-workers also reported an overall slowing on task measuring executive functioning [149] that was not confirmed by others [148,150]. Studies have provided evidence of an impairment in executive processes indicated by a decline in the Grammatical Logical Reasoning task [151,152] associated to an inability to learn a novel task [152]. Working memory is also affected following short-term exposure to hypoxia shown by alteration of performances in a go/no-go discrimination task [153]. These disturbances were not observed in the rapid visual categorical task [154]. In addition, many studies showed disruption of some aspect of attentional processes through various tests [56,155–159]. Episodic memory has not been studied. Although neuropsychological results are highly controversial, more consistent results come from electrophysiological investigations. Most studies have shown EEG changes characterized by increases in delta and theta power and a decrease in alpha activity under hypoxia [158,160–162]. Moreover an identical trend, with regards to ERP P300 assessment, emerges from the literature. Indeed, long- and short-term exposures to hypoxia have induced an increase of P300 latency with minimal effects on P300 amplitude [163–165]. Other parameters such as N100, P200 and N200 seem less sensitive to hypoxia [164].

Using neuroimaging techniques various clinical studies have investigated the cerebrovascular response to hypoxia analyzing regional cerebral blood flow distribution [166–168]. Data demonstrated differential physiological sensitivity of various brain regions. Functional neuroimaging data demonstrated specific sensitivity of frontal lobes [169] supported by the metabolic reduction observed in this region [170]. Thus, hypoxia seems to affect the cerebral network involved in the attention process, which is consistent with neuropsychological deterioration. As for the challenge of rTMS, the effect of symptomatic treatment has not been tested under hypoxia challenge.

Studies of hypoxia in rodents have experienced identical discrepancies regarding cognitive deficits since intensity, frequency and duration of hypoxia exposure modulate the magnitude of cognitive alteration in rodents [171,172]. Findings demonstrated detrimental effects of hypoxia on both acquisition and consolidation processes in different paradigms of cognitive tests. Chronic exposure to hypoxia can alter working and reference spatial memory assessed in MWM task [173–180]. These spatial memory dysfunctions have also been noted after acute exposure of hypoxia beyond a threshold level [172,181]. Using place-discrimination task in a radial arm maze, Titus confirmed these spatial impairments that occur following several days of hypoxia [182]. In addition, Zhang reported dysfunction in acquisition and retention processes in a shuttle box paradigm which models non-declarative memory [183]. In the passive avoidance task, Udayabanu and co-workers revealed that hypoxia could only disrupt retrograde memory suggesting an effect of hypoxia on consolidation [184]. Finally brief exposure to hypoxia led to milder and more transient cognitive alteration in spatial working memory assessed in the Y maze task and in the

NOR task [185–187]. In addition acute exposure interfered with consolidation of memory in the step-through avoidance task [188]. The sensitivity of other markers to hypoxia has not been studied in rodents. The effect of symptomatic drugs on cognitive impairment induced by hypoxia has been explored. For example, Physostigmine and Galantamine has been tested to improve spatial learning and working memory induced by chronic hypoxia [180]. In addition, administration of physostigmine prior to hypoxic exposure was shown to preserve recognition memory sensitive to hypoxia [185].

Strengths and weaknesses of current strategies and identification of translational requirements

Currently, the majority of symptomatic treatments fail due to a lack of efficacy in patients detected during phase II/III clinical studies. This suggests that current preclinical and clinical methods to test drug efficacy are not effective. To improve the current process of drug development, we proposed to introduce new pharmacodynamic biomarkers at each stage of preclinical and clinical development, in addition to current clinical and neuropsychological assessments. These markers are based on neurophysiological and neuroimaging measurements already commonly used in the clinical field (for diagnosis and better understanding of the pathophysiology). They are a direct reflection of cerebral activity patterns and may overcome the frequenly criticized neuropsychological evaluation, which lacks objectivity. Moreover, unlike cognitive enhancement that must be evaluated over a period of six months, drug effects on physiological biomarkers might be detectable over a shorter time. Our detailed review of the literature indicates that these alternative biomarkers display a good sensitivity to symptomatic treatment in AD or MCI patients since in most cases improvement in the pharmacodynamic markers correlates with cognitive gain. In HVT, studies are scarce and do not allow an assessment of the contribution of these biomarkers as predictive tools. However, further studies to assess if their use is pertinent may clarify this issue.

From a preclinical point of view, the development of these biomarkers reflecting brain activity in a physiological resting state might reduce the gap between preclinical and clinical phases. Indeed, some evidence suggests that the limited translatability of animal cognitive tests might explain disappointing results in the development of successful treatments for AD. The use of EEG, PET and MRI in the preclinical assessment protocol of new pharmacological compounds should facilitate the extrapolation of preclinical outcomes to the clinical field. Results obtained from studies on genetically engineered animal models indicate a great sensitivity of these markers to AD pathogenesis supporting their use as translational tools. However their sensitivity to clinically approved symptomatic treatments has not yet been explored. Nevertheless, we cannot overlook the importance of cognitive endpoints in the predictive validity of preclinical models. As evidenced by the review, cognitive tests used today are disparate and poorly comparable to those performed in humans. These limitations are reinforced by the fact that the neuropsychological battery used as a reference standard to assess clinical drug efficacy, the ADAS-Cog, is almost impossible to model in rodents as this specific scale mainly explores unavailable functions in rodents, that is verbal memory, language and praxis. Over the past decade, a new technological instrument specifically designed to facilitate cross-species has emerged. The touchscreen technology relies on a set of straightforward cognitive tasks procedurally similar to clinical neuropsychological tests belonging to the CANTAB battery (computer-based cognitive test procedures becoming more prevalent in the clinical cognitive testing domain). They explore through computer-automated cognitive tests a range of cognitive domains (memory, attention, executive functions) thought to be affected in AD. In addition, this standardized cognitive exploration tool uses the same types of visual stimuli used in the CANTAB tasks. The predictive validity remains to be demonstrated by extensive studies. Validation of translational cognitive tasks may potentially expand the role of EEG and PET in the preclinical models whereby these markers can be measured under specific tasks [189,190]. MRI performed in rodents under anesthesia, cannot benefit from such studies.

