Brain activity of benzoate, a D-amino acid oxidase inhibitor, in patients with mild cognitive impairment in a randomized, double-blind, placebo controlled clinical trial

Hsien-Yuan Lane, M.D., Ph.D. a,b,c,#, Cheng-Hao Tu, Ph.D. d,#, Wei-Che Lin, M.D., Ph.D. e, Chieh-Hsin Lin, M.D., Ph.D. b,f,g*

a Department of Psychiatry & Brain Disease Research Center, China Medical University Hospital, Taichung, Taiwan

b Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan

c Department of Psychology, College of Medical and Health Sciences, Asia University, Taichung, Taiwan

d Graduate Institute of Acupuncture Science, China Medical University, Taichung, Taiwan

e Department of Radiology, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Kaohsiung, Taiwan

f Department of Psychiatry, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Kaohsiung, Taiwan

g School of Medicine, Chang Gung University, Taoyuan, Taiwan

© The Author(s) 2021. Published by Oxford University Press on behalf of CINP. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
# Contribute equally to the paper.

* Corresponding author at: Department of Psychiatry, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, No. 123, Dapi Rd., Niaosong District, Kaohsiung City, 833, Taiwan.

Email cyndi36@gmail.com (C.-H. Lin).
Abstract

Background: Current anti-dementia drugs cannot benefit mild cognitive impairment (MCI). Sodium benzoate (a D-amino acid oxidase [DAO] inhibitor) has been found to improve the cognitive function of patients with early-phase Alzheimer’s disease (AD) (mild AD or MCI). However, its effect on brain function remains unknown. This study aimed to evaluate the influence of benzoate on fMRI in patients with amnestic MCI.

Methods: This was a 24-week randomized, double-blind, placebo-controlled trial, which enrolled 21 patients with aMCI and allocated them randomly to either of 2 treatment groups: (1) benzoate group (250-1500 mg/day); (2) placebo group. We assessed the patients’ working memory, verbal learning and memory, and resting-state fMRI and regional homogeneity (ReHo) maps at baseline and endpoint.

Results: Resting-state ReHo decreased in right orbitofrontal cortex after benzoate treatment, but didn’t change after placebo. Moreover, after benzoate treatment, the change in working memory was positively correlated and the change in ReHo in right precentral gyrus and right middle occipital gyrus; and the change in verbal learning and memory was positively correlated with the change in ReHo in left precuneus. In contrast, after placebo treatment, the change in working memory or in verbal learning and memory was not correlated with the change in ReHo in any brain region.

Conclusion: The current study is the first to demonstrate that a DAO inhibitor, sodium benzoate herein, can alter brain activity as well as cognitive functions in the MCI individuals. The preliminary finding lends supports for DAO inhibition as a novel approach for early dementing processes.

Keywords: Mild cognitive impairment (MCI); Sodium benzoate; D-amino acid oxidase inhibitor; fMRI; Regional homogeneity (ReHo)
The trial was registered on the ClinicalTrials.gov website (NCTNCT02239003):
https://clinicaltrials.gov/ct2/show/NCT02239003

Significance Statement

This is the first study exploring the effect of an N-methyl-D-aspartate receptor (NMDAR) enhancer (sodium benzoate) on brain activity in the individuals with mild cognitive impairment (MCI). The benzoate treatment decreased regional homogeneity (ReHo) in the right orbitofrontal cortex, while placebo treatment did not alter ReHo. These results may indicate the possible brain mechanisms for the treatment of benzoate on MCI patients. The results may contribute to the elucidation of the pathophysiology of MCI and the development of novel therapy to enhance NMDAR.
Introduction

Mild cognitive impairment (MCI) is a slight cognitive impairment that is accompanied with mostly normal function in processes that control the performance of daily activities (Levey et al., 2006). The concept of MCI was developed in an attempt to recognize dementia in its earliest clinically expressed form (Bowen et al., 1997; Boeve, 2012). MCI, particularly amnestic MCI (aMCI), is a risk factor or a prodromal stage of Alzheimer’s disease (AD).

Acetylcholinesterase inhibitors (AChEIs) have been developed for treating AD, especially, mild-moderate AD (Birks, 2006; Burns et al., 2006). However, AChEIs are not recommended for the treatment of MCI because of the weak beneficial effects (Fellgiebel, 2007). Memantine, an N-methyl-D-aspartate (NMDA) receptor (NMDAR) antagonist, has been used to treat moderate-severe AD based on the “glutamate excitotoxicity theory” (Reisberg et al., 2003; Scarpini et al., 2003), but not for mild AD (Schneider et al., 2011). Moreover, MCI is not effectively treated by memantine either (O’Brien et al., 2011). The poor efficacy of AChEIs and memantine for MCI implies that there should be other mechanism(s) underlying the pathogenesis of MCI.

