pharmadvances

doi: 10.36118/pharmadvances.2023.47

Volume **5**, special issue **2023**

AGE AND SEX-DEPENDENT DIFFERENCES IN GABA TRANSMISSION IN MICE HIPPOCAMPAL NERVE TERMINALS

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OBJECTIVE: major depressive disorder (MDD) is a psychiatric disorder characterized by depressed mood, diminished interests, impaired cognitive functions, and vegetative symptoms, such as disturbed sleep or appetite. An episode of major depression may occur only once in a person's lifetime, but it is more likely to recur throughout a person's life. The prevalence of adults with a major depressive episode was highest among individuals aged 18-25 and it occurs about twice as often in women than in men. For years, the monoaminergic system (serotonin, norepinephrine, and dopamine) has received the most attention in the neurobiology of MDD, and most therapeutic drugs have been developed to target these systems. Although the neurobiological basis remains elusive, the hypothesis emerged that altered glutamatergic and GABAergic transmissions are pivotal to the disease. While the role of glutamate transmission has been investigated, the implication of GABAergic network largely remains unknown. In this context we aim at investigating the efficiency of GABA release from nerve endings (synaptosomes) isolated from the hippocampus of 20 day postnatal, (P20), adolescent (40 day postnatal, P40), young adult (3 months old), adult (6 months old), and aged (16 months) male and female mice. In addition, biochemical analyses were performed to compare the density of several proteins involved in the production, degradation, and activity of GABA in synaptosomes isolated from the same experimental groups.
MATERIALS AND METHODS: synaptosomes were isolated from the hippocampus of mice of different ages and from both sexes. They were preloaded with [^{3}H]GABA (30 nM) and the release of the neurotransmitter was investigated with the "up-down superfusion" approach in different depolarizing conditions (12 or 20 mM KCl-enriched solution). In parallel, Western blot analysis was carried out to compare the density of the vesicular GABA transporter (VGAT) and the glutamate decarboxylase 65/67 (GAD 65/67).
RESULTS: the efficiency of [^{3}H]GABA exocytosis from hippocampal synaptosomes increases starting from 20 days postnatal and reaches the maximal level at 6 months in either male and female mice, then decreasing in older animals. Comparing the two sexes, the functional studies demonstrated that [^{3}H]GABA exocytosis in significantly higher in hippocampal synaptosomes from P40 male mice when compared to female ones but it is rapidly recovered in synaptosomes from 3 months old female mice. Western blot analysis unveiled age and sex-dependent differences in the density of VGAT and GAD 65/67 protein expression in hippocampal synaptosomes.
CONCLUSIONS: our study demonstrates the existence of sex and age-dependent changes in the hippocampal GABAergic transmission that might have a role in dictating synaptic plasticity especially in the early developmental ages.