In light of these preliminary results and observations, it appears that these alternative markers – combined with appropriate cognitive assessment- would contribute to improve the predictive and translational value of drug development stages preceding Phase II/III studies. However, the drug discovery process may still suffer from a lack of predictability in early stage experimental medicine studies due to the difficulties in identifying benefits of cognition enhancer compounds in HVT without cognitive impairment. It might be easier to reverse existing deficits than improve normal functioning. In this view, our strategy to improve predictive capacity is to include, in preclinical and clinical (phase 1) trials translational pharmacodynamic models that exhibit cognitive impairment reminiscent of that observed in patients with AD. The choice of non-pharmacological provocation challenge – sleep deprivation, rTMS and hypoxia – with a broader action in the CNS might provide an alternative model to the scopolamine one, limited by its pharmacological nature.

Overall, this review confirms the feasibility of inducing a transient cognitive disorder in human and rodent models. The cognitive deficits relate to multiple functions such as executive functions, working memory and attention. Investigations on neurophysiological and imaging biomarkers are less extensive but seem to show modulations of these parameters in response to the challenges. However, methodological issues and lack of harmonization of clinical assessment

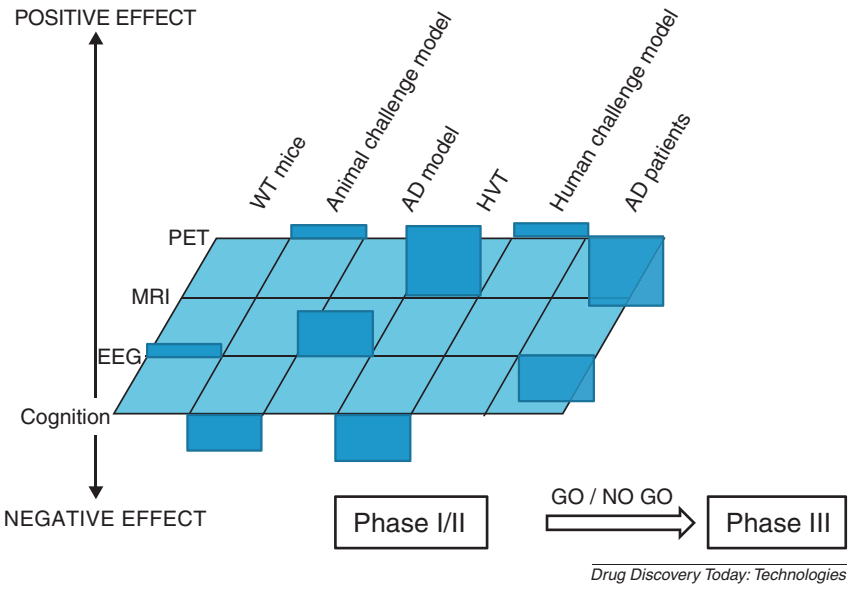

Figure 3. Simulation of an AD drug development using a matrix battery.

tools make it difficult to compare these challenges. It would be interesting to evaluate the intrinsic limits of each model by performing parallel studies with standardized assessment protocols based on a biomarker battery including cognitive, neurophysiological and imaging endpoints. The reversibility of changes in response to symptomatic drug exposure will define their predictability and consequently their own ability to support 'hint of efficacy studies' in early clinical stages as well as in preclinical studies.

The identification of the most sensitive combination of pharmacodynamic markers (as clinical efficacy indices) and pharmacodynamic models (for early prediction of efficacy) will increase the effectiveness of the drug discovery process in AD through a multidimensional matrix approach (Fig. 3).

Conclusion

A fairly abundant literature is now available on: (i) the effect of AD symptomatic drugs on different biomarkers assessing brain activity; (ii) the effect of challenge tests on cognitive function and these similar biomarkers of brain activity. In contrast, the data on the combination of symptomatic drug and challenge test remain poor and with controversial results. We feel that this combination coupled with biomarker assessment could be a new and stringent paradigm to test new symptomatic drugs in the context of AD, in particular at the early stage of disease (Fig. 3). A systematic evaluation and validation of such an approach remains necessary and it is one of the goals of the IMI Pharmacog consortium, which aims to further explore the preclinical ans clinical studies in the context of gold standard symptomatic treatments for AD.

Conflict of interest

The authors have no conflict of interest to declare.

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References

1 Yesavage, J.A. *et al.* (2002) Donepezil and flight simulator performance: effects on retention of complex skills. *Neurology* 59, 123–125
2 FitzGerald, D.B. *et al.* (2008) Effects of donepezil on verbal memory after semantic processing in healthy older adults. *Cogn. Behav. Neurol.* 21, 57–64
3 Gron, G. *et al.* (2005) Cholinergic enhancement of episodic memory in healthy young adults. *Psychopharmacology (Berl.)* 182, 170–179
4 Beglinger, L.J. *et al.* (2004) Neuropsychological test performance in healthy volunteers before and after donepezil administration. *J. Psychopharmacol.* 18, 102–108
5 Beglinger, L.J. *et al.* (2005) Neuropsychological test performance in healthy elderly volunteers before and after donepezil administration: a randomized, controlled study. *J. Clin. Psychopharmacol.* 25, 159–165
6 Balsters, J.H. *et al.* (2011) Donepezil impairs memory in healthy older subjects: behavioural, EEG and simultaneous EEG/fMRI biomarkers. *PLoS ONE* 6, e24126
7 Bentley, P. *et al.* (2008) Cholinesterase inhibition modulates visual and attentional brain responses in Alzheimer's disease and health. *Brain* 131 (Pt 2), 409–424
8 Blin, J. *et al.* (1998) Physostigmine results in an increased decrement in brain glucose consumption in Alzheimer's disease. *Psychopharmacology (Berl.)* 136, 256–263