NMDAR, a subtype of ionotropic glutamate receptor, plays an important role in synaptic plasticity, learning, memory, and cognition (Wu et al., 2007; Amano and Maruyama, 2011). The NMDAR density decreases with age (Segovia et al., 2001). In AD patients, glutamate levels declined in cerebrospinal fluid (Martínez et al., 1993) and brain (Lowe et al., 1990), the number of glutamate terminals decreased in the hippocampus (Cowburn et al., 1988), and D-serine (an NMDAR agonist) levels fell in the serum (Hashimoto et al., 2004). Therefore, dysfunction in the NMDAR neurotransmission may contribute substantially to the pathophysiology of AD.

The classical way for activation of NMDAR is applying agonists, such as D-serine (Esposito et al., 2012) or D-cycloserine (Pitkanen et al., 1995a; Pitkanen et al., 1995b). However, D-cycloserine has failed in the treatment of patients with AD (Laake and Oeksengaard, 2002). A novel way to activate NMDAR is inhibiting the activity of D-amino acids oxidase (DAO) (Lin et al., 2012), which is responsible for degrading D-serine (Vanoni et al., 1997; Sasabe et al., 2012). DAO levels have been
found to play a role in early-phase AD (Lin et al., 2017). Sodium benzoate, a pivotal DAO inhibitor, is generally recognized as safe (GRAS) and widely used as food preservatives in many countries (Joint FAO/WHO Expert Committee on Food Additives, 1973; IPCS, 1993; Department of Health, http://food.doh.gov.tw/chinese/ruler/ingrdient_standard.htm). Sodium benzoate is also used for the treatment of urea cycle enzymopathies; and the therapeutic dose is in the range of 250-500 mg/kg body weight (15,000-30,000 mg for a 60 kg patient) per day (Tremblay and Qureshi, 1993; Feillet and Leonard, 1998). In a 24-week, randomized, double-blind, placebo-controlled clinical trial (Lin et al., 2014) on aMCI or mild AD, benzoate significantly improved cognitive functions (including working memory and verbal learning & memory tests). The elderly patients tolerated sodium benzoate 250-1500 mg/day very well.

Resting-state functional magnetic resonance imaging (rfMRI) is a non-invasive way to investigate the spontaneous brain activity which may reflect the brain dynamics in local cortical tissues or large-scale brain networks (Jiang and Zuo, 2016). Recent study suggested that the changes of local functional connectivity (FC) may affect whole brain dynamics via the change of local excitation-inhibition ratio (Deco et al., 2014). With the treatment of NMDAR antagonist MK801, increased brain responses to olfactory stimuli has been observed in primates using pharmacological fMRI (Zhao et al., 2018). The increased brain responses may be underpinned by the change of local excitation-inhibition ratio with blockade of NMDAR on local inhibitory interneurons and then disinhibit principal neurons. Moreover, long-term exposure of ketamine, another NMDAR antagonist, has been associated the altered local FC in human (Liao et al., 2012). Hence, with treatment of sodium benzoate, the local excitation-inhibition ratio may be changed in terms of the changes in local FC. Regional homogeneity (ReHo) is a reliable method to characterized the local FC (Zang et al., 2004). In the present study, we conducted the rfMRI scans and ReHo analysis to investigate the benzoate treatment-associated local FC changes, which may contribute to the improvement of cognitive function in early-phase AD.
Methodology

This study enrolled the patients with aMCI from the outpatient clinic at the Department of Psychiatry, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, into a 24-week clinical trial. The Institutional Review Board (IRB) of the hospital approved the study in accordance with the current revision of the Declaration of Helsinki.

The trial was registered on the ClinicalTrials.gov website (NCTNCT02239003): https://clinicaltrials.gov/ct2/show/NCT02239003.

Subjects

After a description of the study to the patients, written informed consents were obtained. Patients were evaluated by the research psychiatrist after a thorough medical and neurological workup.

Patients were enrolled into this study if they [1] were aged 50-90, [2] satisfied the criteria for aMCI (McKhann et al., 1984) of a presumably degenerative nature defined as subjective memory complaint corroborated by an informant and insufficient global cognitive and functional impairment to meet NINCDS-ADRDA criteria and had a Clinical Dementia Rating (CDR) (Morris, 1993) score of 0.5, [3] were physically healthy and had all laboratory assessments (including urine/blood routine, biochemical tests, and electrocardiograph) within normal limits, and [4] had sufficient education to communicate effectively and were capable of completing the assessments of the study. For patients who had already been on AChEIs therapy, AChEIs had to be continued for at least three months before enrollment and AChEIs doses had to be kept unchanged during the study duration. For patients who had not yet been on AChEIs therapy, AChEIs or other anti-dementia medications were forbidden during the study duration.