THE INVOLVEMENT OF TRPV2 CHANNELS IN THE NEUROPROTECTIVE EFFECTS OF CANNABIDIOL IN A MODEL OF *IN VITRO* ISCHEMIA

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OBJECTIVE: in the last few years a number of scientific studies have demonstrated a key role for cannabinoids in numerous pathological mechanisms of the central nervous system (CNS), including brain injury following cerebral ischemia. Cerebral ischemia is the second cause of death in industrialized countries with a high incidence and mortality rate. It results from a transient or permanent reduction in cerebral blood flow and restricts the delivery of substrates causing delayed neuronal loss and neurodegeneration. There are two types of ischemia: focal ischemia, which is confined to a specific region of the brain; and global ischemia, which encompasses wide areas of brain tissue. However, the role of cannabinoids in the mechanisms leading to neurodegeneration following cerebral ischemia is yet unclear. Many studies have produced conflicting results on cannabinoids putative protective and/or toxic effects.
MATERIALS AND METHODS: we investigated the effects of the selected cannabinoids, Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabigerol (CBG) in rat organotypic hippocampal slices exposed to oxygen and glucose deprivation (OGD), an *in vitro* model of forebrain global ischemia. Cell death in the CA1 subregion of slices was quantified by propidium iodide fluorescence and morphological analysis and tissue organization were examined by immunohistochemistry and confocal microscopy.
RESULTS: incubation with THC exacerbated, whereas incubation with CBD attenuated CA1 injury induced by OGD. THC toxicity was prevented by CB1 receptor antagonists while the neuroprotective effect of CBD was blocked by TRPV2, 5-HT1A and PPARγ antagonists. Confocal microscopy confirmed that CBD, but not THC, had a significant protective effects toward neuronal damage, tissue disorganization and glia activation caused by OGD in organotypic hippocampal slices mediated, at least in part, by TRPV2 channels, since the TRPV2 antagonist tranilast blocked them. The TRPV2 expression decreased after OGD in CA1 pyramidal neurons, but it increased in activated, phagocytic microglia. CBD increased TRPV2 expression, decreased microglia phagocytosis, and increased rod microglia after OGD.
CONCLUSIONS: our results show that cannabinoids have different effects in ischemia and play different roles in the mechanisms of post-ischemic neuronal death. CBD showed neuroprotective effects while THC worsened the neurodegeneration caused by ischemia. The effect of CBD was related to its agonistic activity on the TRPV2 channel.

PRESYNAPTIC COMPLEMENT, A SYNAPTIC ORGANIZER IN THE CNS: RELEVANCE TO SYNAPTOPATHY

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OBJECTIVE: complement recently emerged as a main player in white and grey matter lesions in experimental autoimmune encephalomyelitis (EAE) mice, an animal model of multiple sclerosis. To improve the knowledge of its activity, we investigated the impact of C1q and C3 proteins in the cortex of EAE mice at the acute stage of disease as promoters of synaptic pruning and modulators of glutamate release from cortical nerve endings (synaptosomes) and astrocytic processes (gliosomes).
MATERIALS AND METHODS: synaptosomes and gliosomes from the cortex of EAE mice at 21 ± 1 day post immunization (d.p.i.) were analysed for C1q and C3 protein content, glutamate release efficiency, viability and ongoing apoptosis.
RESULTS: in EAE mice at 21 ± 1 d.p.i. changes in the viability and in the apoptosis of both cortical synaptosomes and gliosomes did not emerged when compared to control animals, but a significant increase of the C1q and C3 proteins was detected, that was concomitant to microgliosis (quantify as CD11b density), astrocytosis (quantified as GFAP density) and synaptic derangements (measured as SNAP25 and PSD95 density) in cortical homogenates. Glutamate exocytosis was significantly reduced in EAE cortical synaptosomes and gliosomes, while the complement-evoked releasing activity was almost halved in synaptosomes but increased in gliosomes. Interestingly, EAAT2 density in EAE synaptosomes was significantly lower than that in control, while the EAAT1 density in gliosomes was increased.
CONCLUSIONS: our results unveil new mechanisms of actions of the complement in the CNS of EAE mice that could be relevant to disease progression and suggest new therapeutic targets for the management of MS.

HEMOKININ-1 ACTIVATES TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 CHANNELS IN THE SPINAL CORD TO INDUCE NOCICEPTIVE BEHAVIORS

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OBJECTIVE: hemokinin-1, which is a homologue of substance P, mainly acts on the neurokinin-1 (NK1) receptors and is involved in pain transmission in the spinal cord. The transient receptor potential vanilloid 1 (TRPV1) channel plays a key role in pain transmission in the spinal cord. Details of pain transmission by hemokinin-1, including the involvement of TRPV1 channel, have not been explored. Here, we investigated the mechanisms that underlie hemokinin-1 induced nociceptive behaviors.
MATERIALS AND METHODS: hemokinin-1 was administered intrathecally into the spinal cord of mice, and nociceptive behaviors initiated immediately after. A combination of directed biting, licking, and scratching in mice was recorded for the total response time of these nociceptive behaviors in different pharmacological conditions. We used western blotting to confirm that hemokinin-1 activated the TRPV1 channel by measuring the amount of phosphorylated TRPV1 channel.
RESULTS: intrathecal administration of hemokinin-1 evoked nociceptive behaviors consisting of licking and scratching with biting of the hind limbs in mice. Hemokinin-1-induced nociceptive behaviors were decreased by capsazepine, a TRPV1 channel antagonist. Pre-treatment with anti-TRPV1 channel antibody eliminated the hemokinin-1 induced nociceptive behaviors. Moreover, the phosphorylation of the TRPV1 channel in the spinal dorsal horn was increased by intrathecal administration of hemokinin-1.
CONCLUSIONS: hemokinin-1 elicits nociceptive behaviors through the TRPV1 channels as well as the NK1 receptors in the spinal dorsal horn. Hemokinin-1 may regulate pain transmission via TRPV1 channels on primary afferents.