9 Foldi, N.S. *et al.* (2005) Detecting effects of donepezil on visual selective attention using signal detection parameters in Alzheimer's disease. *Int. J. Geriatr. Psychiatry* 20, 485–488

10 Lawrence, A.D. and Sahakian, B.J. (1995) Alzheimer disease, attention, and the cholinergic system. *Alzheimer Dis. Assoc. Disord.* 9 (Suppl 2), 43–49

11 Rockwood, K. (2004) Size of the treatment effect on cognition of cholinesterase inhibition in Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* 75, 677–685

12 Sahakian, B.J. and Coull, J.T. (1993) Tetrahydroaminoacridine (THA) in Alzheimer's disease: an assessment of attentional and mnemonic function using CANTAB. *Acta Neurol. Scand. Suppl.* 149, 29–35

13 Sahakian, B.J. *et al.* (1993) Further analysis of the cognitive effects of tetrahydroaminoacridine (THA) in Alzheimer's disease: assessment of attentional and mnemonic function using CANTAB. *Psychopharmacology (Berl.)* 110, 395–401

14 Balkan, S. *et al.* (2003) Effect of donepezil on EEG spectral analysis in Alzheimer's disease. *Acta Neurol. Belg.* 103, 164–169

15 Brassen, S. and Adler, G. (2003) Short-term effects of acetylcholinesterase inhibitor treatment on EEG and memory performance in Alzheimer patients: an open, controlled trial. *Pharmacopsychiatry* 36, 304–308

16 Reeves, R.R. *et al.* (2002) The effects of donepezil on quantitative EEG in patients with Alzheimer's disease. *Clin. Electroencephalogr.* 33, 93–96

17 Rodriguez, G. *et al.* (2002) Quantitative EEG changes in Alzheimer patients during long-term donepezil therapy. *Neuropsychobiology* 46, 49–56

18 Sneddon, R. *et al.* (2006) QEEG monitoring of Alzheimer's disease treatment: a preliminary report of three case studies. *Clin. EEG Neurosci.* 37, 54–59

19 Schulz, H. *et al.* (1996) The use of diurnal vigilance changes in the EEG to verify vigilance-enhancing effects of memantine in a clinical pharmacological study. *Neuropsychobiology* 33, 32–40

20 Juckel, G. *et al.* (2008) Diagnostic usefulness of cognitive auditory event-related p300 subcomponents in patients with Alzheimers disease? *J. Clin. Neurophysiol.* 25, 147–152

21 Reeves, R.R. *et al.* (1999) The effects of donepezil on the P300 auditory and visual cognitive evoked potentials of patients with Alzheimer's disease. *Am. J. Geriatr. Psychiatry* 7, 349–352

22 Thomas, A. *et al.* (2001) Donepezil, rivastigmine, and vitamin E in Alzheimer disease: a combined P300 event-related potentials/neuropsychologic evaluation over 6 months. *Clin. Neuropharmacol.* 24, 31–42

23 Katada, E. *et al.* (2003) Long-term effects of donepezil on P300 auditory event-related potentials in patients with Alzheimer's disease. *J. Geriatr. Psychiatry Neurol.* 16, 39–43

24 Werber, E.A. *et al.* (2003) The clinical use of P300 event related potentials for the evaluation of cholinesterase inhibitors treatment in demented patients. *J. Neural Transm.* 110, 659–669

25 Hashimoto, M. *et al.* (2005) Does donepezil treatment slow the progression of hippocampal atrophy in patients with Alzheimer's disease? *Am. J. Psychiatry* 162, 676–682

26 Krishnan, K.R. *et al.* (2003) Randomized, placebo-controlled trial of the effects of donepezil on neuronal markers and hippocampal volumes in Alzheimer's disease. *Am. J. Psychiatry* 160, 2003–2011

27 Schmidt, R. *et al.* (2008) Longitudinal multimodal imaging in mild to moderate Alzheimer disease: a pilot study with memantine. *J. Neurol. Neurosurg. Psychiatry* 79, 1312–1317

28 Kircher, T.T. *et al.* (2005) Cortical activation during cholinesterase-inhibitor treatment in Alzheimer disease: preliminary findings from a pharmaco-fMRI study. *Am. J. Geriatr. Psychiatry* 13, 1006–1013

29 Petrella, J.R. *et al.* (2009) Effects of donepezil on cortical activation in mild cognitive impairment: a pilot double-blind placebo-controlled trial using functional MR imaging. *Am. J. Neuroradiol.* 30, 411–416

30 Saykin, A.J. *et al.* (2004) Cholinergic enhancement of frontal lobe activity in mild cognitive impairment. *Brain* 127 (Pt 7), 1574–1583

31 van Wageningen, H. *et al.* (2009) Evidence for glutamatergic neurotransmission in cognitive control in an auditory attention task. *Neurosci. Lett.* 454, 171–175

32 Tune, L. *et al.* (2003) Donepezil HCl (E2020) maintains functional brain activity in patients with Alzheimer disease: results of a 24-week, double-blind, placebo-controlled study. *Am. J. Geriatr. Psychiatry* 11, 169–177

33 Teipel, S.J. *et al.* (2006) Effects of donepezil on cortical metabolic response to activation during (18)FDG-PET in Alzheimer's disease: a double-blind cross-over trial. *Psychopharmacology (Berl.)* 187, 86–94