Exclusion criteria included history of significant cerebrovascular disease, Hachinski Ischemic Score > 4, major neurological, psychiatric or medical conditions other than MCI, substance (including
alcohol) abuse or dependence, delusion, hallucination or delirium symptoms, severe visual or hearing loss, and inability to follow the protocol.

**Treatments**

Eligible patients continued their originally ongoing psychotropic drugs (if any) throughout the study period and were randomly assigned into either of two treatment groups in a double-blind manner: sodium benzoate (250-1500 mg/day) or placebo for 24 weeks. Sodium benzoate (250 mg/capsule) was purchased from Excelsior Biopharma Inc.

To ensure concealment of the randomization assignment, study medication was provided in coded containers with supply of identical-appearing capsules of placebo or benzoate. Patients were randomized through a computer-generated randomization table to receive placebo or benzoate treatment in a 1:1 ratio. Non-blind pharmacists dispensed appropriate medication for treatment according to the randomization table. Benzoate was initiated at 250-500 mg/day. According to the clinical condition, cognitive function assessment and the patients’ tolerance, the dose was adjusted every 8 weeks in each group. If the cognition did not improve, the dose could be titrated by 250-500 mg/day from the 9th week, and another 250-500 mg/day from the 17th week of the study. The total dose range was 250-1500 mg/day. The dosing strategy of benzoate was based upon the doses in our pilot study aforementioned (Lin et al., 2014), where benzoate was effective and safe after 24 weeks of treatment in the elderly patients.

During the study period, limited use of benzodiazepines (up to 4-mg/day lorazepam or equivalent) was allowed as concomitant medication for anxiety or insomnia. No other centrally acting drugs or cytochrome P450 inducers (or inhibitors) were permitted (Lane and Chang, 1998).

Patients, caregivers, and investigators, except the investigational pharmacist, were all blind to the assignment. Patient medical adherence and safety were closely monitored by caregivers and research physicians, and pill-counting was monitored by the study staff.
Evaluation of cognitive function and side effects

Cognitive functions were measured by working memory (Wechsler Memory Scale–Third Edition [WMS-III], Spatial Span) (Wechsler, 1997; Silver et al., 2003) and verbal learning and memory tests (WMS-III, Word Listing) (Wechsler, 1997) at week 0 and week 24, and the Alzheimer’s disease assessment scale-cognitive subscale (ADAS-cog) (Rosen et al., 1984) at weeks 0, 8, 16, and 24.

Clinical ratings were performed by a research psychiatrist (CHL), who was trained and experienced in the rating scales.

Side effect assessments were examined every 8 weeks during the drug treatment period by routine physical and neurological examinations and the Udvalg for Kliniske Undersogelser (UKU) Side-effects Rating Scale (Lingjaerde et al., 1987). Routine laboratory tests, including CBC and biochemistry, were checked at baseline (week 0), week 8, week 16, and endpoint of the drug treatment (week 24).

Methods for the image study

Image Acquisition

Resting-state fMRI was measured at baseline (week 0) and endpoint (week 24). Images were acquired with an 8-channel head coil in a 3.0 Tesla MRI scanner (Signa Excite, GE, USA). The rfMRI sessions were continuously scanned with ascending echo-planar imaging sequence for whole brain scanning (repetition time = 2000 ms; echo time = 30 ms; flip angle = 80°; matrix= 64×64; field of view = 240×240 mm²; slice number = 32; slice thickness = 4 mm) after 10-second blank scans for stabilized the signal. All scans were acquired within a dim-light shielding room. Before scanning, subjects were instructed to keep relaxed and awakened but not to move head or focus on any specific matter.
Preprocessing of resting-state fMRI data

The preprocessing protocol of rfMRI data was reported elsewhere (Wu et al., 2016). In brief, the rfMRI images (without first 10-second blank scans) were preprocessed using Data Processing Assistant for Resting-State fMRI 4.3 (DPARSF 4.3, State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, China). Images were corrected for different slice acquisition times, realigned to correct the head motions occurring alone the session, normalized into Montreal Neurological Institute (MNI) reference space with EPI template, and resampled with the voxel size 2x2x2 mm\(^3\). The time-series activities in each voxel were then linearly detrended, band-pass filtered (0.01–0.08 Hz), and regressed out the confounding variables as six head movement parameters, global mean signal, mean signal of white matter, and mean signal of cerebral spinal fluid.

The ReHo maps were generated by calculating the Kendall’s coefficient of concordance (KCC) voxel-by-voxel on preprocessed time-series activities between a given voxel and its nearest neighbors (26 voxels). The ReHo maps were spatially smoothed using a 3D Gaussian kernel of 8 mm full-width at half-maximum (FWHM) and then standardized by divided with its own global mean.

