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## THERAPIES

### HEADING: Neuropsychopharmacology

## Contributions of animal models of cognitive disorders to neuropsychopharmacology

Cognitive models of brain diseases

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## **Abbreviations**

3-NP: 3-nitropropionic acid

6-OHDA: 6-hydroxydopamine

AChEI: acetylcholinesterase inhibitors

ADHD: attention deficit/hyperactivity disorder

AMPA: alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate

ARB: angiotensin type 1 receptor blocker

BDNF: brain-derived neurotrophic factor

cAMP: cyclic adenosine monophosphate

CAT: catalase

cGMP: cyclic guanosine monophosphate

CNS: central nervous system

COX-1: cyclooxygenase-1

CREB: cAMP response element binding protein

CRP: C-reactive protein

DRD1: dopamine receptor D1

GPx: glutathione peroxidase

H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide

HO·: hydroxyl radical

ICV injection: intracerebroventricular injection

IL-1 $\beta$ : interleukine-1  $\beta$

IL-6: interleukine-6

IVIG: intravenous immunoglobulin

LPS: lipopolysaccharide

MCI: mild cognitive impairment

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

mTOR: mammalian target of rapamycin

NAC: N-acetylcysteine

NMDA receptor: N-methyl-D-aspartate receptor

NSAIDs: non-steroidal anti-inflammatory drugs

O<sub>2</sub><sup>-</sup>: superoxide radical

PCP: phencyclidine

PDE: phosphodiesterase

PolyI:C: polyriboinosinic:polyribocytidylic acid

PPAR  $\gamma$ : peroxisome proliferator-activated receptor  $\gamma$

ROS: reactive oxygen species

SOD: superoxide dismutase

Trk B: tropomyosin receptor kinase B

TNF-  $\alpha$ : tumor necrosis factor-  $\alpha$

## **Summary**

Cognitive disorders and symptoms are key features of many mental and neurological diseases, with a large spectrum of impaired domains. Because of their possible evolution and detrimental functioning impact, they are a major pharmacological target for both symptomatic and disease-modifier drugs, while few cognitive enhancers have been marketed with an insufficient efficiency. It explains the need to model these cognitive disorders beyond the modelization of mental or neurological diseases themselves. According to the experimental strategy used to induce cognitive impairment, three categories of models have been identified: neurotransmission-driven models; pathophysiology-driven models; environment-driven models. These three categories of models reflect different levels of integration of endogenous and exogenous mechanisms underlying

cognitive disorders in humans. Their comprehensive knowledge and illustration of their pharmacological modulation could help to propose a renewing strategy of drug development in central nervous system (CNS) field at a time when the academic and industrial invest seems to be declining despite the medical and social burden of brain diseases.

## **KEYWORDS**

Cognitive disorders; Animal model; Drug development; Neurotransmission; Disease modifier

For a long time outside the scope of therapeutic strategies for central nervous system (CNS) disorders, cognitive deficits are now considered as core feature of many psychiatric and neurological diseases including Alzheimer's disease, Parkinson's disease, schizophrenia, major depression disorder, bipolar disorders... The last decade, awareness of their negative impact on global functional outcomes, quality of life and patient recovery has grown. The spectrum of cognitive manifestations may affect different domains including working memory, episodic memory, visual and verbal memory, attention, cognitive flexibility, and various other aspects of executive functioning [1]. The clinical picture of cognitive deficits is specific for each pathology depending on underpinned cerebral damages, and sometimes difficult to establish because of the confounding influence of coexisting behavioral changes. However, despite this relative specificity, some overlap in the impaired cognitive domains have been emphasized between neuropsychiatric diseases, suggesting common dysregulating patterns.

To date, this cognitive impairment remains untreated and represents a real burden for patients. Development of efficient cognitive enhancing therapies requires the development of validated and reliable animal models whose cognitive deficits can be targeted or corrected by pharmacological intervention. Cognitive disorders are the result of a set of interconnected complex mechanisms associated with a disease, difficult to translate in animals. The objective of animal modeling have long consisted in dissecting complex pathophysiological mechanisms into simple elements easier to mimic in animals. In pharmacological research the objective of animal model is different with the need of reproducible tasks mimicking elemental phenotypes to compare drug candidates in early phase of development.

The first step in the process of building such a model is to define the therapeutic purpose and more specifically the strategy envisaged to normalize cognitive deficits. The symptomatic approach aims to alleviate symptoms by restoring neurochemical brain balance without addressing the causal mechanisms. The other approach aims to counteract the pathogenic mechanisms to slow or reverse the progression of brain damage and relieve the associated symptoms. Both approaches require the use of distinct animal models ranging from experimental manipulations to elicit analogous cognitive symptoms to those observed in humans, to the modeling of a specific pathophysiological process likely to play a direct or indirect role in the onset and the course of the cognitive deficit. However, this distinction between symptomatic and disease-modifying approaches, although relevant in the

modeling strategy, may become arbitrary with respect to therapeutic agents since some agents may have both properties.

It is according to this transnosological approach (focusing on the common neurobiological and pathological mechanisms related to cognitive symptoms in diseases rather than on the specific feature of each) that we wished to address rodent animal modeling dedicated to the development of cognition-enhancing drugs for CNS disorders. We classified these models into three categories according to the experimental strategy used to induce cognitive impairment: 1- Neurotransmission driven models, 2- Pathophysiology driven and 3- Environmental driven models. These three categories of models reflect different levels of integration of endogenous and exogenous mechanisms underlying cognitive disorders in humans.

### **Neurotransmission driven models**

The first category of animal models designed to mimic cognitive impairment mainly derived from clinical observations that pointed out 1- the deleterious effects of neuropsychotropic drugs on cognitive performances and 2- changes in the volume or neurotransmitter content of brain areas associated with cognitive processes. Although the exact neurobiological bases underlying cognitive component of neuropsychiatric disorders remain unclear and the selectivity of pharmacological agents too low or weak to conclude, three main avenues of research have emerged to model the main cognitive disturbances encountered. The experimental design of these models mainly focused on the modulation of cholinergic, glutamatergic and dopaminergic neurotransmission systems, in accordance with the common pathological features clinically described, although many other neurotransmitters participate in the regulation of cognitive functions.