34 Higgins, G.A. and Jacobsen, H. (2003) Transgenic mouse models of Alzheimer's disease: phenotype and application. *Behav. Pharmacol.* 14, 419–438

35 Bryan, K.J. *et al.* (2009) *Transgenic Mouse Models of Alzheimer's Disease: Behavioral Testing and Considerations*.

36 Sturchler-Pierrat, C. *et al.* (1997) Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc Natl Acad Sci U S A* 94, 13287–13292

37 Van Dam, D. *et al.* (2005) Symptomatic effect of donepezil, rivastigmine, galantamine and memantine on cognitive deficits in the APP23 model. *Psychopharmacology (Berl.)* 180, 177–190

38 Van Dam, D. and De Deyn, P.P. (2006) Cognitive evaluation of disease-modifying efficacy of galantamine and memantine in the APP23 model. *Eur. Neuropsychopharmacol.* 16, 59–69

39 Hsiao, K. *et al.* (1996) Correlative memory deficits. Abeta elevation, and amyloid plaques in transgenic mice. *Science* 274, 99–102

40 Dong, H. *et al.* (2005) Acetylcholinesterase inhibitors ameliorate behavioral deficits in the Tg2576 mouse model of Alzheimer's disease. *Psychopharmacology (Berl.)* 181, 145–152

41 Borchelt, D.R. *et al.* (1997) Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. *Neuron* 19, 939–945

42 Minkeviciene, R. *et al.* (2004) Memantine improves spatial learning in a transgenic mouse model of Alzheimer's disease. *J. Pharmacol. Exp. Ther.* 311, 677–682

43 Oddo, S. *et al.* (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron* 39, 409–421

44 Romberg, C. *et al.* (2011) Impaired attention in the 3xTgAD mouse model of Alzheimer's disease: rescue by donepezil (Aricept). *J. Neurosci.* 31, 3500–3507

45 Holcomb, L. *et al.* (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med* 4, 97–100

46 Platt, B. *et al.* (2011) Abnormal cognition, sleep, EEG and brain metabolism in a novel knock-in Alzheimer mouse, PLB1. *PLoS One* 6, e27068

47 Platt, B. *et al.* (2011) FDG-PET imaging, EEG and sleep phenotypes as translational biomarkers for research in Alzheimer's disease. *Biochem. Soc. Trans.* 39, 874–880

48 Nicholson, R.M. *et al.* (2010) Regional cerebral glucose uptake in the 3xTG model of Alzheimer's disease highlights common regional vulnerability across AD mouse models. *Brain Res.* 1347, 179–185

49 Jyoti, A. *et al.* (2010) EEG, activity, and sleep architecture in a transgenic AbetaPPswe/PSEN1A246E Alzheimer's disease mouse. *J. Alzheimers Dis.* 22, 873–887

50 Platt, B. *et al.* (2011) Abnormal cognition, sleep, EEG and brain metabolism in a novel knock-in Alzheimer mouse, PLB1. *PLoS ONE* 6, e27068

51 Scholtzova, H. *et al.* (2008) Memantine leads to behavioral improvement and amyloid reduction in Alzheimer's-disease-model transgenic mice shown as by micromagnetic resonance imaging. *J. Neurosci. Res.* 86, 2784–2791

52 Gerlai, R. (1998) A new continuous alternation task in T-maze detects hippocampal dysfunction in mice. A strain comparison and lesion study. *Behav. Brain Res.* 95, 91–101

53 Gerlai, R. *et al.* (1994) T-maze spontaneous alternation rate is decreased in S100 beta transgenic mice. *Behav. Neurosci.* 108, 100–106

54 Spowart-Manning, L. and van der Staay, F.J. (2004) The T-maze continuous alternation task for assessing the effects of putative cognition enhancers in the mouse. *Behav. Brain Res.* 151, 37–46

