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Homeostasis of serine enantiomers is disrupted in the *post-mortem* caudate putamen and cerebrospinal fluid of living Parkinson's disease patients

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A R T I C L E I N F O

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A B S T R A C T

L-serine generated in astrocytes plays a pivotal role in modulating essential neurometabolic processes, while its enantiomer, D-serine, specifically regulates NMDA receptor (NMDAR) signalling. Despite their physiological relevance in modulating cerebral activity, serine enantiomers metabolism in Parkinson's disease (PD) remains elusive. Using High-Performance Liquid Chromatography (HPLC), we measured D- and L-serine levels along with other amino acids known to modulate NMDAR function, such as L-glutamate, L-aspartate, D-aspartate, and glycine, in the *post-mortem* caudate putamen (CPu) and superior frontal gyrus (SFG) of PD patients. Moreover, we examined these amino acids in the cerebrospinal fluid (CSF) of *de novo* living PD, Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) patients versus subjects with other neurological disorders (OND), used as control. We found higher D-serine and L-serine levels in the CPu of PD patients but not in the SFG, a cerebral region that, in contrast to the CPu, is not innervated by nigral dopaminergic terminals. We also highlighted a significant elevation of both serine enantiomers in the CSF samples from PD but not in those of AD and ALS patients, compared with control subjects. By contrast, none or only minor changes were found in the amount of other NMDAR modulating amino acids. Our findings identify D-serine and L-serine level upregulation as a biochemical signature associated with nigrostriatal dopaminergic degeneration in PD.

Abbreviations: 6-OHDA, 6-hydroxydopamine; Aβ, Amyloid beta; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ASCT1, alanine serine cysteine transporter 1; BDI, Beck Depression Inventory; CNS, central nervous system; CPu, caudate putamen; CSF, cerebrospinal fluid; D-Asp, D-aspartate; D-Ser, D-serine; DAAO, D-amino acid oxidase; Gly, glycine; H&Y, Hoehn & Yahr; HPLC, High-Performance Liquid Chromatography; IQR, interquartile range; L-Asp, L-aspartate; L-Glu, L-glutamate; L-Ser, L-serine; LB, Lewy bodies; MMSE, Mini-Mental State Examination; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NMDAR, ionotropic N-methyl-D-aspartate receptors; NMSS, Non-Motor Symptoms Scale; OND, other neurological disorders; PD, Parkinson's disease; PHGDH, 3-phosphoglycerate dehydrogenase; PMI, *post-mortem* interval; SFG, superior frontal gyrus; SR, serine racemase; TH, tyrosine hydroxylase; UPDRS, Unified Parkinson's Disease Rating Scale..

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1. Introduction

In addition to the canonical neurotransmitters L-glutamate (L-Glu), L-aspartate (L-Asp), and glycine (Gly) (Andersen et al., 2021; Curtis and Watkins, 1960; Zhou and Danbolt, 2014), the activation of glutamatergic ionotropic N-methyl-D-aspartate (NMDA) receptors (NMDAR) is also modulated by the atypical D-amino acids, D-serine (D-Ser) and D-aspartate (D-Asp), which regulate synaptic transmission/plasticity and cognition (Errico et al., 2011; Krashia et al., 2016; Martineau et al., 2006; Sacchi et al., 2012; Sasabe and Suzuki, 2019; Usiello et al., 2020; Wolosker et al., 2016). However, our understanding regarding the role and possible alterations in D-amino acid metabolism in aging and neurodegenerative disorders is still limited and requires comprehensive research. In particular, findings obtained in humans and animal models indicate that abnormally high D-Ser levels may produce either harmful or beneficial effects depending on the neuropathological context (Seckler and Lewis, 2020; Singh and Singh, 2011; Wolosker and Balu, 2020).

D-ser acts as an endogenous obligatory co-agonist at the glycine modulatory site on GluN1 subunit of NMDAR (Sasabe and Suzuki, 2019; Wolosker et al., 2016). Elevated D-Ser levels have been reported to cause motor neurons death (Sasabe et al., 2012, 2007) in the ^{G93A}SOD-1 transgenic mouse model of amyotrophic lateral sclerosis (ALS) (Sasabe et al., 2007; Thompson et al., 2012), and in mice expressing inactive D-amino acid oxidase (DAAO) (Sasabe et al., 2012), the enzyme involved in D-Ser degradation (Pollegioni et al., 2018; Wolosker et al., 2016). Similarly, transgenic mice expressing the R199W mutant DAAO enzyme, previously identified in familial ALS patients (Mitchell et al., 2010), exhibited motor impairments and loss of lumbar spinal cord motor neurons (Kondori et al., 2017).

D-Ser variations have also been documented in the *post-mortem* brain, cerebrospinal fluid (CSF), and serum of individuals diagnosed with Alzheimer's disease (AD) (Madeira et al., 2015; Maffioli et al., 2022; Piubelli et al., 2021), and frontotemporal dementia (FTD) (Palese et al., 2020). However, inconsistencies across independent studies have been revealed, calling for further investigations aimed at addressing the association between central D-Ser variations and AD pathophysiology (Biemans et al., 2016; Nuzzo et al., 2020; Orzylowski et al., 2021). Although the occurrence of D-Ser metabolism modification in the brain of AD patients remains puzzling, a recent study utilizing the 3xTg mouse model of AD showed that oral administration of both D-Ser and L-serine (L-Ser) rescued deficits in hippocampal synaptic plasticity and spatial memory (Le Douce et al., 2020).