### **Cholinergic modulation based models**

The rationale for the use of cholinergic modulation to induce cognitive impairment in rodents mainly arises from the histological description of a substantial loss of cholinergic neurons in the basal forebrain in the early stage of Alzheimer's disease [2]. The resulting changes in cholinergic content within target regions (the cerebral cortex, the amygdala and the hippocampus) have supported the hypothesis that a cholinergic deficit in key brain areas could at least partly be linked to the onset of cognitive decline. This relationship has been further supported by the reversible negative impact of anticholinergic drugs such as scopolamine on cognitive performance in humans, reminiscent of the cognitive changes seen in Alzheimer's disease [3]. Far from being restricted to Alzheimer's disease, clinical data suggested a role of the cholinergic system in cognitive impairment in other CNS pathologies including Parkinson's disease, schizophrenia, mood disorders... In particular, abnormalities of cholinergic systems have been widely associated with attentional deficits observed in schizophrenic patients [4]. More broadly, muscarinic and nicotinic receptors dysregulation might compromise attention, working memory and executive functions [5,6]. These findings were consistent with clinical evidence of a beneficial effect of nicotine consumption on multiple cognitive domains impaired in schizophrenic patients as well as in healthy subjects in particular in those displaying low cognitive performance [7–9].

These clinical data have stimulated the development of animal models of cholinergic disruption using a variety of experimental methods to characterize the spectrum of cognitive deficits related to cholinergic deficit. For example, lesions of the basal forebrain cholinergic system were shown to disrupt attentional processing, spatial reference memory, working memory and recognition memory to varying degrees depending on the extent of cholinergic damage (basocortical pathways and/or septo-hippocampal pathways) [10–15]. Similar patterns of cognitive impairment have been obtained in pharmacological-based animal models using muscarinic or nicotinic cholinergic receptors antagonists (scopolamine and mecamylamine respectively) suggesting the involvement of both cholinergic systems in the regulation of normal cognitive functioning [16–20]. The scopolamine-induced cognitive impairment model has been particularly studied in rodent because of its well documented human counterpart. Interestingly, the use of scopolamine in animals has highlighted that learning and impairment are also triggered by primary effects including attentional deficits and sensory discrimination impairment beyond memory itself [17,21]. This first generation of animal models have contributed to the emergence of acetylcholinesterase inhibitors (AChEI) including donepezil, rivastigmine and galantamine. However, despite the beneficial effects obtained in cholinergic and non-cholinergic animal models of cognitive disorders, AChEI have demonstrated limited therapeutic efficacy in the management of



cognitive symptoms in patients with Alzheimer's disease. Although initially dedicated to Alzheimer's disease patients, the cognitive effects of AChEIs have been studied in other pathologies showing mixed degrees of efficacy [22]. In Parkinson disease's patients with mild cognitive impairment, clinical studies tend to show a beneficial effect on cognitive performances. In patients with schizophrenia, the majority of clinical studies have demonstrated no significant improvement suggesting that AChEIs have at most only a marginal effect on neurocognitive functions. In addition, in patients with bipolar disorder efficacy of AChEIs remains uncertain due to methodological concerns and clinical trials with larger sample sizes should be considered.

Supported by clinical imaging advances and experimental knowledge, new drug candidates targeting specific nicotinic ( $\alpha 4\beta 2$  or  $\alpha 7$ ) and muscarinic (M1) receptors subtypes have been developed and tested in animal models. Despite promising effect on animal models and in early clinical trials, to date, full or partial agonists developed to target subtypes of cholinergic receptor (encenicline, xanomeline...) failed to meet the primary cognitive endpoint in larger phase 3 trials in Alzheimer's and schizophrenic patients [23,24]. However, some authors raised the critical issue of receptor desensitization and inadequate doses to achieve therapeutic efficacy, supporting the assessment of a wide dose range in further clinical studies [23]. Another approach has been developed based on an amplification of cholinergic transmission through glial connexin modulation [25]. Research has gradually moved towards the development of positive allosteric modulators, suspected to be less prone to cause receptor desensitization.

### **Glutamatergic modulation-based models**

As one of the alternative approaches to cholinergic modulation, cognitive disorder resulting from changes in glutamatergic neurotransmission have been extensively studied in animals. The prominent role of glutamate receptors in the neurobiological mechanisms of synaptic plasticity underlying learning and memory processes, prompted the construction of animal models with glutamatergic manipulations. In addition, a large number of clinical studies highlighted dysfunction of the glutamatergic system in psychiatric and neurodegenerative diseases related to alteration in glutamate clearance and metabolism or dysregulation of glutamate receptors in a number of brain areas mediating cognitive behavior. The relevance of using N-methyl-D-aspartate receptor

(NMDA) receptor antagonists to model cognitive symptoms in animals has been further supported by clinical evidence suggesting NMDA receptor hypofunction in many of these disorders (schizophrenia, depression, Alzheimer's disease ...) and memory loss observed following the administration of certain types of anesthetics (i.e. phencyclidine [PCP] and ketamine ) that act as NMDA receptor channel blockers [26,27].