55 McCoy, J.G. and Strecker, R.E. (2011) The cognitive cost of sleep lost. *Neurobiol. Learn. Mem.* 96, 564–582
56 Blogg, S.L. and Gennser, M. (2006) Cerebral blood flow velocity and psychomotor performance during acute hypoxia. *Aviat. Space Environ. Med.* 77, 107–113
57 Chee, M.W. and Choo, W.C. (2004) Functional imaging of working memory after 24 hr of total sleep deprivation. *J. Neurosci.* 24, 4560–4567
58 Gottselig, J.M. *et al.* (2006) Random number generation during sleep deprivation: effects of caffeine on response maintenance and stereotypy. *J. Sleep Res.* 15, 31–40
59 Killgore, W.D. *et al.* (2009) Executive functions and the ability to sustain vigilance during sleep loss. *Aviat. Space Environ. Med.* 80, 81–87
60 Chee, M.W. *et al.* (2010) Sleep deprivation and its effects on object-selective attention. *Neuroimage* 49, 1903–1910
61 Tucker, M.A. and Fishbein, W. (2009) The impact of sleep duration and subject intelligence on declarative and motor memory performance: how much is enough? *J. Sleep Res.* 18, 304–312
62 Yoo, S.S. *et al.* (2007) A deficit in the ability to form new human memories without sleep. *Nat. Neurosci.* 10, 385–392
63 Chee, M.W. *et al.* (2006) Functional imaging of working memory following normal sleep and after 24 and 35 h of sleep deprivation: correlations of fronto-parietal activation with performance. *Neuroimage* 31, 419–428
64 Sagaspe, P. *et al.* (2006) Effects of sleep deprivation on Color-Word, Emotional, and Specific Stroop interference and on self-reported anxiety. *Brain Cogn.* 60, 76–87
65 Van Dongen, H.P. *et al.* (2003) The cumulative cost of additional wakefulness: dose–response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep* 26, 117–126
66 Thomas, M. *et al.* (2000) Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. *J. Sleep Res.* 9, 335–352
67 Chee, M.W. and Chuah, Y.M. (2007) Functional neuroimaging and behavioral correlates of capacity decline in visual short-term memory after sleep deprivation. *Proc. Natl. Acad. Sci. U. S. A.* 104, 9487–9492
68 Choo, W.C. *et al.* (2005) Dissociation of cortical regions modulated by both working memory load and sleep deprivation and by sleep deprivation alone. *Neuroimage* 25, 579–587
69 Mu, Q. *et al.* (2005) Decreased brain activation during a working memory task at rested baseline is associated with vulnerability to sleep deprivation. *Sleep* 28, 433–446
70 Mu, Q. *et al.* (2005) Decreased cortical response to verbal working memory following sleep deprivation. *Sleep* 28, 55–67
71 Drummond, S.P. *et al.* (2001) Increased cerebral response during a divided attention task following sleep deprivation. *J. Sleep Res.* 10, 85–92
72 Jackson, M.L. *et al.* (2011) The effect of sleep deprivation on BOLD activity elicited by a divided attention task. *Brain Imaging Behav.* 5, 97–108
73 Borbely, A.A. *et al.* (1981) Sleep deprivation: effect on sleep stages and EEG power density in man. *Electroencephalogr. Clin. Neurophysiol.* 51, 483–495
74 Dijk, D.J. *et al.* (1993) Dynamics of electroencephalographic sleep spindles and slow wave activity in men: effect of sleep deprivation. *Brain Res.* 626, 190–199
75 Landolt, H.P. *et al.* (2000) Zolpidem and sleep deprivation: different effect on EEG power spectra. *J. Sleep Res.* 9, 175–183
76 Brunner, D.P. *et al.* (1993) Repeated partial sleep deprivation progressively changes in EEG during sleep and wakefulness. *Sleep* 16, 100–113
77 Lee, H.J. *et al.* (2004) Auditory event-related potentials and psychological changes during sleep deprivation. *Neuropsychobiology* 50, 1–5
78 Morris, A.M. *et al.* (1992) The P300 event-related potential. The effects of sleep deprivation. *J. Occup. Med.* 34, 1143–1152
79 Ray, K. *et al.* (2012) Modafinil improves event related potentials P300 and contingent negative variation after 24 h sleep deprivation. *Life Sci.* 91, 94–99
80 Chuah, L.Y. and Chee, M.W. (2008) Cholinergic augmentation modulates visual task performance in sleep-deprived young adults. *J. Neurosci.* 28, 11369–11377
81 Chuah, L.Y. *et al.* (2009) Donepezil improves episodic memory in young individuals vulnerable to the effects of sleep deprivation. *Sleep* 32, 999–1010
82 Dodds, C.M. *et al.* (2011) Effects of donepezil on cognitive performance after sleep deprivation. *Hum. Psychopharmacol.* 26, 578–587
83 Chang, H.M. *et al.* (2009) Melatonin preserves longevity protein (sirtuin 1) expression in the hippocampus of total sleep-deprived rats. *J. Pineal Res.* 47, 211–220
84 Guan, Z. *et al.* (2004) Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus. *Brain Res.* 1018, 38–47
85 Xu, Z.Q. *et al.* (2010) The mechanism and characterization of learning and memory impairment in sleep-deprived mice. *Cell Biochem. Biophys.* 58, 137–140
86 Hairston, I.S. *et al.* (2005) Sleep restriction suppresses neurogenesis induced by hippocampus-dependent learning. *J. Neurophysiol.* 94, 4224–4233
87 Tartar, J.L. *et al.* (2006) Hippocampal synaptic plasticity and spatial learning are impaired in a rat model of sleep fragmentation. *Eur. J. Neurosci.* 23, 2739–2748
88 Ward, C.P. *et al.* (2009) Experimental sleep fragmentation impairs spatial reference but not working memory in Fischer/Brown Norway rats. *J. Sleep Res.* 18, 238–244
89 Hagewoud, R. *et al.* (2010) Sleep deprivation impairs spatial working memory and reduces hippocampal AMPA receptor phosphorylation. *J. Sleep Res.* 19, 280–288
90 Pierard, C. *et al.* (2007) Modafinil restores memory performance and neural activity impaired by sleep deprivation in mice. *Pharmacol. Biochem. Behav.* 88, 55–63
91 Anagnostaras, S.G. *et al.* (2001) Hippocampus and contextual fear conditioning: recent controversies and advances. *Hippocampus* 11, 8–17
92 Graves, L.A. *et al.* (2003) Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. *Learn. Mem.* 10, 168–176
93 Hagewoud, R. *et al.* (2010) Sleep deprivation impairs contextual fear conditioning and attenuates subsequent behavioural, endocrine and neuronal responses. *J. Sleep Res.* 20, 259–266
94 Ruskin, D.N. and Lahoste, G.J. (2008) Aspects of learned fear related to the hippocampus are sleep-dependent. *Behav. Brain Res.* 191, 67–71
95 Dere, E. *et al.* (2004) Higher order memories for objects encountered in different spatio-temporal contexts in mice: evidence for episodic memory. *Rev. Neurosci.* 15, 231–240
96 Halassa, M.M. *et al.* (2009) Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron* 61, 213–219
97 Palchykova, S. *et al.* (2009) Sleep deprivation in the dark period does not impair memory in OF1 mice. *Chronobiol. Int.* 26, 682–696
98 Cordova, C.A. *et al.* (2006) Sleep deprivation in rats produces attentional impairments on a 5-choice serial reaction time task. *Sleep* 29, 69–76
99 McCoy, J.G. *et al.* (2007) Experimental sleep fragmentation impairs attentional set-shifting in rats. *Sleep* 30, 52–60
100 Friedman, L. *et al.* (1979) Effects of sleep deprivation on sleepiness, sleep intensity, and subsequent sleep in the rat. *Sleep* 1, 369–391
101 Huber, R. *et al.* (2000) Topography of EEG dynamics after sleep deprivation in mice. *J. Neurophysiol.* 84, 1888–1893
102 Tobler, I. and Borbely, A.A. (1990) The effect of 3-h and 6-h sleep deprivation on sleep and EEG spectra of the rat. *Behav. Brain Res.* 36, 73–78
103 Guse, B. *et al.* (2010) Cognitive effects of high-frequency repetitive transcranial magnetic stimulation: a systematic review. *J. Neural Transm.* 117, 105–122
104 Sparing, R. and Mottaghy, F.M. (2008) Noninvasive brain stimulation with transcranial magnetic or direct current stimulation (TMS/tDCS) – from insights into human memory to therapy of its dysfunction. *Methods* 44, 329–337