ROLE OF SPINAL CHOLECYSTOKININ-8, NOCICEPTIN, AND HEMOKININ-1 IN DIABETIC ALLODYNIA

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OBJECTIVE: lack of effective drugs for allodynia, a painful neuropathy caused by diabetes mellitus, decreases the quality of life of patients. It is assumed that various neurotransmitters are involved in the spinal cord during the development of allodynia caused by hyperglycemia-induced neuropathy, but studies have not yet been conducted on the details of this phenomenon. Therefore, neurotransmitters of the spinal cord in streptozotocin-induced diabetic allodynia were examined.
MATERIALS AND METHODS: allodynia was strongly developed from day 3 to day 14 after streptozotocin administration. In this study, the von Frey filament technique was performed using a mouse model of diabetic neuropathic pain 7 days after intravenously administration of streptozotocin. Antibodies of representative neurotransmitter peptides were intrathecally administered to allodynia-induced mice.

RESULTS: streptozotocin-induced allodynia was reduced by intrathecally administered antibodies of cholecystokinin-8, nociceptin, and hemokinin-1. In contrast, intrathecally administered antibodies of substance P, somatostatin, and angiotensin-2 did not affect streptozotocin-induced diabetic allodynia. Diabetic allodynia was decreased by intrathecally administered CI-988, a cholecystokinin CCK-B receptor antagonist, and JTC-801, a nociceptin receptor antagonist. The amount of mRNA of CCK-B receptors increased in the spinal cord, but not dorsal root ganglion, of streptozotocin-induced diabetic allodynia mice.
CONCLUSIONS: these results suggest that diabetic allodynia is expressed by transmitting it to the spinal dorsal hone by cholecystokinin-8, nociceptin, and hemokinin-1 released from primary afferent neurons of the spinal cord.

NUTRACEUTICAL APPROACHES FOR THE MANAGEMENT OF AGING: PROMISING ANTIAGING ACTIVITY OF A NEW FORMULATION OF ELLAGIC ACID

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OBJECTIVE: aging is a progressive physio-pathological process that causes both central and peripheral complications. Ellagic acid (EA) is a nutraceutical product, contained in pomegranate fruit, that has been shown to be beneficial in peripheral disorders, but also in central neuroinflammatory and neurodegenerative disorders. In this study, we analyzed the impact of the oral administration of an EA micro-dispersion (EAm), a new formulation that increase the EA solubility and therefore the bioavailability, in young and old mice.
MATERIALS AND METHODS: young (3 months) and old (20 months) mice were chronically (14 days) administrated with vehicle (mineral water) or with the EAm suspension, dissolved in the drinking water. Weight, beverage, and behavioural skills of both young and old mice were monitored. Cortical nerve endings (synaptosomes) were incubated with [^{3}H]NA and release experiments were performed to evaluate changes in noradrenaline exoxytosis. PCR and western blot analysis were performed to support by a biochemical point of view the functional observations.
RESULTS: oral EAm did not modify animal weight and motor and anxiety behavioral skills in young and old mice, but significantly recovered changes in *ex-vivo, in vitro* parameters in old animals. The exocytosis of noradrenaline, which has an important role in the control of mood and anxiety, but also in contrasting inflammation, is decreased in the cortex of old mice. EAm administration did not modify noradrenaline release in young animals but recovered it in old mice. Furthermore, the content of GFAP (astrocytosis marker) was increased in the cortex of the old mice, while IBA-1 (marker of microglia activation) and CD45 (marker of lymphocytes) immunopositivities were unchanged when compared to young ones. EAm treatment significantly reduced CD45 signal in both young and old mice cortical lysates, it diminished GFAP immunopositivity in young mice, but failed to affect IBA-1 expression in both young and old animals. Finally, EAm treatment significantly reduced IL1b expression in old mice.
CONCLUSIONS: these results suggest that EAm is beneficial to aging and could represent a promising nutraceutical ingredient for elders.