Such antigitamatergic pharmacological agents produce a myriad of behavioral effects ranging from selective memory deficits in response to low doses to a wide range of behavioral changes evoking core schizophrenia-like symptoms for higher doses [28,29]. In rodent, acute and sub-chronic administration of non-competitive NMDA antagonists including PCP and ketamine, have been shown to produce deficits in spatial memory, recognition memory, working memory, attention and cognitive flexibility [30,31]. Local infusion of NMDA antagonists AP-5 in specific brain regions have highlighted the crucial role of NMDA signaling within 1) amygdala, hippocampus, medial septum and entorhinal cortex in memory processes and 2) medial prefrontal cortex, dorsomedial striatum and nucleus accumbens in working memory, attention and cognitive flexibility [32–34]. Other methods modulating glutamatergic activity via genetic deletion of NMDA receptor subunits as well as the administration of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) antagonists (e.g. NBQX, LY293558) or selective modulators of certain metabotropic receptor subtypes (e.g. MPEP, mGlu5-receptor antagonist) have all been shown to induce cognitive impairment in rodents [35]. These models and in particular NMDA receptor antagonist-based models have been widely used to assess symptomatic therapeutic strategies aiming to rescue glutamate function. Due to risks of seizure or excitotoxicity therapeutic strategies early focused on the modulation of allosteric or co-agonist binding sites to facilitate NMDA receptor activity [36]. Various pharmacological agents stimulating the glycine site either directly via agonist/partial agonist or indirectly through the glycine transporter GlyT-1, obtained convincing results in animals that failed to be confirmed in Alzheimer's disease and schizophrenic patients. However, some studies suggested a promising effect on cognitive function in major depressive disorder [37–39].

The targeting of other ionotropic or metabotropic glutamatergic receptors exhibiting close functional coupling with NMDA receptor subtypes within the synapse such as the AMPA receptor and mGluR5 receptor, has opened new therapeutic avenue for the indirect stimulation of NMDA receptor activity. While the clinical evaluation of positive modulators of AMPA-type glutamate receptors with AMPAkinases has not demonstrated any major improvement in cognitive performance in patients, the mGluR5 positive allosteric modulators are still in the preclinical validation stage.

However, the preclinical results currently available show a good propensity to alleviate cognitive impairment induced by glutamatergic or non-glutamatergic modulation [40,41]

A new line of research is currently underway targeting the kynurenin pathway of tryptophan metabolism. This signaling pathway is altered in various disorders including schizophrenia, autism spectrum disorders, Huntington's disease and dementia, and is suspected to be involved in the cognitive symptoms. The kynurenin pathway generates a series of neuroactive compounds, including quinolinic acid and kynurenic acid, which can accumulate in the brains of patients and participate in cognitive impairment by altering the degree of activation or blockade of NMDA receptors. The preclinical works have demonstrated the relevance of pharmacological modulation of the kynurenin pathway, primarily by enzymatic inhibition, for the treatment of cognitive dysfunction in CNS disorders [42].

It is important to remember that both a deficit and an excess of glutamate can induce cognitive impairment through different neurochemical mechanisms. In animals, excessive levels of glutamate in the synaptic cleft, triggered by the blockade of glutamate transporters, compromises the phasic activation of NMDA receptors in favor of tonic activation, which leads to an alteration of synaptic plasticity processes and memory performance. Memantine, a non-competitive NMDA receptor antagonist currently prescribed in advanced stages of Alzheimer's disease, has been shown to decrease the level of cognitive decline in Alzheimer's disease patients by counteracting NMDA receptor overstimulation without affecting the physiological glutamatergic activity required for synaptic plasticity [43]. In rodent, Bali et al. demonstrated that memantine could alleviate spatial working memory deficit induced by scopolamine, however the authors suggested that this effect may rely on a direct action on  $\alpha 7$  nicotinic acetylcholine receptors [44].

### **Dopaminergic modulation-based models**

The frequent occurrence of cognitive disorders in pathologies where the dopaminergic system is damaged including schizophrenia, attention deficit/hyperactivity disorder (ADHD) and Parkinson's disease, has drawn particular attention to this system. In these pathologies, disturbances in cognitive functions may include executive functions, working memory, attention and visuospatial processing depending on the dopaminergic pathway mainly affected. The dopaminergic theory of cognitive

disorder has also been supported by the observation of a deficit in visuospatial and executive functions in humans intoxicated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a parkinsonian syndrome resulting from a pure dopamine deficiency [45]. In addition, in a genetic association studies performed on a large postmortem cohort of cases with Alzheimer's disease and schizophrenia, Tsang et al. highlighted a relationship between the dopamine receptor D1 (DRD1) polymorphism and the cognitive performance [46].

Based on this clinical evidence, animal models have been developed using neurotoxins capable of selectively destroying dopaminergic neurons such as 6-hydroxydopamine (6-OHDA) or MPTP. Animal models of nigro-striatal denervation induced by bilateral stereotaxic injections of 6-OHDA in striatum have been shown to recapitulate most of cognitive impairment observed in Parkinson's disease patients affecting spatial memory, working memory, sustained attention, and cognitive flexibility while unilateral 6-OHDA-induced medial forebrain bundle lesion only partially replicate cognitive deficits [47,48]. On the other hand, bilateral lesion of the dopaminergic input to the prefrontal cortex has been shown to impair attention, working memory and cognitive flexibility [49,50]. These findings highlighted the crucial modulatory role of dopamine within the prefrontal and striatal regions, receiving abundant innervation from dopaminergic neurons, which together through the frontostriatal loops participate in the regulation of executive functions and working memory. However, a limit in efficacy of dopamine modulation is explained by the observation that the corrective action of pharmacological agents on dopaminergic deficits within the injured brain area may cause simultaneously excessive dopaminergic stimulation in relatively spared regions resulting in related cognitive impairment. This phenomenon is particularly related to cognitive functions regulated by D1 receptors appear to be particularly concerned. Yet, D1 dopaminergic receptors have been shown to play a key role in modulating the functioning of the prefrontal cortex network and related cognitive functions [51]. Preclinical evidence highlighted a dose-dependent beneficial effect of D1 receptor agonists on cognitive performance following an inverted U-shaped response curve [52]. Thus, only an intermediate concentration is optimal, while a deficit or excess has a deleterious effect on cognition. Therefore, the experimental approach to develop new treatments modulating the dopaminergic system favoured the use of healthy animals with poor cognitive performance or elderly animals with natural diffuse dopaminergic deficiency. In such model, D1 receptor agonists have been shown to compensate for prefrontal dopamine deficiency and to alleviate associated working memory impairment [53]. More recently a direct role for D<sub>5</sub> dopamine receptors in prefrontal cortex-dependent cognitive functions have been demonstrated in transgenic mice models [54]. These preclinical data paved the way for the development of partial

D1/D5 receptor agonists to improve working memory performance. However, to date, these drug candidates have not provided clinical evidence of efficacy in healthy volunteers [55].