105 Rudiak, D. and Marg, E. (1994) Finding the depth of magnetic brain stimulation: a re-evaluation. *Electroencephalogr. Clin. Neurophysiol.* 93, 358–371

106 Grafman, J. *et al.* (1994) Induction of a recall deficit by rapid-rate transcranial magnetic stimulation. *Neuroreport* 5, 1157–1160

107 Rami, L. *et al.* (2003) Effects of repetitive transcranial magnetic stimulation on memory subtypes: a controlled study. *Neuropsychologia* 41, 1877–1883

108 Rossi, S. *et al.* (2001) Prefrontal cortex in long-term memory: an 'interference' approach using magnetic stimulation. *Nat. Neurosci.* 4, 948–952

109 Rossi, S. *et al.* (2006) rTMS for PTSD: induced merciful oblivion or elimination of abnormal hypermnesia? *Behav. Neurol.* 17, 195–199

110 Rossi, S. *et al.* (2004) Age-related functional changes of prefrontal cortex in long-term memory: a repetitive transcranial magnetic stimulation study. *J. Neurosci.* 24, 7939–7944

111 Sandrini, M. *et al.* (2003) The role of prefrontal cortex in verbal episodic memory: rTMS evidence. *J. Cogn. Neurosci.* 15, 855–861

112 Gagnon, G. *et al.* (2010) Paired-pulse transcranial magnetic stimulation over the dorsolateral prefrontal cortex interferes with episodic encoding and retrieval for both verbal and non-verbal materials. *Brain Res.* 1344, 148–158

113 Floel, A. *et al.* (2004) Prefrontal cortex asymmetry for memory encoding of words and abstract shapes. *Cereb. Cortex* 14, 404–409

114 Mottaghy, F.M. (2006) Interfering with working memory in humans. *Neuroscience* 139, 85–90

115 Mottaghy, F.M. *et al.* (2002) Segregation of areas related to visual working memory in the prefrontal cortex revealed by rTMS. *Cereb. Cortex* 12, 369–375

116 Oliveri, M. *et al.* (2001) Parieto-frontal interactions in visual-object and visual-spatial working memory: evidence from transcranial magnetic stimulation. *Cereb. Cortex* 11, 606–618

117 Sligte, I.G. *et al.* (2011) Magnetic stimulation of the dorsolateral prefrontal cortex dissociates fragile visual short-term memory from visual working memory. *Neuropsychologia* 49, 1578–1588

118 Turatto, M. *et al.* (2004) The role of the right dorsolateral prefrontal cortex in visual change awareness. *Neuroreport* 15, 2549–2552

119 Wagner, M. *et al.* (2006) Repetitive transcranial magnetic stimulation of the dorsolateral prefrontal cortex affects divided attention immediately after cessation of stimulation. *J. Psychiatr. Res.* 40, 315–321

120 Feredoes, E.A. and Sachdev, P.S. (2006) Differential effects of transcranial magnetic stimulation of left and right posterior parietal cortex on mental rotation tasks. *Cortex* 42, 750–754

121 Postle, B.R. *et al.* (2006) Repetitive transcranial magnetic stimulation dissociates working memory manipulation from retention functions in the prefrontal, but not posterior parietal, cortex. *J. Cogn. Neurosci.* 18, 1712–1722

122 Terao, Y. and Ugawa, Y. (2006) Studying higher cerebral functions by transcranial magnetic stimulation. *Suppl. Clin. Neurophysiol.* 59, 9–17

123 Kessels, R.P. *et al.* (2000) Spatial working memory performance after high-frequency repetitive transcranial magnetic stimulation of the left and right posterior parietal cortex in humans. *Neurosci. Lett.* 287, 68–70

124 Szczepanski, S.M. and Kastner, S. (2009) Transcranial magnetic stimulation studies of visuospatial attentional control. *F1000 Biol. Rep.* 1, 81

125 Desmond, J.E. *et al.* (2005) Cerebellar transcranial magnetic stimulation impairs verbal working memory. *Ann. Neurol.* 58, 553–560

126 Minks, E. *et al.* (2010) Transcranial magnetic stimulation of the cerebellum. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.* 154, 133–139

127 Ilmoniemi, R.J. *et al.* (1999) Transcranial magnetic stimulation – a new tool for functional imaging of the brain. *Crit. Rev. Biomed. Eng.* 27, 241–284

128 Bohning, D.E. *et al.* (2000) BOLD-f MRI response to single-pulse transcranial magnetic stimulation (TMS). *J. Magn. Reson. Imaging* 11, 569–574

129 Fox, P. *et al.* (1997) Imaging human intra-cerebral connectivity by PET during TMS. *Neuroreport* 8, 2787–2791

130 Ilmoniemi, R.J. *et al.* (1997) Neuronal responses to magnetic stimulation reveal cortical reactivity and connectivity. *Neuroreport* 8, 3537–3540

131 Komssi, S. *et al.* (2002) Ipsi- and contralateral EEG reactions to transcranial magnetic stimulation. *Clin. Neurophysiol.* 113, 175–184