Data analysis

Chi-square test (or Fisher’s exact test) was used to compare differences of categorical variables and Student two-sample t test (or Mann-Whitney U test if the distribution was not normal) was used for continuous variables between two treatment groups.

The statistical analysis of standardized ReHo maps were conducted by statistical parametric mapping 12 (SPM12, Wellcome center for Human Neuroimaging, University College London, UK). The 2-sample t test and paired t tests were performed to probe the possible difference between different groups at baseline and different time points (i.e., before and after treatment) in each group, respectively. The correlation analysis was also conducted between change of cognitive measurements
and change of ReHo maps in each group. As an exploratory study, a less stringent significant threshold (uncorrected p<0.005 at voxel-level with the cluster size > 50, corresponding to the uncorrected cluster-level p<0.080) was applied in the present study to reduce the chance of Type II error (Cremers et al., 2017).

Results

A total of 24 patients with aMCI were enrolled into this study. Among them, 3 patients were excluded (1 alcohol use and poor medical adherence, 1 comorbid with delusional disorder, 1 diagnosed as Parkinson disease). The remaining 21 patients completed the 24-week clinical trial and brain MRI measurement.

The demographic and clinical characteristics are shown in Table 1. Patients in the sodium benzoate group appeared younger (p=0.042) and had lower education level (p=0.003) than patients in the placebo group.

At week 0 (baseline), patients in the benzoate group had higher ADAS-cog score than the placebo group (15.1±3.9 vs. 10.8±3.6, p=0.023, Mann-Whitney U test). At endpoint, there was no significant difference between the two groups in ADAS-cog (9.1±3.5 vs. 6.8±2.7, p=0.113, t test). There was no significant difference in ADAS-cog score change from baseline to endpoint between the two groups (6.0±2.2 vs. 4.0±4.0, p=0.196, t test).
ReHo at baseline and after treatment

At baseline, the benzoate group showed higher ReHo than in the placebo group in right middle frontal gyrus (recognized as part of orbitofrontal cortex [OFC]) and bilateral medial frontal gyrus (recognized as supplementary motor area) (Supplemental Table S1).

After benzoate treatment, decreased ReHo was found in right middle frontal gyrus, while no increased ReHo was found. After placebo treatment, no significant changes of ReHo were found (Figure 1, Table 2).

Correlations between the cognitive changes and ReHo alterations

Regarding the relationship between the change in working memory (assessed by WMS-III, Spatial Span) and the change in ReHo, a positive correlation was found in right precentral gyrus (recognized as primary motor cortex) and right middle occipital gyrus (recognized as primary visual cortex), but no negative correlation was detected in any brain region in the benzoate group. There was neither positive nor negative correlation observed in any brain region in the placebo group (Figure 2, Table 3).

Regarding the relationship between the change in verbal learning and memory (measured by WMS-III, Word Listing) and the change in ReHo, a positive correlation was shown in left precuneus, but no negative correlation was revealed in any brain region in the benzoate group. There was neither positive nor negative correlation discovered in any brain region in the placebo group (Figure 2, Table 3).
Safety

Both sodium benzoate and placebo were well-tolerated. The side effect was mild and did not warrant medical treatment. All the 21 patients completed the trial, without dropout. The routine blood cell count and chemistry were all within the normal ranges and remained unchanged after treatment (data not shown).

Discussion

To our knowledge, this is the first study exploring the effect of an NMDAR enhancer on brain activity in the individuals with MCI or dementia. The benzoate treatment decreased ReHo in the right OFC (which consists of Brodmann area 10, 11 and 47) (Figure 1), while placebo treatment did not alter ReHo (Table 2). These results may indicate the possible brain mechanisms for the treatment of benzoate on MCI patients.

Anatomically, OFC is interconnected with parahippocampus (Haber and Behrens, 2014). It has been indicated that increased parahippocampal-prefrontal functional connectivity is predictive of impaired episodic memory in aMCI (Zhang et al., 2016). Further, the MCI patients with increased ReHo in the orbital part of the inferior frontal gyrus (which is cytoarchitectonically most closely represented by Brodmann area 47) showed a trend of deteriorating into AD or remaining in MCI, rather than reverting to a cognitively intact state (Cai et al., 2018). Accordingly, in the present study, at baseline, the benzoate group also displayed greater ReHo in the right OFC and more cognitive impairment (as shown by the higher ADAS-cog score) than the placebo group.

After treatment, the mean ADAS-cog score decreased by 6.0±2.2 in the benzoate group and 4.0±4.0 in the placebo group; however, the group difference was insignificant in this small-sized study (p=0.196). Of interest, as aforementioned, after benzoate treatment, decreased ReHo was found in right middle frontal gyrus, while, after placebo treatment, no significant ReHo change was found (Figure 1, Table 2). Therefore, decreased ReHo in OFC with benzoate treatment may be potentially
beneficial for the outcome of the cognitive aging process. In accordance, transcranial direct current stimulation (tDCS), with its potential to enhance NMDAR-related neurotransmission and neuroplasticity (Chang et al., 2018), significantly reduced prefrontal hyperactivity and resulted in “normalization” of abnormal network configuration during fMRI (Meinzer et al., 2015).