### **Critical approach of neurotransmission-driven models**

For the sake of clarity, the animal modeling approach described above appears somewhat monolithic that is not. In fact, animal models based on the alteration of a particular neurotransmission system are not exclusively dedicated to the development of pharmacological agents specifically targeting the deregulated system that generated the cognitive symptoms.

The homeostasis of cognitive functions is ensured by complex neural networks involving different neurotransmission systems. The deregulation of one of the actors in this equilibrium can compromise, when not physiologically compensated, this homeostasis and consequently disrupt the overall functioning of the network and the related functions. As an example, in a comprehensive review which compiled preclinical data from pharmacological modulation and central neurochemical lesion studies, Robbins highlighted the close interplay of monoaminergic and cholinergic systems, in the control of distinct aspects of attention processes [56]. In the same line of evidence, some authors have found that the detrimental effect of ketamine on working memory in rodents may result from 1) a direct effect on the glutamatergic system resulting in an increased glutamate release within the prefrontal cortex and 2) an indirect effect via the alteration in glutamatergic firing rates, on the dopaminergic transmission [57]. As a consequence, both glutamatergic and dopaminergic (by D1 receptor agonists) therapies have been shown to improve ketamine-induced working memory deficits [58].

Furthermore, in a recent study, a novel pharmacological modulator of the NMDA receptor, NYX-458, demonstrated its efficacy in improving performance in several cognitive domains (attention, working memory, and executive function) of MPTP-treated non-human primates [59]. These few examples, and many others, show that the deficit of one system can be compensated by pharmacological intervention on another system.

This also implies that a seemingly similar cognitive symptom may be underpinned by distinct neurobiological alterations and a drug candidate will have all the more chances of clinical success if it shows an ability to restore cognitive impairments induced by distinct mechanisms. In a

comparative study, Bali et al. examined the cognitive enhancing potential of a selective  $\alpha 7$  nicotinic receptor agonist on working memory deficits induced by scopolamine or MK-801 [60]. They pointed to better efficacy on working memory impairment caused by cholinergic dysfunction rather than glutamatergic receptor blockade. Furthermore, Unal et al. demonstrated a differential efficacy profile of the  $\alpha 7$  nicotinic receptor agonist, positive allosteric modulators, and antipsychotic drug (clozapine) on the range of cognitive deficits induced by MK-801 administration [61]. These results suggest that pharmacological agents might exhibit a specific pattern of cognitive improvement rather than a complete cognitive benefit over the entire spectrum of cognitive deficits associated with a model and could therefore be complementary in the management of cognitive disorders.

### **Pathophysiology driven models**

Another experimental approach to build animal models dedicated to the development of cognition-enhancing drugs consists in reproducing in animals the pathogenic processes highlighted in neurologic and psychiatric pathologies likely to participate in brain tissue damage. Irrespective to their origin, some common pathogenic mechanisms involved in the onset and course of the diseases have been identified which may partly underpin neuropsychiatric diseases-relevant cognitive impairments. Among these, inflammation, oxidative stress and neuroplasticity failure involved as factors or correlates in the pathophysiology of many CNS disorders have been commonly implicated as cognitive modulators. In most cases biomarkers related to these pathological processes which participate in the deterioration of neuronal circuits, coexist and are interconnected. For example, the longitudinal epigenetic study lead on at-risk mental state individuals (from the ICAAR cohort) has demonstrated that specific dysmethylation in genes involved in axon guidance, redox metabolism and inflammation could contribute to the risk of conversion to psychosis [62]. By replicating these common pathogenic mechanisms in animals, the objective has been to develop molecules that target the mechanisms that cause cognitive disorders and not just the symptoms. The neuroprotective strategy may involve interfering with processes that negatively affect brain functioning and neuronal integrity and/or promoting protective and repair mechanisms to slow disease progression and, ideally, reverse it for regeneration. We propose to distinguish three kinds of pathophysiology-driven models but it should keep in mind that many molecules tested have pleiotropic effects on the different pathophysiological pathways.

## **Inflammatory challenge models**

Inflammation have been strongly implicated in the development and pathogenesis of various neuropsychiatric disorders such as major depression, bipolar disorders, schizophrenia and dementia. Clinical evidence have pointed to several links between inflammatory markers and cognitive status of patients. For example, clinical studies in patients with schizophrenia supported a positive correlation between the severity of cognitive deficits and levels of inflammatory markers [63]. In addition, this link has been demonstrated in preclinical studies which provided evidence of the major role of pro-inflammatory cytokine such as interleukine-1  $\beta$  (IL-1 $\beta$ ), interleukine-6 (IL-6), tumor necrosis factor-  $\alpha$  (TNF- $\alpha$ ) and C-reactive protein (CRP) in molecular and cellular mechanisms subserving cognitive processes including synaptic plasticity, neurogenesis and neuromodulation [64]. The development of animal models of inflammation are based on the induction of a peripheral immune challenge or central inflammatory stress triggered by administration of endotoxin (lipopolysaccharide [LPS] and polyriboinosinic:polyribocytidylic acid [PolyI:C]) or neurotoxin (streptozotocin, okadaic acid, and colchicine) respectively. These pro-inflammatory agents induce the production of proinflammatory mediators resulting in neuronal damage and astrogliosis associated with neuroplasticity impairment and cognitive decline. LPS-induced neuroinflammation model is the most commonly used in rodent and is associated with oxidative stress and microglial activation, which stimulate inflammatory cytokine signaling acting as neurotoxic factors. In this model, animals develop cognitive impairment at variable time point after induction of neuroinflammatory stress [65]. Repeated systemic administration of LPS have a negative impact on cognitive processes that depend upon the hippocampus including contextual memory, spatial memory, and recognition memory as well as the prefrontal cortex-dependent attentional function. In some models, LPS is injected stereotaxically into lateral ventricle or directly in specific brain areas to assess regional difference in susceptibility to LPS-induced neurotoxicity and assess related cognitive deficit.