In contrast to its D-enantiomer, which is selectively involved in NMDAR occupancy, L-Ser regulates a plethora of metabolic processes in the central nervous system (CNS) (Holm and Buschard, 2019; Murtas et al., 2020), as a precursor of sphingolipids, purine nucleotides, antioxidants, and endogenous NMDAR co-agonists, such as Gly and D-Ser (Metcalf et al., 2018; Murtas et al., 2020; Zhou et al., 2017). Consistent with its essential role in the biosynthesis of vital molecules, a deficiency in L-Ser levels, resulting from a genetic mutation in one of the enzymes involved in its production or trafficking (Damseh et al., 2015; El-Hattab, 2016; Heimer et al., 2015; Srour et al., 2015), compromises brain development and functioning, leading to severe neurological impairments in infants (e.g. microcephaly, psychomotor retardation, and seizures) (De Koning et al., 2003; Maugard et al., 2021; Metcalf et al., 2018). Recently, L-Ser supplementation has demonstrated remarkable anti-inflammatory, and neuroprotective properties in various animal models of brain injury, neuropathies, and neurodegenerative diseases (Le Douce et al., 2020; Handzlik et al., 2022; Ye et al., 2021), and preliminary clinical trials (Bradley et al., 2018; Fridman et al., 2019; Levine et al., 2017).

Structural and functional changes impacting NMDAR have been documented in patients and animal models of Parkinson's disease (PD), contributing to the development of motor and non-motor symptoms associated with this neurological condition (Campanelli et al., 2022; Gardoni et al., 2006; Gardoni and Di Luca, 2015; Hallett et al., 2005). However, the precise involvement of D-Ser and D-Asp metabolism in these NMDAR-related alterations remains unknown. Despite this knowledge gap, the pharmacological blockade of the glycine transporter-1 and the subsequent increased stimulation at GluN1 NMDAR subunit was found to improve several parkinsonian features in dopamine-depleted 6-hydroxydopamine (6-OHDA)-lesioned mice and rats, and in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated marmosets (Frouni et al., 2022, 2021; Schmitz et al., 2013).

Consistent with preclinical findings, a clinical investigation carried out on a small cohort of PD patients has shown that D-Ser supplementation, given as an adjuvant to PD medications, ameliorates both motor and non-motor symptoms, as indicated by significantly improved UPDRS, Starkstein Apathy Scale, and positive and negative syndrome scale (PANS) scores (Gelfin et al., 2012; Heresco-Levy et al., 2013).

In the present study, to gain insight into the occurrence of D-Ser and D-Asp, as well as of the primary neuroactive amino acids modulating glutamatergic transmission, including L-Glu, Gly, and L-Asp, in the PD brain, we measured by High-Performance Liquid Chromatography (HPLC) the levels of these molecules in the *post-mortem* caudate putamen (CPu) and Superior Frontal Gyrus (SFG) of PD patients compared to non-demented controls. Moreover, we determined the concentrations of these amino acids in the CSF samples of a large cohort of *de novo* living patients diagnosed with PD, AD and ALS compared to controls with other neurological disorders (OND). Our findings identified increased D-Ser and L-Ser concentrations in the *post-mortem* CPu and in the CSF of *de novo* living PD patients as a biochemical signature associated with nigrostriatal dopaminergic degeneration.

2. Material and methods

2.1. Human post-mortem tissue collection

Post-mortem CPu and SFG tissue samples were obtained from The Netherlands Brain Bank (Netherlands Institute for Neuroscience, Amsterdam, open access: www.brainbank.nl, accessed on 27 January 2022) and derived from two different cohorts of PD patients and relative non-demented control subjects. We used *post-mortem* CPu tissues from 27 PD patients, characterized by Braak LB stage 3–4 ($n = 13$) and Braak Lewy bodies (LB) stage 6 ($n = 14$). Due to the limited number of available CPu samples of non-demented controls from the Netherlands Brain Bank, PD patients were compared to 6 non-demented controls which were matched for age, sex, and *post-mortem* interval (PMI) to limit the effects of confounding factors in the statistical analysis. The *post-mortem* SFG samples collected from PD patients (Braak LB stage ≥ 4) ($n = 10$) were comparable in number to the samples obtained from non-demented controls ($n = 10$). These two groups were also age, sex, and PMI matched.

Non-demented controls were selected from adult individuals without cognitive decline and Braak LB ≤ 3 in accordance with the Braak and Braak criteria (Braak and Braak, 1991). Demographic characteristics of PD patients and non-demented controls are described in Table 1 (CPu) and Table 2 (SFG). Control subjects had no known clinical history of neurological disorders as confirmed by the neuropathological evaluation of their samples. Clinical diagnosis of PD was based on diagnostic procedure according to the UK Brain Bank criteria for PD (Hughes et al., 1992) and confirmed by neuropathological findings (Braak and Braak, 1995). CPu and SFG frozen tissues were pulverized in liquid nitrogen and stored at −80 °C for subsequent processing.

2.2. Patients enrollment and cerebrospinal fluid collection

A group of 33 consecutive *de novo* PD patients was enrolled in the study between 2017 and 2019. PD patients were admitted to the Neurology Unit of IRCCS Neuromed in Pozzilli (IS, Italy) and later diagnosed. After their admittance, all patients underwent blood tests for

Table 1
Demographic and clinical characteristics of Parkinson's disease patients and non-demented control subjects from *post-mortem* caudate putamen tissue collection.

Characteristics	Control		Parkinson's disease	
	N	Median [IQR]	N	Median [IQR]
Sex (male/female)	1 / 5		16 / 11	
Age (years)		83.00 [74.50–86.00]		74.50 [67.00–81.00]
PMI (hours)		5.42 [4.86–6.69]		6.00 [5.09–7.37]
Braak LB stage (0-II/ III/IV/V/VI)	0/0/ 0/0/ 0		0/3/ 10/0/ 14	

Number of subjects is indicated as (n). Values are expressed as median (interquartile range, IQR). Abbreviations: PMI = *post-mortem* interval; Braak LB = Braak Lewy body.

Table 2
Demographic and clinical characteristics of Parkinson's disease patients and non-demented control subjects from *post-mortem* superior frontal gyrus tissue collection.