2-PENTADECYL-2-OXAZOLINE (PEA-OXA) TREATMENT REDUCES THE COGNITIVE DAMAGE AND IMPAIRED NEURAL PLASTICITY IN VITAMIN D DEFICIENCY

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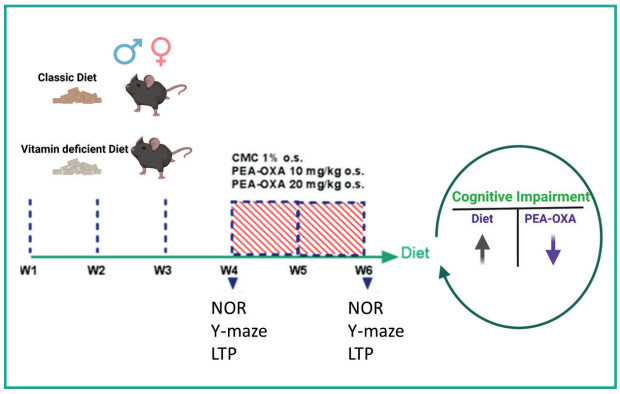
OBJECTIVE: 2-pentadecyl-2-oxazoline (PEA-OXA) is a natural compound, that is a secondary metabolite, found in green and roasted coffee beans, with anti-inflammatory and neuroinflammatory properties. We have previously shown that PEA-OXA is a α2 adrenergic receptor (α2AR) antagonist and a putative protean agonist on histamine H3 receptors. Vitamin D deficiency is considered a risk factor for the development of several immune-mediated pathological condition, including aging and dementia.
MATERIALS AND METHODS: in this study, we used a vitamin D deficiency condition induced by diet in male and female mice and evaluated the possible protective effects of PEA-OXA on the cognitive damage associated with low vitamin D levels.
RESULTS: we found that vitamin D deficiency induced a deficit of discriminative and spatial memory deficits, as revealed by the Novel Object Recognition (NOR) test and Y Maze Forced Alternation test. Consistently, electrophysiological studies revealed an impaired neural plasticity (Long-term potentiation LTP) in the hippocampus (Lateral Entorhinal Cortex-Dentate Gyrus pathway). Chronic treatment with PEA-OXA (10 mg/kg, os) restored cognitive damage and electrophysiological changes in both male and female mice.
CONCLUSIONS: our findings may suggest PEA-OXA as a novel compound for the treatment of cognitive decline in different neurodegenerative diseases.

Graphical abstract



ANXIOLYTIC-LIKE/RELAXANT EFFECTS OF BEO ARE USEFUL TO MANAGE ANXIETY AND AGITATION SYMPTOMS OBSERVED IN PATIENTS WITH DEMENTIA