Supported by epidemiological studies showing a lower rates of Alzheimer's disease among people chronically treated by non-steroidal anti-inflammatory drugs (NSAIDs), numerous therapeutic strategies aimed at reducing the neuroinflammatory process to improve cognitive performances have been developed in animals. In addition to selective or non-selective

cyclooxygenase (COX) I and II inhibitors, other approaches have been deployed to assess the beneficial effects of glucocorticoids, TNF- $\alpha$  inhibitors, interferon- $\beta$ 1a or intravenous immunoglobulin (IVIG) therapies on cognitive impairment [66,67]. However, despite convincing results in animals, clinical trials have failed to report significant efficacy to slow the progression of cognitive symptoms in patients with mild to moderate Alzheimer's disease. It is interesting to note that several drugs, initially considered symptomatic drugs, have shown neuroprotective properties in animal models of neuroinflammation. These include AChE inhibitors, glutamatergic modulators and nicotinic  $\alpha$ 7 agonists. For example, Liu et al. indicated that galantamine may prevent the spatial and associative memory deficit induced by intracerebroventricular (ICV) injection of LPS, by preventing microglial and astrocyte activation in the hippocampus and the downstream pro-inflammatory signaling cascade [68]. Similarly, using a model of bilateral hippocampal injection of LPS, it has been shown that minocycline, a tetracycline antibiotic, can alleviate disorders of spatial reference memory and working memory according to a similar mechanism [69]. Based on the promising anti-inflammatory and pro-cognitive effects described in animals, minocycline was then evaluated in humans with a view to mitigate cognitive deterioration in pathologies such as Alzheimer's disease and schizophrenia. In a meta analysis, Cho et al. highlighted a cognitive enhancing effect of minocycline in various cognitive domains i.e. attention, memory and executive function, in early stage schizophrenic patients [70]. In contrast no cognitive improvement have been observed in mild Alzheimer's disease patients [71].

### **Oxidative challenge model**

Often associated with inflammation process, cerebral oxidative stress occurs when the production of prooxidants such as reactive oxygen species (ROS) exceed the antioxidant capacity. This imbalance leads to the accumulation of oxidative damage like oxidized proteins, glycated products, and lipid peroxidation which can trigger degenerative processes, neurotransmitter dysregulation and functional decline including cognitive impairment. Clinical evidence suggested a role of free radicals and antioxidants in the pathogenesis and progression of numerous psychiatric and neurodegenerative disorders [72]. Some brain regions have been described as particularly susceptible to oxidative stress including hippocampus, amygdala, and prefrontal cortex which may explain functional consequences in particular cognitive and emotional phenotypes. In these regions,



high ROS concentrations reportedly decrease LTP and synaptic signalling mechanisms leading to a reduction in brain plasticity [73]. As oxidative stress can result either from depletion of the antioxidant system including superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), or from overproduction of pro-oxidants such as hydroxyl radical (HO·), superoxide radical (O<sub>2</sub>·-) or hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), animal models have tried to mimic these two phenomena.

Both the suppression of antioxidant defense using chemical or genetic tools (e.g. buthionine-sulfoximine or heterozygous SOD2<sup>+/-</sup> knockout mice) and the brain induction of ROS through the injection of pro-oxidant agents (e.g. menadione, 3-nitropropionic acid [3-NP], paraquat) or intracerebral administration of oxidizing agent (e.g. OH·, H<sub>2</sub>O<sub>2</sub>) are able to induce cognitive disorders in animals. In addition, certain metals such as iron and copper may act as catalysts in the oxidative reactions of biological macromolecules leading to increased production of ROS. Well known to participate in pathogenic mechanisms in neurodegenerative diseases, the role of brain iron accumulation has also been suggested in a subset of neuropsychiatric pathologies and more broadly in cognitive dysfunction [74,75]. Recently post-mortem tissue analysis and imaging studies have reported a correlation between iron load in regions of the brain and cognitive decline in Alzheimer's patients [76,77]. Furthermore, iron-mediated redox stress in animal is associated with memory impairment related to dysregulation of synaptic, mitochondrial and cytoskeletal proteins [78].

From these animal models several strategies have been tested to either manage excess ROS levels through ROS-scavenging mechanisms or to reduce accumulation of redox-active transition metals using chelators. Most of free radical scavengers that demonstrated a beneficial effect on oxidative stress and cognitive deficit in animal models have yielded mostly disappointing results in clinical studies [79]. However, some compounds such as N-acetylcysteine (NAC) an antioxidant precursor molecule which has been shown to exhibit anti-oxidant and anti-inflammatory properties, have provided conflicting results in patients with neurological or psychiatric disease and continue to attract interest [80]. A recent clinical study have suggested that NAC may be a promising adjunctive treatment option to manage cognitive symptoms of schizophrenia [81]. Although the evidence linking iron accumulation to neurodegenerative processes goes beyond its ability to generate ROS, iron chelators have been shown to reduce oxidative damage and cognitive impairment in various animal models of neurodegenerative diseases. Deferiprome, an orally active iron-chelating agent, has been shown to have a beneficial effect on the cognitive abilities of rodents subjected to oxidative stress induced by iron overload as well as in animal models of Alzheimer's disease or Parkinson's disease [82–84]. A phase 2 study is ongoing to investigate the safety and efficacy of

deferiprone in participants with prodromal Alzheimer's disease and mild Alzheimer's (Disease ClinicalTrials.gov Identifier: NCT03234686).