Characteristics	Control		Parkinson's disease	
	N	Median [IQR]	N	Median [IQR]
Sex (male/female)	10/0		10/0	
Age (years)		85.00 [79.00–88.25]		85.00 [81.75–86.00]
PMI (hours)		5.87 [5.62–6.58]		5.79 [4.42–6.45]
Braak LB stage (0-II/ III/IV/V/VI)	4/0/ 0/0/0		0/0/ 2/2/6	

Number of subjects is indicated as (n). Values are expressed as median (interquartile range, IQR). Abbreviations: PMI = *post-mortem* interval; Braak LB = Braak Lewy body.

diagnostic purposes, complete neurological evaluation, brain and spinal MRI scan and CSF withdrawal within 24 h. Patients were drug-free before CSF withdrawal and neurophysiological assessment. PD-specific therapies were initiated later.

The diagnosis of PD was performed according to current diagnostic criteria by a neurologist expert in movement disorders (Postuma et al., 2015). All of patients had been submitted to TC/MRI during the diagnostic workup.

The following features of PD patients were considered: (a) sex, (b) age at lumbar puncture (LP), (c) disease duration, calculated as the time interval between the first motor symptoms and the time of diagnosis, (d) disease stage, according to the Hoehn & Yahr (H&Y) scale (Hoehn and Yahr, 1967), (e) parts II and III of the Unified Parkinson's Disease Rating Scale (UPDRS-II, to evaluate patient perceptions of motor experiences of daily living, and UPDRS-III, to evaluate motor activities) (Antonini et al., 2013), Non-Motor Symptoms Scale (NMSS) to evaluate non-motor symptoms of PD (Chaudhuri et al., 2007), the Mini-Mental State Examination (MMSE) to evaluate global cognitive functioning (Measso et al., 1993), Beck Depression Inventory–Second Edition (BDI-II) was used to assess the presence of depressive symptom (Sica and Ghisi, 2007).

For AD patients, the diagnosis of probable Alzheimer's Disease was based on the current criteria of the International Working Group (Dubois et al., 2014). For ALS patients, the diagnosis of ALS was made according to the revised El Escorial criteria (Brooks et al., 2000).

Demographic and clinical characteristics of the AD, PD, ALS and OND controls are summarized in Table 3. PD, AD, ALS and OND patients differed for age ($p = 0.001$, Kruskal Wallis test), while sex was equally distributed among groups ($\chi^2 = 3.45$; $p = 0.327$; Chi-square test).

We also collected CSF samples from OND control group comprised 43 patients with non- inflammatory/non-degenerative central nervous system disorders or peripheral nervous system disorders (**Supplemental table 3**).

The study was conducted according to the Declaration of Helsinki and with informed written consent provided by all subjects. The study was approved by the ethics committee of the IRCCS NEUROMED (Approval N. 06/17). All procedures were carried out in accordance with approved guidelines.

CSF samples were collected according to international guidelines (Del Campo et al., 2012; Teunissen et al., 2009; Vanderstichele et al., 2012). LP was performed between 8:00 to 10:00, after an overnight fasting. CSF was immediately collected in sterile polypropylene tubes (Sarstedt® tubes, codes: 62.610.210) and gently mixed to avoid possible gradient effects. All samples were centrifuged at 2000 x g for 10 min at room temperature and then aliquoted in 0.5 ml aliquots in sterile polypropylene tubes (Sarstedt® tubes, codes: 72.730.007). Aliquots were frozen at −80 °C pending analysis, avoiding freeze/thaw cycles. CSF Amyloid beta 1–40 (Aβ40) and Amyloid beta 1–42 (Aβ42) were

Table 3
Demographic and clinical characteristics of Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis patients, and control subjects enrolled in the cerebrospinal fluid sample collection.

Demographic/clinical characteristic	OND (N = 43)		Parkinson's disease (N = 33)		Alzheimer's disease (N = 50)		Amyotrophic lateral sclerosis (N = 29)	
	N	Median [IQR]	N	Median [IQR]	N	Median [IQR]	N	Median [IQR]
Sex (male/female)	24 / 19		23 / 10		25 / 25		18 / 11	
Age (years)	43	58.00 [49.00–66.00]	33	61.00 [55.50–67.00]	50	69.00 [58.75–73.00]	29	63.00 [59.00–71.00]
Disease duration (months)			32	12.00 [8.50–24.00]				
MMSE			28	26.49 [25.35–28.25]	46	17.01 [8.99–21.11]		
UPRDS-I			28	9.00 [6.00–13.00]				
UPRDS-II			28	6.00 [3.00–8.00]				
UPRDS-III			33	19.00 [12.50–25.50]				
H&Y (1/1.5/2/2.5/3/4/5)			32	16 / 1 / 15 / 0 / 0 / 0 / 0				
Non-motor symptoms			25	27.00 [20.00–36.50]				
BDI-II			29	13.00 [4.00–17.50]				
Amyloid beta p40 (pg/ml)					37	186.50 [136.90–304.40]		
Amyloid beta p42 (pg/ml)					50	284.30 [191.50–423.10]		
TAU (pg/ml)					50	454.30 [285.80–670.60]		

Number of subjects is indicated as (n). Values are expressed as median (interquartile range, IQR). Abbreviations: OND = other neurological disorders; MMSE = Mini-Mental State Examination; UPDRS = Unified Parkinson's Disease Rating Scale; H&Y = Hoehn-Yahr scale; BDI = Beck Depression Inventory; CSF = cerebrospinal fluid.

measured with commercially available enzyme-linked immunosorbent assays (ELISAs) purchased from Euroimmun (EUROIMMUN AG, Lübeck, Germany), while total tau was detected using INNOTEST kits (Fujirebio Europe, Gent, Belgium). Internal quality controls were assayed in each run. Operators blinded to the diagnosis performed the measurements. Blood-contaminated samples were excluded from the analysis (cutoff of 50 red blood cells per microliter).