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OBJECTIVE: preclinical results indicate that essential oil obtained by *Citrus Bergamia* (Risso et Poiteau) (BEO) shown interesting anxiolytic-relaxant effects useful to treat behavioral and psychological symptoms of dementia (BPSDs), including agitation, depression and anxiety.
MATERIALS AND METHODS: to study the involvement of GABAergic and 5-HTergic transmissions in BEO behavioral effects, male Wistar rats (250-300 g) (n = 3-8) were systemically pretreated with diazepam (1.2 and 5 mg/kg, i.p.) and 8-hydroxy-2-(di-n-propylamino)tetralin ((±)8-OH-DPAT) (1 mg/kg, i.p.), selective agonists of GABA-A and 5-HT1A receptors, respectively, or with the selective antagonists flumazenil (3 mg/kg, i.p.) and WAY-100635 (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)cyclohexane-carboxamide) (1 mg/kg, i.p.),. The effects of BEO (250 and 500 µl/kg, i.p.) were analyzed in open-field, elevate plus-maze and forced swimming tasks.
RESULTS: the data yielded show that anxiolytic-relaxant effects of BEO are not superimposable to that observed with the benzodiazepine and that 5-HT1A receptor is indirectly involved.
CONCLUSIONS: further experiments are needed to deeply elucidate the mechanism of action of the essential oil that will allow a rational use in management of early BPSDs observed in patients with dementia.

ROLE OF mGlu5 AND mGlu2/3 RECEPTORS IN BEHAVIOURAL EFFECTS OF BERGAMOT ESSENTIAL OIL

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OBJECTIVE: bergamot essential oil (BEO) shows an interesting anxiolytic/relaxant action useful in the management of agitation, aggression and anxiety, typical behavioural and psychological symptoms of dementia (BPSD). Pharmacological results indicate that the behavioural effects of BEO do not completely overlap with those of benzodiazepines and are indirectly modulated by the 5-HT1A receptor. It is known that serotonin modulates glutamatergic neurotransmission particularly by metabotropic receptors. Among glutamatergic receptors, the mGluR5 and mGluR2/3 subtypes seems to play a key role in the control of anxiety.
MATERIALS AND METHODS: Male Wistar rats (250-300 g) (n = 3-8) were systemically pretreated with MPEP (3 and 10 mg/kg, i.p.), selective antagonist of the mGlu5 receptor, and with LY-341495 (3 and 10 mg/kg, i.p.), selective antagonist of mGlu2/3 receptor. Behavioral effects of BEO (500 µl/kg, i.p.) were analyzed in open-field, elevate plus-maze and rotarod tasks.
RESULTS: the results show that the antagonists differently modulate BEO effect. Particularly, the effects observed in the EPM seem to suggest that the mGlu5 receptor is more involved in the anxiolytic action of the phytocomplex.
COCLUSIONS: these data indicate that complex and fine mechanisms underlying behavioral effects of BEO that however need to be further elucidated.

PAIN ASSESSMENT FOR BETTER PHARMACOLOGICAL TREATMENT OF PAIN AND AGITATION IN SEVERE DEMENTIA: I-MOBID2 VALIDATION

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OBJECTIVE: it is estimated that some 55 million people are affected by dementia and 97% develops neuropsychiatric symptoms (NPS), among which the most resistant is agitation. Its treatment consists in the use of atypical antipsychotics, often off-label and increasing of 1.6-1.7-fold the risk of death for cardiocerebrovascular accidents. Agitation is, at least in part, caused by unrelieved pain because of lack of self-reporting, with consequent assessment difficulties. Thus, the purpose of the present clinical trial is to validate for the clinical use in the Italian setting the Mobilization-Observation-Behaviour-Intensity-Dementia (MOBID2) tool that unravels concealed musculoskeletal inflammatory and neuropathic pain, through active guided movements, and also pain from head, skin and internal organs.
MATERIALS AND METHODS: according to the current international guidelines the validation trial, approved by Calabria Region Ethics Committee protocol No. 31/2017, consisted in three phases: translation, cross-cultural adaptation and validation has been conducted in a cohort of 11 non-verbal, severe demented patients aged ≥65. The psychometric properties of the validat-

ed Italian I-MOBID2 were measured through content validity index, Spearman's rank order correlation and intraclass correlation coefficient.
RESULTS: the I-MOBID2 allowed to disclose pain in 63.6% patients. The psychometric analysis demonstrated good scale content validity index (0.89), high construct validity (Spearman rank order correlation Rho = 0.748) and good-to-excellent inter-rater (Intraclass correlation coefficient, ICC = 0.778) and test-retest (ICC = 0.902) reliability with 5.8 min average execution time.
CONCLUSIONS: this validation trial provided, for the first time, the Italian setting with I-MOBID2 a valid and reliable tool, that proved to unravel pain in over a half of patients suffering from severe dementia. This pain assessment scale is suitable also for future development in community setting. The effect of establishment of appropriate pain treatment on reduction of the Cohen-Mansfield Agitation Inventory score and on as-need agitation rescue medications deserves investigation.