Furthermore, after benzoate treatment, the change in nonverbal (spatial) working memory was positively correlated and the change in ReHo in right precentral gyrus and right middle occipital gyrus; and the change in verbal learning and memory was positively correlated with the change in ReHo in left precuneus (Figure 2). On the other hand, after placebo treatment, the change in nonverbal working memory or in verbal learning and memory was not correlated with the change in ReHo in any brain region (Table 3). In accordance, previous study has shown that, in the nonverbal working memory, regions of activation included the precentral gyrus and precuneate gyrus in the left parietal lobe and the occipital cortex in the right hemisphere (Binder and Urbanik, 2006). In addition, in the MCI patients, perfusion in precunei, parietal cortex, and left hippocampus was correlated with verbal memory (Nobili et al., 2008).

Dysregulation of NMDAR is implicated in the pathogenesis of AD (Lin et al., 2019b; Lin et al., 2019a; Chang et al., 2020). DAO can regulate the NMDAR function. In a recent study in 397 individuals (including aMCI, mild AD, moderate to severe AD, and healthy elderly), DAO levels in the serum increased with the severity of the cognitive deficits (Lin et al., 2017); this is the first study indicating that the peripheral DAO levels may increase with age-related cognitive decline. The finding supports the hypofunction of NMDAR hypothesis in AD (Lin et al., 2020). It is critical to identify and treat AD as early as possible, potentially to arrest its progression (Hsu et al., 2018). Following the pilot study on sodium benzoate for the treatment of cognitive function of early-phase AD (Lin et al., 2014), the current study further supports that DAO could serve as a novel target of drug development for early stage of cognitive decline.

This study is limited by its small sample size. In addition, whether the finding in Han Taiwanese can be extrapolated to other populations is unclear.
In conclusion, the preliminary results may contribute to the elucidation of the pathophysiology of MCI and the development of novel therapy to enhance NMDAR. Future larger-sized studies in subjects with various ethnicities are warranted. In addition, whether neuroimaging biomarkers, as shown in the current study, can be applied for early detection and individualized medicine also deserves further investigation.
Acknowledgments

This work was supported by Ministry of Science and Technology, Taiwan (MOST 107-2628-B-182A-002; 109-2312-B-039-001), National Health Research Institutes (NHRI-EX108-10816NC), Kaohsiung Chang Gung Memorial Hospital (CMRPG8E1041), and China Medical University (CMU108-MF-03). The sponsors were not involved in study design; collection, analysis and interpretation of data; writing of the report; and the decision to submit the article for publication.

Conflict of Interest

All authors declare that they have no conflicts of interest.

Author Contributions

CH Lin and HY Lane involved in study conception and design, literature review, participants enrollment, data interpretation, and manuscript writing; CH Tu involved in literature review, data analysis and interpretation, and manuscript writing; WC Lin involved in brain image data collection and processing; all authors reviewed and approved the manuscript.
References

Amano H, Maruyama IN (2011) Aversive olfactory learning and associative long-term memory in Caenorhabditis elegans. Learn Mem 18:654-665.

Binder M, Urbanik AS (2006) Material-dependent activation in prefrontal cortex: working memory for letters and texture patterns--initial observations. Radiology 238:256-263.

Birks J (2006) Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev:CD005593.

Boeve BF (2012) Mild cognitive impairment associated with underlying Alzheimer's disease versus Lewy body disease. Parkinsonism Relat Disord 18 Suppl 1:S41-44.

Bowen J, Teri L, Kukull W, McCormick W, McCurry SM, Larson EB (1997) Progression to dementia in patients with isolated memory loss. Lancet 349:763-765.

Burns A et al. (2006) Clinical practice with anti-dementia drugs: a consensus statement from British Association for Psychopharmacology. J Psychopharmacol 20:732-755.

Cai S, Wang Y, Kang Y, Wang H, Kim H, von Deneen KM, Huang M, Jiang Y, Huang L (2018) Differentiated Regional Homogeneity in Progressive Mild Cognitive Impairment: A Study With Post Hoc Label. Am J Alzheimers Dis Other Demen 33:373-384.

Chang CH, Lane HY, Lin CH (2018) Brain Stimulation in Alzheimer's Disease. Front Psychiatry 9:201.

Chang CH, Lin CH, Lane HY (2020) d-glutamate and Gut Microbiota in Alzheimer's Disease. Int J Mol Sci 21.