2.3. Western blotting

Frozen, powdered samples from *post-mortem* tissues were sonicated in 1% sodium dodecyl sulfate and boiled for 10 min. Aliquots (2 µl) of the homogenate were used for protein determination using a Bio-Rad Protein Assay kit. Equal amounts of total proteins (30 µg) for each sample were loaded on precast 4–20% gradient gels (BioRad Laboratories, Hercules, CA, USA). Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to PVDF membranes (GE Healthcare, Chicago, IL, USA) via the Trans Blot Turbo System (BioRad Laboratories). A primary antibody against tyrosine hydroxylase (1:2000, MAB318; Merck Sigma) was used to assess the dopaminergic neurons degeneration. Immunodetections were accomplished by using the following primary antibodies: serine racemase (1:500, sc-5751; Santa Cruz), D-amino acid oxidase (1:1000, EB11100; Everest Biotech), 3-phosphoglycerate dehydrogenase (1:1000, 13428S; Cell Signaling), alanine serine cysteine transporter 1 (1:1000, 8442; Cell Signaling) and β-actin (1:10000, A5441; Merk Sigma). Optical density values were normalized to β-actin for variations in loading and transfer. Blots were then incubated with anti-mouse or anti-goat horseradish peroxidase conjugated secondary antibodies. Immunoreactivity was detected by enhanced chemiluminescence (GE-Healthcare) and quantified by Quantity One software (Bio-Rad).

2.4. HPLC analysis of amino acids content

Brain tissue samples were homogenized in 1:20 (*w/v*) 0.2 M TCA, sonicated (3 cycles, 10 s each) and centrifuged at 13,000 ×g for 20 min. All the precipitated protein pellets from brain samples were stored at −80 °C for protein quantification. CSF samples (100 µl) were mixed in a 1:10 dilution with HPLC-grade methanol (900 µl) and centrifuged at 13,000 ×g for 10 min; supernatants were dried and then suspended in 0.2 M TCA. TCA supernatants from brain and CSF samples were then neutralized with 0.2 M NaOH and subjected to precolumn derivatization with o-phthaldialdehyde /N-acetyl-L-cysteine in 50% methanol. Amino acids derivatives were resolved on a UHPLC Nexera X3 system (Shimadzu) by using a Shim-pack GIST C18 3-µm reversed-phase column (Shimadzu, 4.0 × 150 mm) under isocratic conditions (0.1 M sodium acetate buffer, pH 6.2, 1% tetrahydrofuran, and 1 ml/min flow rate). A washing step in 0.1 M sodium acetate buffer, 3% tetrahydrofuran and 47% acetonitrile, was performed after every run. Identification and quantification of amino acids were based on retention times and peak areas, compared with those associated with external standards. The identity of the D-Asp peak was further evaluated by selective degradation catalyzed by a recombinant human D-aspartate oxidase enzyme (hDDO) (Katane et al., 2018). hDDO enzyme (12.5 µg) was added to the samples, incubated at 30 °C for 3 h, and subsequently derivatized. For tissue samples, total protein content of homogenates was determined by Bradford assay method, after re-solubilization of the TCA precipitated protein pellets. The detected amino acids concentration in tissue homogenates was normalized by the total protein content and expressed as nmol/mg protein; amino acids levels in the CSF were expressed as µM.

2.5. Statistical analyses

The normality distribution was tested using the Kolmogorov–Smirnov test. Continuous variables were shown as median (interquartile range, IQR). HPLC data from CSF samples were analyzed by ANCOVA considering the effect of age as confounding factor. In the CPu and SFG, differences in continuous variables between PD patients and control subjects were evaluated by non-parametric Mann-Whitney test. Spearman's non-parametric correlations were used to test possible associations between continues variables. Benjamini-Hochberg procedure was used to decrease the false discovery rate and avoid type I errors (false positives).

3. Results

3.1. L-aspartate and D-serine concentrations are increased in the post-mortem caudate putamen of PD patients

First, we determined by HPLC the levels of the neuroactive amino acids modulating glutamatergic NMDAR neurotransmission, namely L-Glu, D-Ser, Gly, L-Asp and D-Asp, in the *post-mortem* CPu of PD subjects and non-demented controls (Fig. 1; Table 1). The *post-mortem* PD brains were divided into two groups according to their Braak LB score (Braak LB 3–4, $n = 13$; Braak LB 6, $n = 14$).

Mann-Whitney test revealed no significant changes in L-Glu, D-Asp and Gly levels between PD patients and control subjects, although a clear trend towards D-Asp increase was observed in PD brains (L-Glu: Ctrl *vs* PD Braak LB 3–4, $p = 0.179$; Ctrl *vs* PD Braak LB 6, $p = 0.178$; D-Asp: Ctrl *vs* PD Braak LB 3–4, $p = 0.071$; Ctrl *vs* PD Braak LB 6, $p = 0.152$; Gly: Ctrl *vs* PD Braak LB 3–4, $p = 0.179$; Ctrl *vs* PD Braak LB 6, $p = 0.178$; Fig. 1C,E,G). Interestingly, L-Asp content was significantly increased in the CPu of both Braak LB 3–4 and LB 6 PD patients (median [IQR] of nmol/mg protein, L-Asp: Ctrl = 12.59 [8.13–21.67] *vs* PD Braak LB 3–4 = 24.25 [16.34–31.52], $p = 0.028$; Ctrl = 12.59 [8.13–21.67] *vs* PD Braak LB 6 = 29.80 [17.17–36.03], $p = 0.015$; Fig. 1D). Similarly, we found significantly higher levels of the NMDAR co-agonist, D-Ser, in the CPu of PD patients, independently of their disease severity (median [IQR] of nmol/mg protein, Ctrl = 1.38 [0.81–1.71] *vs* PD Braak LB 3–4 = 1.80 [1.54–2.29], $p = 0.046$; Ctrl = 1.38 [0.81–1.71] *vs* PD Braak LB 6 = 1.91 [1.41–2.58], $p = 0.041$; Fig. 1F).

Taken together, our HPLC measurements show a marked elevation of L-Asp and D-Ser in the *post-mortem* CPu of PD patients, irrespective of the stage of disease.