NEED FOR A CLINICAL TRIAL INVESTIGATING EFFICACY AND SAFETY OF ORAL SPRAY NAbiximols FOR PAIN AND AGITATION TREATMENT IN SEVERE DEMENTIA PATIENTS

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OBJECTIVE: some 55 million people are affected by dementia and up to 80% of residents in long-term care facilities suffer from chronic pain, contributing to the development of fluctuant neuropsychiatric symptoms, and agitation in particular. Evidence is accumulating in favor of the involvement of the endocannabinoid system in pain and behavior processing. Therefore, the purpose of the present study is to propose a double-blind, placebo-controlled, randomized trial to investigate efficacy and safety of oral spray nabiximols, containing Δ9-tetrahydrocannabinol and cannabidiol (Sativex®), for pain and agitation treatment in severe dementia patients (Mini-Mental State Examination ≤12) over 65.
MATERIALS AND METHODS: the trial NACTOPAISD (NAbiximols Clinical Translation To the treatment of Pain and Agitation In Severe Dementia) is approved by Calabria Region Ethical Committee (protocol n. 118 of April 21st, 2022). It is planned to be conducted in agreement with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) and the Consolidated Standards of Reporting Trials (CONSORT) statements.
RESULTS: the trial has two coprimary endpoints, consisting in efficacy on pain and agitation, assessed through the recently validated Italian Mobilization-Observation-Behavior-Intensity-Dementia and the Cohen-Mansfield Agitation Inventory. The secondary endpoint is the evaluation of efficacy duration after wash-out and the assessment of quality of life through the Dementia Quality of Life measure DEMQOL. The results obtained will be subjected to a statistical analysis plan. The assessors are going to note down the occurrence of any adverse events.
CONCLUSIONS: NACTOPAISD, following the way paved by studies assessing the different effects of cannabinoids on neuropsychiatric symptoms, might provide sound rational evidence supporting the efficacy and safety of nabiximols in the treatment of agitation and pain during severe dementia. Since the therapy of nuropsychiatric symptoms of dementia consists in potentially harmful, often off-label, antipsychotics, nabiximols may represent a safer treatment able to improve the quality of life of these fragile patients.

AUTOMATED DIAGNOSIS OF ALZHEIMER'S DISEASE WITH NEUROPSYCHOLOGICAL MEASURES: A META-ANALYTICAL REVIEW

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OBJECTIVE: today it is still difficult to predict patients at risks of Alzheimer's disease (AD) and whether and when individuals at risk (with Mild Cognitive Impairment, MCI) will progress to AD-type dementia and how much time will lapse for progression. Thus, the current challenge is to identify markers that capture MCI and discriminate between patients with MCI who will be convert (MCI converters, MCIc) and who will not convert (MCI not converters, MCInc) to AD-type dementia. In this study we performed a meta-analytic evaluation of the contribution of machine learning (ML) and neuropsychological measures for the automated classification of AD and MCI patients and the prediction of MCIs' conversion to AD-type dementia
MATERIALS AND METHODS: this systematic review was conducted on papers published on the use of ML applied to neuropsychological assessment for the automatic classification of AD, MCI and prediction of conversion of MCI to Alzheimer's type dementia, in accordance with the PRISMA statement. Meta-analytic accuracy, sensitivity and specificity and heterogeneity index were calculated using the MADA package.
RESULTS: evaluating data from 59 published studies, the majority (70%) with low risks of bias, we demonstrated that neuropsychological measures alone can lead to a successful automatic classification of prodromal AD phenotypes regardless of the employment of different ML algorithms. The contrasts MCI *vs* HC, MCIc *vs* MCInc and AD *vs*. HC were automatically recognized with a pooled accuracy of 0.9, 0.759 and 0.914, respectively. Moreover, ML algorithms are able to extract relevant categories of neuropsychological tests that maximize the classification accuracy. In particular: a)