Cowburn R, Hardy J, Roberts P, Briggs R (1988) Regional distribution of pre- and postsynaptic glutamatergic function in Alzheimer's disease. Brain Res 452:403-407.

Cremers HR, Wager TD, Yarkoni T (2017) The relation between statistical power and inference in fMRI. PLoS One 12:e0184923.

Deco G, Ponce-Alvarez A, Hagmann P, Romani GL, Mantini D, Corbetta M (2014) How local excitation-inhibition ratio impacts the whole brain dynamics. J Neurosci 34:7886-7898.

Esposito S, Pristera A, Maresca G, Cavallaro S, Felsani A, Florenzano F, Manni L, Ciotti MT, Pollegioni L, Borsello T, Canu N (2012) Contribution of serine racemase/d-serine pathway to neuronal apoptosis. Aging Cell 11:588-598.

Feillet F, Leonard JV (1998) Alternative pathway therapy for urea cycle disorders. J Inherit Metab Dis 21 Suppl 1:101-111.

Fellgiebel A (2007) [Alzheimer drugs for mild cognitive impairment]. Neuropsychiatr 21:230-233.

Haber SN, Behrens TE (2014) The neural network underlying incentive-based learning: implications for interpreting circuit disruptions in psychiatric disorders. Neuron 83:1019-1039.

Hashimoto K, Fukushima T, Shimizu E, Okada S, Komatsu N, Okamura N, Koike K, Koizumi H, Kumakiri C, Imai K, Iyo M (2004) Possible role of D-
serine in the pathophysiology of Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 28:385-388.

Hsu WY, Lane HY, Lin CH (2018) Medications Used for Cognitive Enhancement in Patients With Schizophrenia, Bipolar Disorder, Alzheimer's Disease, and Parkinson's Disease. Front Psychiatry 9:91.

Jiang L, Zuo XN (2016) Regional Homogeneity: A Multimodal, Multiscale Neuroimaging Marker of the Human Connectome. Neuroscientist 22:486-505.

Laake K, Oeksengaard AR (2002) D-cycloserine for Alzheimer's disease. Cochrane Database Syst Rev:CD003153.

Lane HY, Chang WH (1998) Risperidone-carbamazepine interactions: is cytochrome P450 3A involved? J Clin Psychiatry 59:430-431.

Levey A, Lah J, Goldstein F, Steenland K, Blwise D (2006) Mild cognitive impairment: an opportunity to identify patients at high risk for progression to Alzheimer's disease. Clin Ther 28:991-1001.

Liao Y, Tang J, Fornito A, Liu T, Chen X, Chen H, Xiang X, Wang X, Hao W (2012) Alterations in regional homogeneity of resting-state brain activity in ketamine addicts. Neurosci Lett 522:36-40.

Lin CH, Lane HY, Tsai GE (2012) Glutamate signaling in the pathophysiology and therapy of schizophrenia. Pharmacol Biochem Behav 100:665-677.

Lin CH, Yang HT, Lane HY (2019a) D-glutamate, D-serine, and D-alanine differ in their roles in cognitive decline in patients with Alzheimer's disease or mild cognitive impairment. Pharmacol Biochem Behav 185:172760.

Lin CH, Yang HT, Chiu CC, Lane HY (2017) Blood levels of D-amino acid oxidase vs. D-amino acids in reflecting cognitive aging. Sci Rep 7:14849.

Lin CH, Chiu CC, Huang CH, Yang HT, Lane HY (2019b) pLG72 levels increase in early phase of Alzheimer's disease but decrease in late phase. Sci Rep 9:13221.

Lin CH, Yang HT, Chen PK, Wang SH, Lane HY (2020) Precision Medicine of Sodium Benzoate for the Treatment of Behavioral and Psychological Symptoms of Dementia (BPSD). Neuropsychiatr Dis Treat 16:509-518.

Lin CH, Chen PK, Chang YC, Chuoj LJ, Chen YS, Tsai GE, Lane HY (2014) Benzoate, a D-amino acid oxidase inhibitor, for the treatment of early-phase Alzheimer disease: a randomized, double-blind, placebo-controlled trial. Biol Psychiatry 75:678-685.

Lingjaerde O, Ahlfors UG, Bech P, Dencker SJ, Elgen K (1987) The UKU side effect rating scale. A new comprehensive rating scale for psychotropic drugs and a cross-sectional study of side effects in neuroleptic-treated patients. Acta Psychiatr Scand Suppl 334:1-100.

Lowe SL, Bowen DM, Francis PT, Neary D (1990) Antemortem cerebral amino acid concentrations indicate selective degeneration of glutamate-enriched neurons in Alzheimer's disease. Neuroscience 38:571-577.

Martinez M, Frank A, Diez-Tejedor E, Hernanz A (1993) Amino acid concentrations in cerebrospinal fluid and serum in Alzheimer's disease and vascular dementia. J Neural Transm Park Dis Dement Sect 6:1-9.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA...
Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34:939-944.