3.2. Excitatory D- and L-amino acids levels are unaltered in the post-mortem superior frontal gyrus of PD patients

We next explored whether the upregulation of D-Ser and L-Asp levels observed in the *post-mortem* CPu of PD patients also occurs in the SFG, a region that is not innervated by nigral dopaminergic terminals but receives dopaminergic afferences from the ventral tegmental area (Coenen et al., 2018). Accordingly, we performed the HPLC analysis in another cohort of PD patients ($n = 10$) and non-demented control subjects ($n = 10$) (Fig. 2, Table 2). We selected the SFG due to its critical involvement in the regulation of response inhibition and impulsive behaviors (Sharp et al., 2010), which are impaired in a considerable subset of PD patients (Gatto and Aldinio, 2019), and because structural and functional abnormalities have been recently reported in this brain area in PD patients (Guimarães et al., 2017; Li et al., 2022; Shen et al., 2020).

We found no significant difference in D-Ser and L-Asp levels between PD patients and non-demented controls (L-Asp, $p = 0.353$; D-Ser, $p = 0.684$; Fig. 2D, F). Similarly, we detected comparable L-Glu, D-Asp and Gly concentrations between PD patients and controls (L-Glu, $p = 0.970$; D-Asp, $p = 0.684$; Gly: $p = 0.912$; Fig. 2C,E,G).

Our results are consistent with a previous report indicating that there were no significant changes in excitatory amino acids levels in cortical regions (i.e. precentral gyrus, postcentral gyrus, and frontal cortex) of the PD brain (Gerlach et al., 1996), and indicate that the alterations of D-Ser and L-Asp levels observed under DA denervation are located in a dopaminoceptive region innervated by nigral dopaminergic neurons, specifically the CPu.

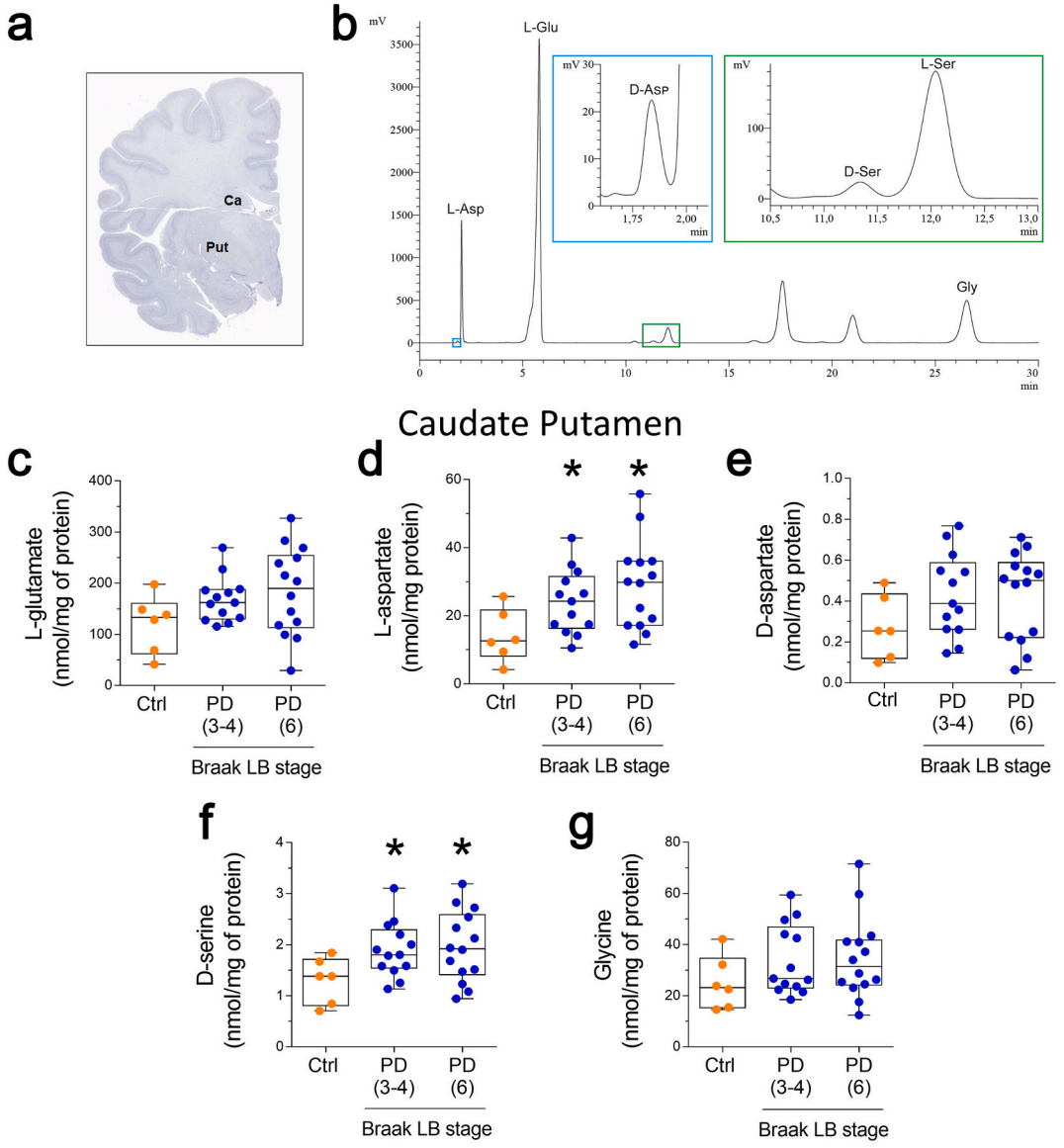

Fig. 1. Analysis of free L-glutamate, L-aspartate, D-aspartate, D-serine and glycine levels in the *post-mortem* caudate putamen of PD patients. (**a**) Representative image showing a Nissl-stained coronal human brain section evidencing the caudate (Ca) and putamen (Put) regions (from the open-source Allen Adult Human Brain Atlas and Allen Reference Atlas – atlas.brain-map.org (Ding et al., 2016)). (**b**) Representative HPLC chromatogram illustrating L-glutamate (L-Glu), L-aspartate (L-Asp), D-aspartate (D-Asp), L-serine (L-Ser), D-serine (D-Ser), and glycine (Gly) peaks obtained from a *post-mortem* caudate putamen sample. (**c-g**) Content of (**c**) L-Glu, (**d**) L-Asp, (**e**) D-Asp, (**f**) D-Ser and (**g**) Gly detected by HPLC analysis in the caudate putamen of PD patients divided into two groups according to their Braak Lewy bodies (LB) scores: Braak LB stage 3–4 (PD 3–4, $n = 13$) and Braak LB stage 6 (PD 6, $n = 14$), compared to non-demented controls (Ctrl, $n = 6$). In each sample, free amino acids were detected in a single run and expressed as nmol/mg of protein. Dots represent the single subjects' values while bars illustrate the median with interquartile range. *$p < 0.05$, compared to Ctrl (Mann-Whitney test).