### **Neuroplasticity challenge models**

The idea of harnessing neuroplasticity mechanisms to prevent cognitive decline naturally arose from a growing body of evidence pointing to a loss of adaptive capacity and repair in brain of patients with neuropsychiatric disorders. Neuroplasticity concept involve different phenomenon including synaptic plasticity, cellular growth and remodeling, and neurogenesis that have been shown to participate in key aspects of CNS development and repair. These processes also subserve memory formation as well as higher order cognitive functions allowing appropriate adaptation of neuronal systems to environmental changes. Markers of altered neuroplasticity have been found in most of CNS pathologies. As a result, the occurrence of failure of neuroplasticity basic mechanisms that underly psychiatric and neurodegenerative diseases might represent a context of vulnerability to environmental injuries conducive to the emergence of brain damage but also directly interfere with cognitive processes. In this regard, animal models of neuroplasticity impairment may be useful for investigating whether pro-neurogenic compounds might hold promise for ameliorating cognitive manifestations.

The cellular and molecular processes controlling neuroplasticity have been widely studied in animal models in particular those regulating cognitive processing. Among these, brain-derived neurotrophic factor (BDNF) has proven to promote a wide variety of neuroplastic mechanisms such as neurogenesis, synaptic strengths, axonal and dendritic growth and neuronal survival. Changes in BDNF levels is a common feature to many neurological and psychiatric disorders [85]. Clinical data have reported impairment in cognitive status associated with an increased psychiatric disorder susceptibility in individual with BDNF single-nucleotide polymorphism (BDNF Val66Met) leading to decreased BDNF secretion [86]. In addition, in patients with MDD, higher levels of miR-132 (miRNA known to affect the expression of BDNF) were significantly correlated with visual memory performance [87]. These data provided evidence of a functional role of BDNF in human memory functions, in particular those depending from brain regions expressing highest levels of BDNF including the prefrontal cortex and hippocampus. In a recent article compiling clinical and

preclinical data, Miranda et al. have proposed that BDNF expression level could be a marker that specifically relates to the occurrence and/or progression of the mnemonic symptoms in those diseases [88]. In vivo and in vitro experimental studies have highlighted the role of cAMP response element binding protein (CREB) family transcription factors in the regulation of BDNF level and the involvement of tropomyosin receptor kinase B (Trk B) receptors signaling in mediating cellular effects. On these bases, transgenic mouse models with modified levels of BDNF or TrkB receptors signaling have been created and have contributed to establish a link between dysregulation of BDNF signaling trafficking and cognitive performances [89]. While BDNF heterozygous knock out mice investigate the developmental effect of general lower of BDNF levels (relevant to certain psychiatric disorders), animal models using infection with a BDNF-expressing lentivirus have been designed to investigate the consequences of regional deletion of BDNF in adult animals [90]. Although these models express cognitive impairment, they have been mainly used as mood disorders model to test the beneficial effect of antidepressant drugs on neuroplasticity mechanisms and associated symptoms [91].

Nevertheless, these models have contributed to stimulate research on growth factor therapy applied to neurodegenerative and psychiatric pathologies, in an attempt to both slow down cell death and improve the functional state of remaining neurons by restoring appropriate neurotrophic support. However, despite the therapeutic potential demonstrated by preclinical studies using neurodegenerative animal models, clinical research has been faced with the challenge of CNS delivery [92]. As an alternative some neuropsychiatric drugs belonging to the family of antidepressants, mood stabilizers, antipsychotics but also ampakines have been shown to increase endogenous BDNF level in the brain and improve memory performances in animal models of cognitive disorders [93–100]. The beneficial effects of lithium on hippocampal neurogenesis and neurotrophic factor regulation in animal models have led to the initiation of a clinical trial currently underway to test the preventive effect of lithium in mild cognitive impairment (MCI) subjects. On the other hand, the latter did not provide any significant improvement in Alzheimer's disease patients (ClinicalTrials.gov Identifier: NCT03185208).

## **Environmental driven models**

The last decade has seen the emergence of a new class of models mainly inspired from environmental conditions likely to generate transient or permanent cognitive disorders in humans. The use of environmental factors has the major advantages of not focusing on a single mechanism and avoiding the use of pharmacological tools as inducer. On the other hand, these models have an interesting translational value since these factors have been identified in humans before being transferred to animals. In this section, we will discuss only some examples of factors that have been experimentally applied to induce 1- transient cognitive impairment suitable to evaluate symptomatic molecules 2- permanent cognitive impairment suitable to develop treatments with disease-modifying aims.

### **Models of transient cognitive impairment**

Like pharmacologically induced cognitive deficits, certain experimental conditions are capable of triggering an imbalance in neurotransmitters levels leading to transient cognitive impairment. Among them, acute hypoxia (defined as a brief exposure to low oxygen levels in the air) and acute sleep deprivation (sleep deprivation for a short period of time) could represent relevant challenge models. These environmental conditions can induce, both in humans and in animal models, transient cognitive deficit that share some similarities with the pattern of cognitive impairment observed in Alzheimer's disease. Neurophysiological underpinnings of transient cognitive impairment triggered by hypoxia or sleep deprivation mainly involve reversible change in neurotransmitter levels.

Brief changes in environmental oxygen in rodents have been shown to early generate alterations in brain acetylcholine levels and to some extent monoamine synthesis through the negative regulation of limiting enzymes requiring O<sub>2</sub> supply to be active [101,102]. These biochemical changes underlie the spatial working memory impairment that have been shown to correlate with hypoxia levels [103]. This finding is supported by the beneficial effects of AChE inhibitors such as physostigmine, which reverses working memory impairment induced by hypoxia [104].