Meinzer M, Lindenberg R, Phan MT, Ulm L, Volk C, Floel A (2015) Transcranial direct current stimulation in mild cognitive impairment: Behavioral effects and neural mechanisms. Alzheimers Dement 11:1032-1040.

Morris JC (1993) The Clinical Dementia Rating (CDR): current version and scoring rules. Neurolgy 43:2412-2414.

Nobili F, Frisoni GB, Portet F, Verhey F, Rodriguez G, Caroli A, Touchon J, Calvini P, Morbelli S, De Carli F, Guerra UP, Van de Pol LA, Visser PJ (2008) Brain SPECT in subtypes of mild cognitive impairment. Findings from the DESCRIPA multicenter study. J Neurol 255:1344-1353.

O'Brien JT, Burns A, Group BAPDC (2011) Clinical practice with anti-dementia drugs: a revised (second) consensus statement from the British Association for Psychopharmacology. J Psychopharmacol 25:997-1019.

Pitkanen M, Sirvio J, MacDonald E, Ekonsalo T, Riekkinen P, Sr. (1995a) The effects of d-cycloserine, a partial agonist at the glycine binding site, on spatial learning and working memory in scopolamine-treated rats. J Neural Transm Park Dis Dement Sect 9:133-144.

Pitkanen M, Sirvio J, MacDonald E, Niemi S, Ekonsalo T, Riekkinen P, Sr. (1995b) The effects of D-cycloserine and MK-801 on the performance of rats in two spatial learning and memory tasks. Eur Neuropsychopharmacol 5:457-463.

Reisberg B, Doody R, Stoffler A, Schmitt F, Ferris S, Mobiush HJ (2003) Memantine in moderate-to-severe Alzheimer's disease. N Engl J Med 348:1333-1341.

Rosen WG, Mohs RC, Davis KL (1984) A new rating scale for Alzheimer's disease. Am J Psychiatry 141:1356-1364.

Sasabe J, Miyoshi Y, Suzuki M, Mita M, Konno R, Matsuoka M, Hamase K, Aiso S (2012) D-amino acid oxidase controls motoneuron degeneration through D-serine. Proc Natl Acad Sci U S A 109:6267-632.

Scarpini E, Scheltens P, Feldman H (2003) Treatment of Alzheimer's disease: current status and new perspectives. Lancet Neurol 2:539-547.

Schneider LS, Dagerman KS, Higgins JP, McShane R (2011) Lack of evidence for the efficacy of memantine in mild Alzheimer's disease. Arch Neurol 68:991-998.

Segovia G, Porras A, Del Arco A, Mora F (2001) Glutamatergic neurotransmission in aging: a critical perspective. Mech Ageing Dev 122:1-29.

Silver H, Feldman P, Bilker W, Gur RC (2003) Working memory deficit as a core neuropsychological dysfunction in schizophrenia. Am J Psychiatry 160:1809-1816.

Tremblay GC, Qureshi IA (1993) The biochemistry and toxicology of benzoic acid metabolism and its relationship to the elimination of waste nitrogen. Pharmacol Ther 60:63-90.

Vanoni MA, Cosma A, Mazzeo D, Mattevi A, Todone F, Curti B (1997) Limited proteolysis and X-ray crystallography reveal the origin of substrate specificity and of the rate-limiting product release during oxidation of D-
amino acids catalyzed by mammalian D-amino acid oxidase. Biochemistry 36:5624-5632.

Wechsler D (1997) Wechsler Memory Scale, 3rd ed. Psychological Association, San Antonio, TX.

Wu CL, Xia S, Fu TF, Wang H, Chen YH, Leong D, Chiang AS, Tully T (2007) Specific requirement of NMDA receptors for long-term memory consolidation in Drosophila ellipsoid body. Nat Neurosci 10:1578-1586.

Wu TH, Tu CH, Chao HT, Li WC, Low I, Chuang CY, Yeh TC, Cheng CM, Chou CC, Chen LF, Hsieh JC (2016) Dynamic Changes of Functional Pain Connectome in Women with Primary Dysmenorrhea. Sci Rep 6:24543.

Zang Y, Jiang T, Lu Y, He Y, Tian L (2004) Regional homogeneity approach to fMRI data analysis. Neuroimage 22:394-400.

Zhang Y, Simon-Vermot L, Araque Caballero MA, Gesierich B, Taylor ANW, Duering M, Dichgans M, Ewers M, Alzheimer's Disease Neuroimaging I (2016) Enhanced resting-state functional connectivity between core memory-task activation peaks is associated with memory impairment in MCI. Neurobiol Aging 45:43-49.