3.3. *Abnormally high D-serine levels in the cerebrospinal fluid of de novo living PD patients*

Next, we investigated the levels of L-Glu, L-Asp, Gly, D-Ser and D-Asp, in the CSF samples collected from *de novo* living PD patients ($n = 33$) and control subjects with OND ($n = 43$) (Fig. 3; Table 3). Additionally, given that prior investigations measuring CSF D-Ser levels in AD patients have provided contrasting results (Biemans et al., 2016; Madeira et al., 2015; Nuzzo et al., 2020), and that mutations in genes regulating D-Ser catabolism have been causally associated with a familiar form of ALS (Mitchell et al., 2010), here we also examined CSF samples of *de novo* living patients affected by AD ($n = 50$) and ALS ($n = 29$) as comparative groups with distinct neurodegenerative pathologies.

Considering a significant main effect of the age among PD, AD, ALS, and OND control patients (Table 3), we used a multivariate ANCOVA model to analyse our data. This analysis demonstrated significant alterations in the CSF levels of L-Glu and D-Ser among the different disease conditions (L-Glu: $F_{(3,154)} = 3.075$, $p = 0.030$; D-Ser: $F_{(3,\ 154)} = 8.305$, $p < 0.0001$; Fig. 3B,D; **Supplemental Table 1**). Bonferroni *post-hoc* multiple comparisons indicated a significant reduction of L-Glu levels in PD patients, compared to OND controls (median [IQR] of nmol/mg protein, OND = 8.10 [6.68–9.93] *vs* PD = 6.16 [5.37–7.28], $p = 0.018$; Fig. 3B; **Supplemental Table 1**). This result is consistent with those of previous investigations (Jiménez-Jiménez et al., 2020; Kuiper et al., 2000; Mally et al., 1997; Tohgi et al., 1991; Wu et al., 2016); nonetheless, the precise neurochemical and metabolic implications of this alteration remain to be fully elucidated, given the multifaceted roles of L-Glu in energy homeostasis and excitatory neurotransmission (Andersen et al., 2021; Yelamanchi et al., 2016).