On the other hand, sleep deprivation leads to elevated levels of excitatory neurotransmitters and dysregulation in other neuromodulators that affect neuronal functioning within the cerebral

networks involved in attentional processes [105,106]. In human, it has been shown that one night of sleep deprivation reduced several specific components of basic cognitive processes, including components of attention and executive functions [107,108]. Sleep deprivation-induced cognitive deficits can be reversed by a wide range of psychotropic drugs with distinct mechanisms of action including donepezil, nicotine, modafinil and melatonin, supporting the involvement of several neurotransmission systems [109]. In addition, caffeine have been shown to prevent sleep deprivation-induced cognitive impairment in rodents that are consistent with the positive effects of caffeine administration in animal models of neuropsychiatric diseases as well as in sleep deprived healthy volunteers [110]. A few days of exposure to sleep restriction or hypoxia were also used experimentally to model cognitive impairment associated with structural and functional changes resulting from neuroplastic and vascular damage. Such sleep restriction-induced alteration has been shown to be reversed by rapamycin, a selective inhibitor of the mammalian target of rapamycin (mTOR) protein kinase [111]. Taken together, these results suggest that the acute sleep deprivation model could be useful to early identify agents possessing pro-cognitive potential.

Interestingly, longer-term exposure to sleep deprivation/restriction becomes a potent stressor that triggers pathological pathways including oxidative stress, neuroinflammation, neuroplasticity impairment which can lead to a more permanent cognitive deficit [112]

### **Models of permanent cognitive impairment**

According to current knowledge, the complex interaction of genetic and environmental factors may influence the etiology and pathogenesis of neuropsychiatric diseases. Among the environmental risk factors common to several neuropsychiatric diseases, chronic stress and diet seem to be particularly implicated in the occurrence of cognitive disorders. Clinical studies suggested that both factors yielded detrimental effect on cognitive functions and may be associated with the development of premature cognitive decline and dementia. The biological mechanisms suspected to be involved in chronic stress- or western diet-related brain damage include those mentioned i.e. neuroinflammation, oxidative stress and alterations of neuroplasticity. These pathological mechanisms trigger degenerative processes and changes in neurotransmitter metabolism that may account for behavioral symptoms.

Experimental study modeling repeated stress exposure reported cognitive deficits affecting spatial reference memory, spatial working memory and attention shifting performance that have been linked to neuroplastic structural changes within the hippocampus and medial prefrontal cortex [113]. In addition, neuroimaging analyses of functional connectivity in patients with various neuropsychiatric disorders and in animal models of chronic stress has revealed converging evidence of a common pattern of stress-related changes in functional connectivity that can be directly correlated with structural changes in these two regions [113]. These findings give this model an interesting translational value for the evaluation of drug candidates aimed at restoring normal cognitive performance.

On the other hand, animal models of metabolic syndromes induced by a single type of diet such as high-fructose, high-sucrose, high-fat, or a combination of diets, such as high-sucrose/high-fat diets, were also associated with cognitive dysfunction and brain damage [114]. In terms of pathogenic mechanisms, studies on animal models of high-fat diet have shown a coordinated action of oxidative stress, inflammation and vascular dysfunction leading to plasticity abnormalities in the cortical and hippocampal areas associated with cognitive dysfunction [115–117].

Overall, the preclinical evidence suggests that these environmental conditions are relevant experimental tools for generating integrated animal models of cognitive impairment. As a result, these models have been used to test original therapeutic approaches based on molecules with pleiotropic action. Thus, telmisartan, an angiotensin type 1 receptor blocker (ARB) with peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ )-stimulating activity has been shown to attenuate cognitive impairment caused by chronic stress and metabolic disorder in rodents [118,119]. The cognitive enhancing effects are likely to, at least partially, relate to anti-inflammatory and neurotrophic mechanisms mediated by PPAR $\gamma$  activation. Additional evidence of telmisartan's efficacy in other animal models of cognitive impairment led to its clinical evaluation in patients with mild cognitive impairment or Alzheimer's disease and as adjunctive therapy in patients with schizophrenia [120]. These preliminary clinical studies have provided encouraging results that need to be confirmed in larger clinical trials.

Another approach has been to modulate phosphodiesterase (PDE) enzyme on the basis of their involvement in the mechanisms of neuronal plasticity through the metabolic regulation of cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) signaling cascades [121]. Due to the high expression of PDE2 within brain structures such as cortex, amygdala, and hippocampus, this enzyme has emerged as an interesting target to regulate cognitive

processes The administration of a PDE2 inhibitor (Bay 60-7550) in chronically stressed animals alleviated cognitive impairment by restoring the neuroplasticity mechanisms [122]. Further evidence resulting from multiple preclinical models of cognitive impairment (aging, Alzheimer's disease...) have supported the beneficial effect of PDE2 inhibitors on several cognitive components [123–125]. However, to date, clinical studies evaluating PDE2 inhibitors or other PDEs inhibitors failed to demonstrate significant improvement of cognitive symptoms in Alzheimer's disease or schizophrenia patients [126]. Recent research suggests that broad-spectrum PDE inhibitors such as caffeine and propentofylline may be better candidates [127].

## Conclusion

Cognitive disorders are associated with many CNS pathologies. Directly related to pathophysiology or in some cases aggravated by treatment, they are polymorphic and although debilitating for patients, are not therapeutically managed due to a lack of effective drugs. In order to define potential drug targets, animal models have been designed based on accumulated histopathological, functional and pharmacological clinical knowledge. The categories of models presented in this article are based on a transological approach focusing on the common aspects of pathogenesis of cognitive symptoms across diseases rather than specific feature to each. With this regard, three main classes of animal models of cognitive disorders can be distinguished (Fig. 1). It explains that this review has not the objective to be exhaustive and exclude models of diseases, such as depression, lesion-based models or neurodevelopmental models.

The first class of models results from the alteration in animals of neurotransmission systems. Whether induced by a neuronal lesion, a pharmacological modulation, or a genetic mutation, these models express a set of symptoms that have been shown to overlap, whatever the neurotransmission system impacted. Indeed, the main cognitive functions are regulated by interconnected networks of neurons, whose equilibrium maintains the integrity. It is therefore not surprising to observe, for example, an improvement in cognitive symptoms resulting from dopaminergic impairment by molecules acting on cholinergic receptors. Although these types of models have contributed to the emergence of new anticholinergic or anti-glutamatergic symptomatic treatments, it seems unlikely that an action on a single neurotransmission system can provide a significant clinical improvement

in cognitive disorders which are most often the result of multiple biochemical dysfunctions. In any case, it seems essential that a molecule developed with the aim of improving cognitive impairment require to be tested in parallel in distinct preclinical models of memory deficits.