132 Paus, T. *et al.* (2001) Cortico-cortical connectivity of the human mid-dorsolateral frontal cortex and its modulation by repetitive transcranial magnetic stimulation. *Eur. J. Neurosci.* 14, 1405–1411

133 Paus, T. *et al.* (1997) Transcranial magnetic stimulation during positron emission tomography: a new method for studying connectivity of the human cerebral cortex. *J. Neurosci.* 17, 3178–3184

134 Siebner, H.R. *et al.* (2000) Lasting cortical activation after repetitive TMS of the motor cortex: a glucose metabolic study. *Neurology* 54, 956–963

135 Strafella, A.P. and Paus, T. (2001) Cerebral blood-flow changes induced by paired-pulse transcranial magnetic stimulation of the primary motor cortex. *J. Neurophysiol.* 85, 2624–2629

136 Mottaghy, F.M. *et al.* (2000) Modulation of the neuronal circuitry subserving working memory in healthy human subjects by repetitive transcranial magnetic stimulation. *Neurosci. Lett.* 280, 167–170

137 Mottaghy, F.M. *et al.* (2003) Modulation of a brain-behavior relationship in verbal working memory by rTMS. *Brain Res. Cogn. Brain Res.* 15, 241–249

138 Thut, G. *et al.* (2005) A new device and protocol for combining TMS and online recordings of EEG and evoked potentials. *J. Neurosci. Methods* 141, 207–217

139 Ives, J.R. *et al.* (2006) Electroencephalographic recording during transcranial magnetic stimulation in humans and animals. *Clin. Neurophysiol.* 117, 1870–1875

140 Cooper, N.R. *et al.* (2008) Effects of rTMS on an auditory oddball task: a pilot study of cortical plasticity and the EEG. *Clin. EEG Neurosci.* 39, 139–143

141 Iwahashi, M. *et al.* (2009) Effect of transcranial magnetic stimulation on P300 of event-related potential. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2009, 1359–1362

142 Okamura, H. *et al.* (2001) EEG modification induced by repetitive transcranial magnetic stimulation. *J. Clin. Neurophysiol.* 18, 318–325

143 Schutter, D.J. *et al.* (2003) High frequency repetitive transcranial magnetic over the medial cerebellum induces a shift in the prefrontal electroencephalography gamma spectrum: a pilot study in humans. *Neurosci. Lett.* 336, 73–76

144 Li, W. *et al.* (2007) Effect of chronic and acute low-frequency repetitive transcranial magnetic stimulation on spatial memory in rats. *Brain Res. Bull.* 71, 493–500

145 Ahmed, Z. and Wieraszko, A. (2006) Modulation of learning and hippocampal, neuronal plasticity by repetitive transcranial magnetic stimulation (rTMS). *Bioelectromagnetics* 27, 288–294

146 Kling, J.W. *et al.* (1990) Memory for conditioned taste aversions is diminished by transcranial magnetic stimulation. *Physiol. Behav.* 48, 713–717

147 Li, L. *et al.* (2007) The influence of low-frequency rTMS on EEG of rats. *Neurosci. Lett.* 412, 143–147

148 van der Post, J. *et al.* (2002) Evaluation of tests of central nervous system performance after hypoxemia for a model for cognitive impairment. *J. Psychopharmacol.* 16, 337–343

149 Noble, J. *et al.* (1993) Cognitive function during moderate hypoxaemia. *Anaesth. Intensive Care* 21, 180–184

150 Berry, D.T. *et al.* (1989) Isocapnic hypoxemia and neuropsychological functioning. *J. Clin. Exp. Neuropsychol.* 11, 241–251

151 Green, R.G. and Morgan, D.R. (1985) The effects of mild hypoxia on a logical reasoning task. *Aviat. Space Environ. Med.* 56, 1004–1008

152 Paul, M.A. and Fraser, W.D. (1994) Performance during mild acute hypoxia. *Aviat. Space Environ. Med.* 65 (Pt 1), 891–899

153 Kida, M. and Imai, A. (1993) Cognitive performance and event-related brain potentials under simulated high altitudes. *J. Appl. Physiol.* 74, 1735–1741

154 Tsarouchas, N. *et al.* (2008) Effects of moderate hypobaric hypoxia on evoked categorical visuocognitive responses. *Clin. Neurophysiol.* 119, 1475–1485

155 Elmenhorst, D. *et al.* (2009) Performance impairment during four days partial sleep deprivation compared with the acute effects of alcohol and hypoxia. *Sleep Med.* 10, 189–197

156 Fowler, B. *et al.* (1987) The effects of hypoxia on reaction time and movement time components of a perceptual-motor task. *Ergonomics* 30, 1475–1485

157 Fowler, B. *et al.* (1982) The effects of hypoxia on serial response time. *Ergonomics* 25, 189–201

158 Saletu, B. *et al.* (1996) Effects of the novel neuroprotective agent, riluzole, on human brain function and behavior: II. Double-blind, placebo-controlled EEG mapping and psychometric studies under hypoxia. *Methods Find. Exp. Clin. Pharmacol.* 18, 67–81

159 Van Dorp, E. *et al.* (2007) Inspired carbon dioxide during hypoxia: effects on task performance and cerebral oxygen saturation. *Aviat. Space Environ. Med.* 78, 666–672

160 Kraaier, V. *et al.* (1988) Quantitative EEG changes due to hypobaric hypoxia in normal subjects. *Electroencephalogr. Clin. Neurophysiol.* 69, 303–312

161 Saletu, B. *et al.* (1990) Brain protection of nicergoline against hypoxia: EEG brain mapping and psychometry. *J. Neural Transm. Park. Dis. Dement. Sect.* 2, 305–325