In contrast, a significant increase of D-Ser content was found in the

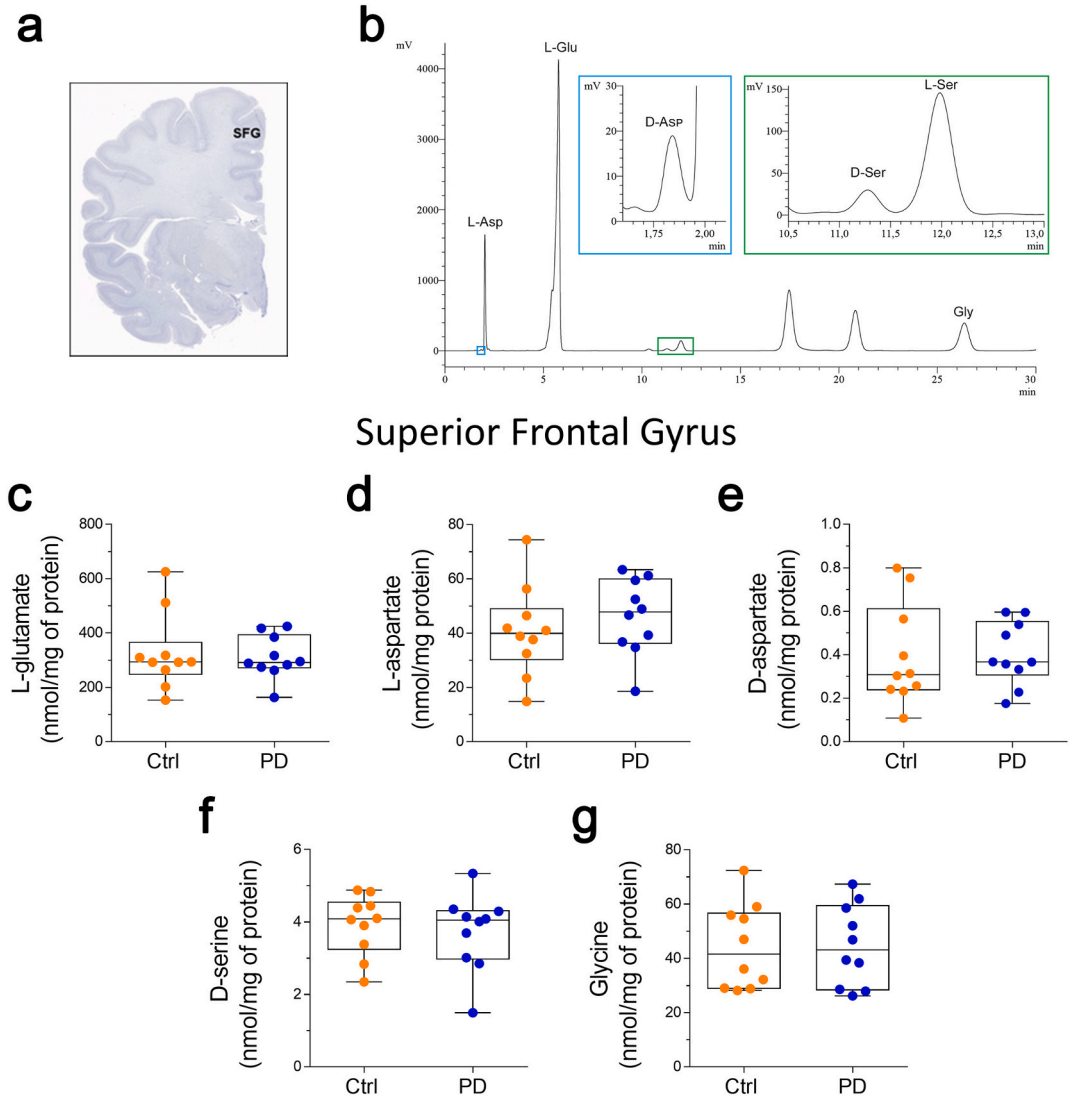

Fig. 2. Analysis of free L-glutamate, L-aspartate, D-aspartate, D-serine and glycine levels in the *post-mortem* superior frontal gyrus of PD patients. (**a**) Representative image showing a Nissl-stained coronal human brain section evidencing the Superior Frontal Gyrus (SFG) (from the open-source Allen Adult Human Brain Atlas and Allen Reference Atlas – atlas.brain-map.org (Ding et al., 2016)). (**b**) Representative HPLC chromatogram illustrating L-glutamate (L-Glu), L-aspartate (L-Asp), D-aspartate (D-Asp), L-serine (L-Ser), D-serine (D-Ser), and glycine (Gly) peaks obtained from a *post-mortem* superior frontal gyrus sample. (**c-g**) Levels of (**c**) L-Glu, (**d**) L-Asp, (**e**) D-Asp, (**f**) D-Ser and (**g**) Gly in the SFG of PD patients ($n = 10$) and non-demented control subjects (Ctrl, n = 10). In each sample, free amino acids were detected in a single run and expressed as nmol/mg of protein. Data points represent values of each subject while bars illustrate the median with interquartile range.

CSF of *de novo* living patients diagnosed with PD compared to OND, AD, and ALS subjects (median [IQR] of nmol/mg protein, OND = 4.07 [3.52–4.67] *vs* PD = 5.05 [4.76–5.68], $p < 0.0001$; AD = 4.22 [3.68–4.88] *vs* PD = 5.05 [4.76–5.68], $p = 0.0011$; ALS = 4.18 [3.48–5.14] *vs* PD = 5.05 [4.76–5.68], $p = 0.0077$; Bonferroni *post-hoc* multiple comparisons; Fig. 3D). In support of the involvement of the observed neurochemical variations in PD, CSF D-Ser levels observed in the current cohort of OND subjects from the Neuromed Hospital align with those reported in previous investigations conducted by our research group on OND subjects from other hospitals, such as the Center for Memory Disturbances, University Hospital of Perugia (Nuzzo et al., 2019), and the Brescia Hospital (Palese et al., 2020).

Moreover, ANCOVA analysis indicated no significant difference in the content of L-Asp ($F_{(3, 154)} = 2.869$, $p = 0.038$; OND *vs* PD, $p = 1.000$, OND *vs* AD $p = 0.087$, OND *vs* ALS $p = 1.000$; Bonferroni *post-hoc* multiple comparisons; Fig. 3C; **Supplemental Table 1**) and Gly ($F_{(3, 87)} = 0.209$, $p = 0.890$; Fig. 3E; **Supplemental Table 1**) among PD, AD, ALS patients and OND controls. D-Asp levels were found below the limit of HPLC detection (Nuzzo et al., 2019).

The unaltered CSF D-Ser levels observed in *de novo* living AD patients corroborate previous findings in independent cohorts of AD patients (Biemans et al., 2016; Nuzzo et al., 2020). Therefore, in contrast to recent observations in the serum (Piubelli et al., 2021), our results suggest that, at least in the CSF, the content of D-Ser may not serve as a reliable biomarker for AD and cognitive deterioration, as postulated in a previous report conducted on a limited cohort of patients receiving various medications (Madeira et al., 2015). Finally, our results indicating unaltered D-Ser levels in the CSF of *de novo* living ALS patients suggest that central alterations in its metabolism may not be a pathological feature common to all ALS patients.

Overall, HPLC findings highlighted a substantial and selective upregulation in the levels of D-Ser in CSF samples of *de novo* living PD patients compared to OND controls, and *de novo* patients with other neurodegenerative diseases, such as AD and ALS.