Zhao F, Holahan MA, Wang X, Uslaner JM, Houghton AK, Evelhoch JL, Winkelmann CT, Hines CDG (2018) fMRI study of the role of glutamate NMDA receptor in the olfactory processing in monkeys. PLoS One 13:e0198395.
Figure Legend

**Figure 1. The changes of regional homogeneity after benzoate treatment in schizophrenia patients.** After 24 weeks benzoate treatment, decreased regional homogeneity (ReHo) has been found in right middle and medial frontal gyrus, where belong to right orbitofrontal cortex. The warm and cold color denotes to increased and decreased ReHo, respectively. Abbreviation: R, right; Med: medial; Mid, middle; G, gyrus.

**Figure 2. Correlation between the changes of regional homogeneity and the changes of memory tests with benzoate treatment in schizophrenia patients.** The changes of regional homogeneity were positively correlated with (left) the changes of working memory in right precentral gyrus and right middle occipital gyrus while with (right) the changes of verbal learning and memory in left precuneus. The warm and cold color denotes to positive and negative correlation, respectively. Abbreviation: R, right; Mid, middle; G, gyrus.
Table 1. Demographic and clinical characteristics of patients with amnestic mild cognitive impairment

| Treatment Groups | Sodium benzoate | Placebo | P Value |
|------------------|-----------------|---------|---------|
| (n = 9)          | (n = 12)        |         |         |

Demographics

| Demographics | Sodium benzoate | Placebo | P Value |
|--------------|-----------------|---------|---------|
| Gender, female, n (%) | 7 (77.8) | 5 (41.7) | 0.184<sup>a</sup> |
| Age, year, mean (SD) | 66.1 (3.2) | 69.2 (3.6) | 0.058<sup>b</sup> |
| Age at illness onset, year, mean (SD) | 65.4 (3.6) | 68.9 (3.6) | 0.042<sup>b</sup> |
| Education, Year, Mean (SD) | 5.0 (1.8) | 8.8 (2.7) | 0.003<sup>c</sup> |
| Body Mass Index (BMI), Mean (SD) | 22.7 (5.8) | 24.0 (2.4) | 0.466<sup>b</sup> |

No. of Patients Using Anti-dementia Drugs

| No. of Patients Using Anti-dementia Drugs | Sodium benzoate | Placebo | P Value |
|-----------------------------------------|-----------------|---------|---------|
| Total | 0 | 1 | 1.000<sup>a</sup> |
| Donepezil (dose, mean ± SD) | 0 | 1 (5.0 ± 0.0) | 1.000<sup>a</sup> |

<sup>a</sup>Fisher’s Exact test; <sup>b</sup>independent t-test; <sup>c</sup>Mann-Whitney U test, if the distribution was not normal.
Table 2. The changes of regional homogeneity after treatment with benzoate or placebo

| Increased ReHo | Decreased ReHo |
|----------------|----------------|
| Anatomic area  | BA  | Size | t Score | Coordinates (mm) | Anatomic area | BA  | Size | t Score | Coordinates (mm) |
|                |     |      |         | x       | y        | z       | x       | y       | z       |
| Benzoate       |      |      |         |         |          |          |         |          |          |
| No significant |      |      |         |         |          |          |         |          |          |
| cluster        |      |      |         |         |          |          |         |          |          |
| Placebo        |      |      |         |         |          |          |         |          |          |
| No significant |      |      |         |         |          |          |         |          |          |
| cluster        |      |      |         |         |          |          |         |          |          |

ReHo: regional homogeneity; BA: Brodmann area; Size: number of voxels in the cluster; R: right; Mid: middle; Med: medial; G: gyrus.
Table 3. Correlation between the changes of cognitive tests and the changes of regional homogeneity (ReHo) with benzoate or placebo treatment

| Anatomic area | BA | Size (mm) | t | x   | y   | z     |
|---------------|----|-----------|---|-----|-----|-------|
| **Positive correlation** |     |           |   |     |     |       |
| Wechsler Memory, spatial span | Benzoate | R Precentral G | 6 | 99  | 4.19| 63    |
| | | R Mid Occipital G | 18 | 93  | 4.10| 24  |
| Placebo | No significant cluster | No significant cluster |
| Wechsler Memory, word listing | Benzoate | L Precuneus | 7 | 103 | 4.80| -21  |
| Placebo | No significant cluster | No significant cluster |

| Anatomic area | BA | Size (mm) | t | x   | y   | z     |
|---------------|----|-----------|---|-----|-----|-------|
| **Negative correlation** |     |           |   |     |     |       |

BA: Brodmann area; Size: number of voxels in the cluster; B: bilateral; L: left; R: right; Sup: superior; Mid: middle; Inf: inferior; N: nucleus; G: gyrus; EC: Entorhinal cortex; PCC: posterior cingulate cortex.