162 Van der Worp, H.B. *et al.* (1991) Quantitative EEG during progressive hypocarbia and hypoxia. Hyperventilation-induced EEG changes reconsidered. *Electroencephalogr. Clin. Neurophysiol.* 79, 335–341

163 Fowler, B. and Prlic, H. (1995) A comparison of visual and auditory reaction time and P300 latency thresholds to acute hypoxia. *Aviat. Space Environ. Med.* 66, 645–650

164 Thakur, L. *et al.* (2011) Event related potential (ERP) P300 after 6 months residence at 4115 meter. *Indian J. Med. Res.* 134, 113–117

165 Wesensten, N.J. *et al.* (1993) Effects of simulated high altitude exposure on long-latency event-related brain potentials and performance. *Aviat. Space Environ. Med.* 64, 30–36

166 Binks, A.P. *et al.* (2008) Gray matter blood flow change is unevenly distributed during moderate isocapnic hypoxia in humans. *J. Appl. Physiol.* 104, 212–217

167 Buck, A. *et al.* (1998) Changes of cerebral blood flow during short-term exposure to normobaric hypoxia. *J. Cereb. Blood Flow Metab.* 18, 906–910

168 Pagani, M. *et al.* (2000) Effects of acute hypobaric hypoxia on regional cerebral blood flow distribution: a single photon emission computed tomography study in humans. *Acta Physiol. Scand.* 168, 377–383

169 Pagani, M. *et al.* (2011) Impact of acute hypobaric hypoxia on blood flow distribution in brain. *Acta Physiol. (Oxf.)* 202, 203–209

170 Hochachka, P.W. *et al.* (1991) Metabolic and work efficiencies during exercise in Andean natives. *J. Appl. Physiol.* 70, 1720–1730

171 Shukitt-Hale, B. *et al.* (1996) Morphological alterations in the hippocampus following hypobaric hypoxia. *Hum. Exp. Toxicol.* 15, 312–319

172 Shukitt-Hale, B. *et al.* (1994) Hypobaric hypoxia impairs spatial memory in an elevation-dependent fashion. *Behav. Neural Biol.* 62, 244–252

173 Barhwal, K. *et al.* (2009) Acetyl-l-carnitine (ALCAR) prevents hypobaric hypoxia-induced spatial memory impairment through extracellular related kinase-mediated nuclear factor erythroid 2-related factor 2 phosphorylation. *Neuroscience* 161, 501–514

174 Barhwal, K. *et al.* (2007) Acetyl-L-carnitine ameliorates hypobaric hypoxic impairment and spatial memory deficits in rats. *Eur. J. Pharmacol.* 570, 97–107

175 Hota, S.K. *et al.* (2009) Bacopa monniera leaf extract ameliorates hypobaric hypoxia induced spatial memory impairment. *Neurobiol. Dis.* 34, 23–39

176 Hota, S.K. *et al.* (2008) Ceftriaxone rescues hippocampal neurons from excitotoxicity and enhances memory retrieval in chronic hypobaric hypoxia. *Neurobiol. Learn. Mem.* 89, 522–532

177 Hota, S.K. *et al.* (2007) Differential temporal response of hippocampus, cortex and cerebellum to hypobaric hypoxia: a biochemical approach. *Neurochem. Int.* 51, 384–390

178 Jayalakshmi, K. *et al.* (2007) N-acetyl cysteine supplementation prevents impairment of spatial working memory functions in rats following exposure to hypobaric hypoxia. *Physiol. Behav.* 92, 643–650

179 Maiti, P. *et al.* (2008) Hypobaric hypoxia induces dendritic plasticity in cortical and hippocampal pyramidal neurons in rat brain. *Behav. Brain Res.* 189, 233–243

180 Muthuraju, S. *et al.* (2009) Acetylcholinesterase inhibitors enhance cognitive functions in rats following hypobaric hypoxia. *Behav. Brain Res.* 203, 1–14

181 Shukitt-Hale, B. *et al.* (1996) Tyrosine administration prevents hypoxia-induced decrements in learning and memory. *Physiol. Behav.* 59, 867–871

182 Titus, A.D. *et al.* (2007) Hypobaric hypoxia-induced dendritic atrophy of hippocampal neurons is associated with cognitive impairment in adult rats. *Neuroscience* 145, 265–278

183 Zhang, J.X. *et al.* (2006) Intermittent hypoxia impairs performance of adult mice in the two-way shuttle box but not in the Morris water maze. *J. Neurosci. Res.* 84, 228–235

184 Udayabanu, M. *et al.* (2008) Nitric oxide associated with iNOS expression inhibits acetylcholinesterase activity and induces memory impairment during acute hypobaric hypoxia. *Brain Res.* 1230, 138–149

185 Bekker, A. *et al.* (2007) Physostigmine reverses cognitive dysfunction caused by moderate hypoxia in adult mice. *Anesth. Analg.* 105, 739–743

186 Bekker, A. *et al.* (2006) Isoflurane preserves spatial working memory in adult mice after moderate hypoxia. *Anesth. Analg.* 102, 1134–1138

187 Haile, M. *et al.* (2009) Nimodipine prevents transient cognitive dysfunction after moderate hypoxia in adult mice. *J. Neurosurg. Anesthesiol.* 21, 140–144

188 Viu, E. *et al.* (2000) Glycine(B) receptor antagonists and partial agonists prevent memory deficits in inhibitory avoidance learning. *Neurobiol. Learn. Mem.* 74, 146–160

189 Platt, B. *et al.* (2010) Epidural EEG recordings using microchips in behavioural context. *Proceedings of Measuring Behavior*

190 Ravasi, L. *et al.* (2011) Use of [18F]fluorodeoxyglucose and the ATLAS small animal PET scanner to examine cerebral functional activation by whisker stimulation in unanesthetized rats. *Nucl. Med. Commun.* 32, 336–342