The second category of models reproduce in animals key pathological mechanisms known to play a deleterious role on neuronal integrity and cognitive functioning. These pathological processes, although presented separately in this paper, are not independent of each other. Both oxidative stress and inflammation are interdependent cellular consequences of a biological defense system. Furthermore chronic inflammation and oxidative stress trigger neuroplasticity impairments. Moreover, even if the experimental approach seems to specifically engage a pathological pathway, these models actually trigger a much more complex physiopathological cascade. These models have made it possible to develop and test different disease-modifying approaches whose transposition to humans has not been as successful as expected. These results suggest that the pathogenic cascade underlying cognitive disorders in patients is fuelled by multiple concomitant pathological events that should be treated independently or with multi-targets drugs to counter the progression of cognitive decline.

The third modeling method uses exposure to environmental situations known to generate transient or permanent cognitive impairment suitable to assess symptomatic or disease modifying strategies. While exposure to moderate hypoxia or sleep deprivation leads to transient changes in neurotransmission underlying cognitive symptoms, chronic exposure to stress or a high-fat or high-sucrose diets can trigger a myriad of pathological mechanisms leading to brain damage that compromise neuronal function and neurochemical homeostasis. From this point of view, animal models based on chronic exposure to environmental risk factors appears to be more integrative and could reproduce with more accuracy the pathophysiological cascade associated with cognitive impairment in humans. These models, still little exploited in the development of cognitive enhancing compounds, may provide a partial response to the limitations of other models. Models of combinations of multiple risk factors are also to be developed in this field of research.

Thus, animal models of cognitive disorders represent an indispensable tool for the evolution of knowledge and the understanding of the pathological mechanisms underlying them. In a perspective of development of new treatments improving cognition, it seems more relevant to evaluate drug candidates on different models belonging to these three categories and meeting different and complementary criteria of validity.



## Disclosure of interest

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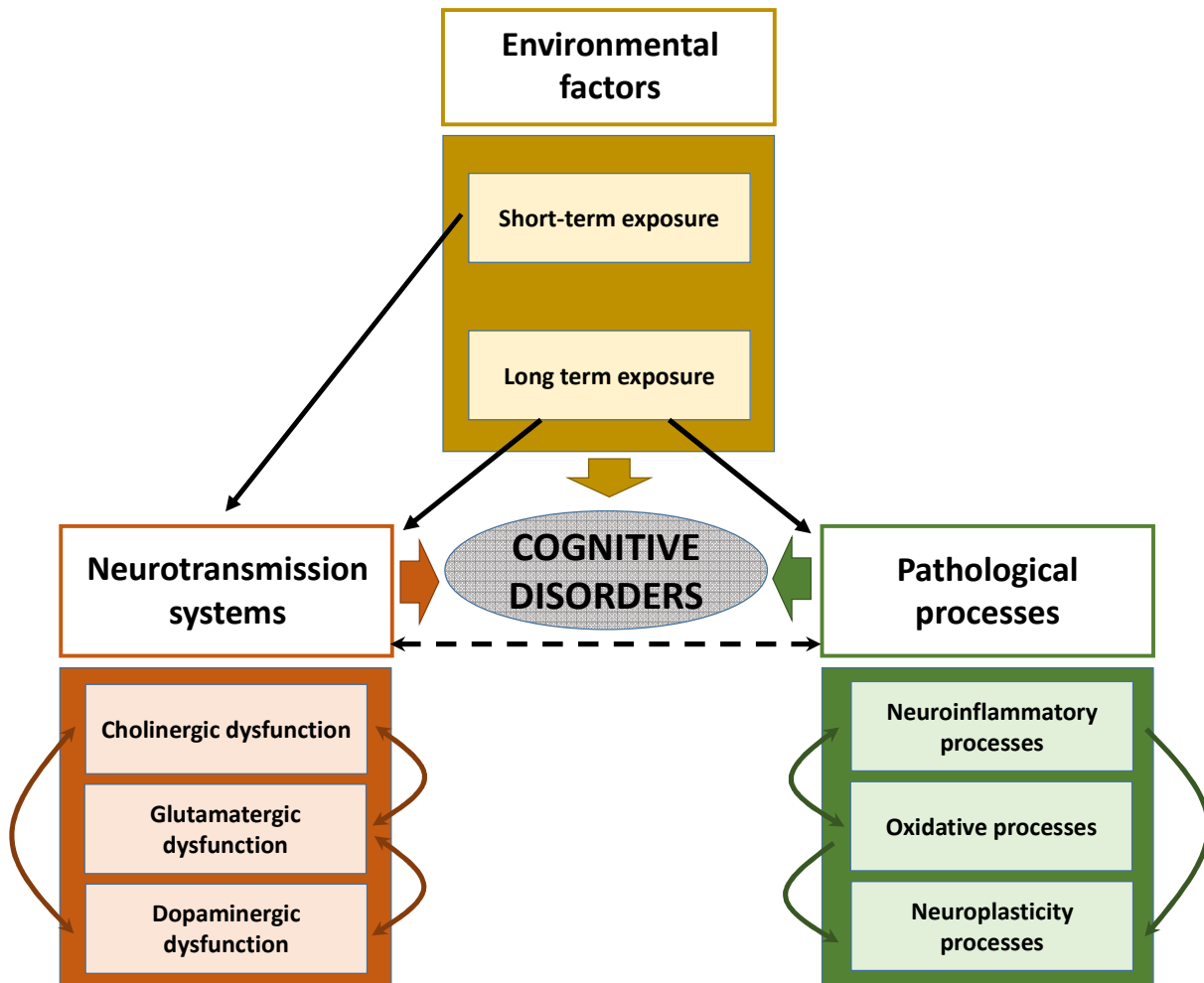


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**Figure 1.** Synthesis scheme of the animal modeling strategy for cognitive impairment.