MMSE, for evaluating the global cognitive status; b) AVLT, for evaluating the long-term memory performance; c) Category Fluency Test, for evaluating the language ability; and d) Digit Span Forward and Backward, for evaluating verbal short-term memory, sustained attention and working memory capacities (**figure 1**).

CONCLUSIONS: this meta-analytic review demonstrates that ML applied on neuropsychological measures can be useful to automatically classify AD patients, even at an early stage of the disease, and to identify a combination of optimal neuropsychological predictors.

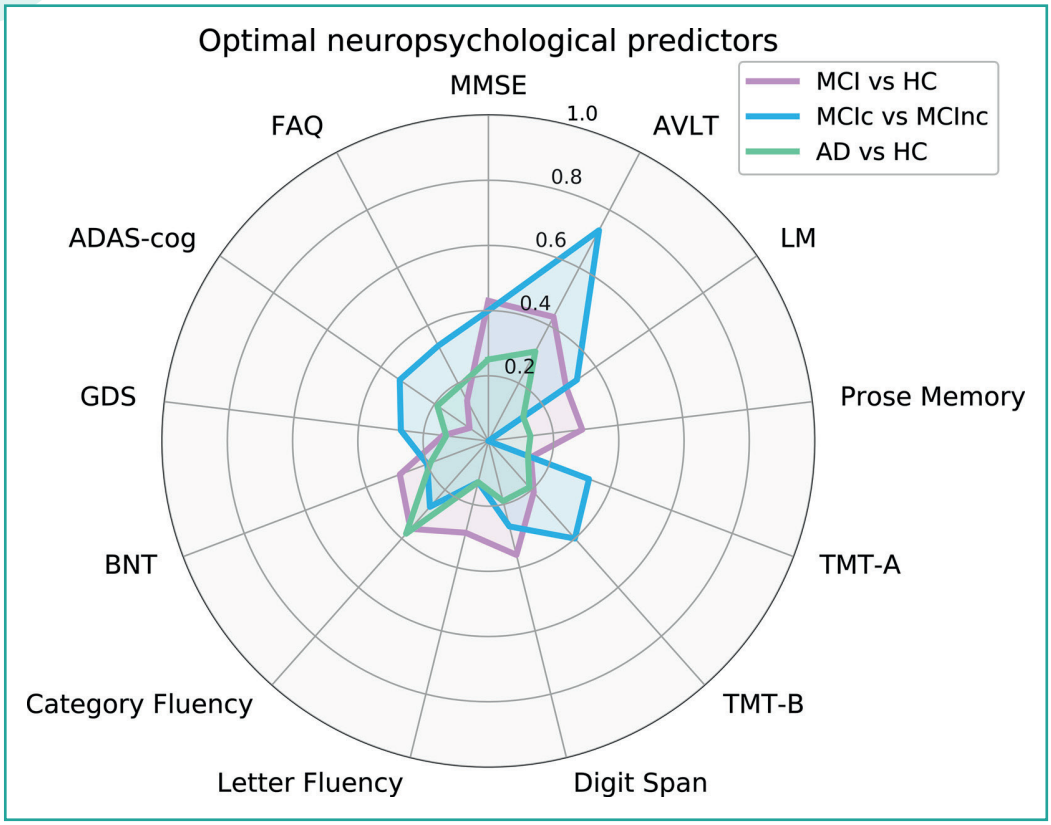

Figure 1. Radar plot of the most frequent optimal predictors (≥25 % frequency), for the different comparisons, MCI *vs*. HC (violet), MCIc *vs*. MCInc (blue), and AD *vs*. HC (green).