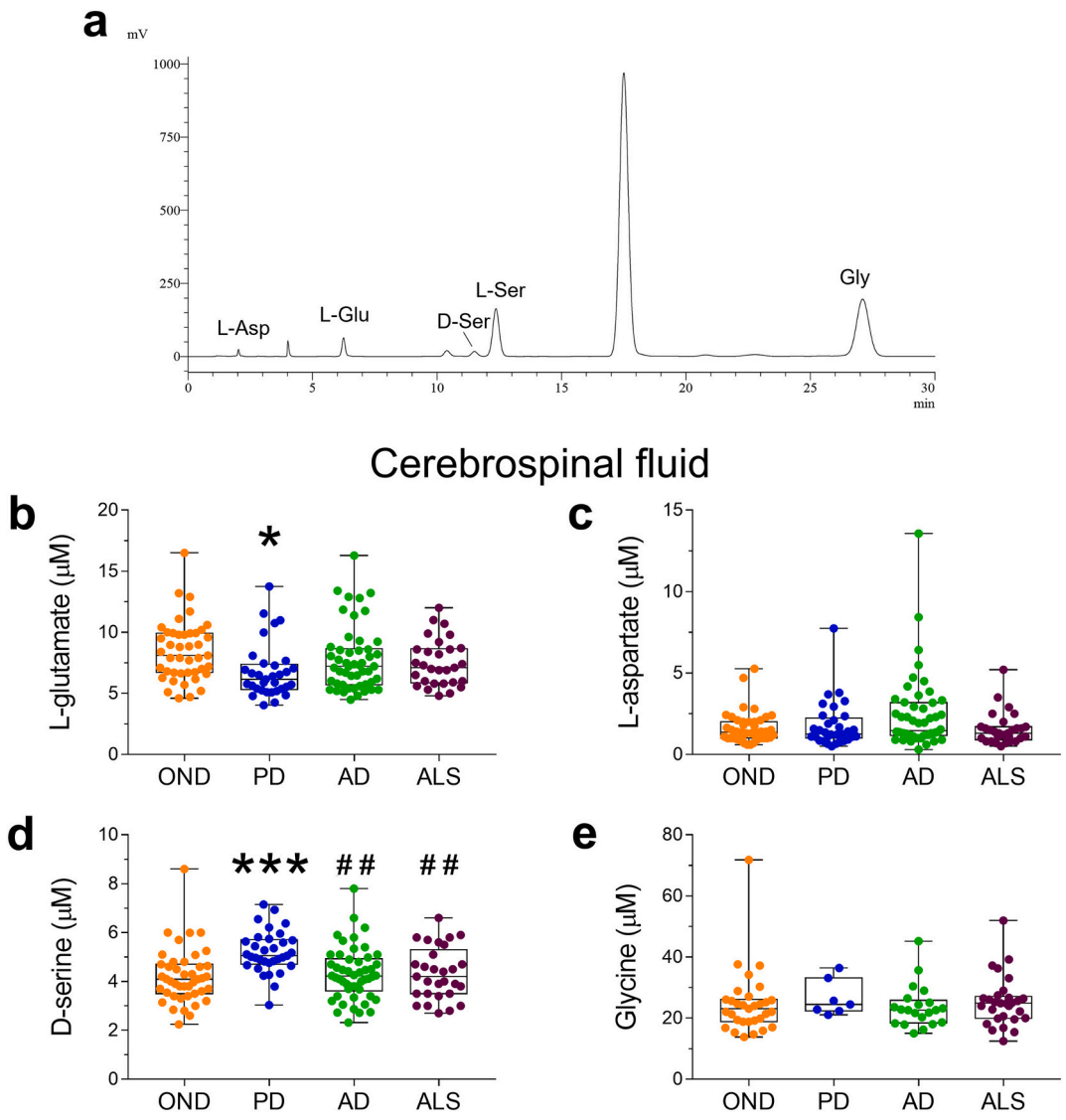

Fig. 3. Analysis of free L-glutamate, L-aspartate, D-serine and glycine in the cerebrospinal fluid of patients affected by PD and other neurological diseases. (**a**) Representative HPLC chromatogram illustrating L-glutamate (L-Glu), L-aspartate (L-Asp), D-serine (D-Ser), L-serine (L-Ser) and glycine (Gly) peaks obtained from a cerebrospinal fluid sample. (**b-e**) Levels of (**b**) L-Glu, (**c**) L-Asp, (**d**) D-Ser and (**e**) Gly in the cerebrospinal fluid of patients affected by PD ($n = 33$; $n = 7$ for Gly detection), Alzheimer's disease (AD, $n = 50$; $n = 21$ for Gly detection), and amyotrophic lateral sclerosis (ALS, $n = 29$) compared to control subjects affected by other neurological disorders (OND, $n = 43$; $n = 31$ for Gly detection). In each sample, free amino acids were detected in a single run. Dots represent the single subjects' values expressed as μM concentration, while bars illustrate the median with interquartile range. $^{*}p < 0.05$, $^{***}p < 0.0001$, compared to OND; $\#\#p < 0.01$, compared to PD (Bonferroni *post-hoc* multiple comparison).

3.4. Cerebrospinal fluid levels of D-serine do not correlate with demographic and clinical features of PD patients

We investigated whether the variations in D-Ser levels are correlated with specific demographic and clinical features characterizing our cohort of *de novo* living PD patients. We performed a linear regression between D-Ser levels and disease duration, and motor and non-motor symptoms evaluated by UPDRS (Antonini et al., 2013), NMSS (Chaudhuri et al., 2007), BDI (Sica and Ghisi, 2007), and MMSE (Measso et al., 1993). Spearman's correlation analyses followed by Benjamini-Hochberg correction for multiple comparisons indicated that CSF D-Ser levels of *de novo* living PD patients were not correlated with any assessed demographic or clinical parameters (Table 4). A similar lack of correlation was also found when the linear regression analysis was extended to the other neuroactive amino acids detected in PD patients' CSF (**Supplemental Table 2**). Similar to PD, in *de novo* AD patients, we observed no significant correlation between the CSF levels of D-Ser or the other NMDAR-related amino acids with the clinical and biochemical features of AD patients, such as MMSE, levels of Aβ40, Aβ42 or the microtubule-associated protein tau (Table 4; **Supplemental Table 2**).

3.5. Unaltered expression of serine racemase in the caudate putamen of PD patients

We investigated whether the increased D-Ser levels observed in the CPu of PD patients depend on the altered striatal expression of enzymes involved in its metabolism. Hence, we analyzed the protein levels of the enzymes regulating D-Ser biosynthesis and catabolism, serine racemase (SR) and DAAO, respectively (Martineau et al., 2006; Pollegioni and Sacchi, 2010; Sasabe and Suzuki, 2019; Wolosker et al., 2016). Previous investigations demonstrated a remarkable DAAO gene and protein expression in the hindbrain but not in the forebrain regions of mice, monkeys, and humans (Cuomo et al., 2019; Gonda et al., 2022; Keller et al., 2018; Nuzzo et al., 2019; Suzuki et al., 2017; Wang and Zhu,

Table 4
Correlation analysis between cerebrospinal fluid D-serine content and clinical characteristics of Parkinson's disease and Alzheimer's disease patients.

Diagnosis	Clinical parameter	D-Serine		
		r	*p* value	N
Parkinson's disease	Months of disease	−0.190	0.299	32
	UPDRS-I	0.006	0.977	28
	UPDRS-II	−0.118	0.550	28
	UPDRS-III	0.009	0.962	33
	Non-motor symptoms	0.128	0.542	25
	BDI-II	−0.269	0.158	29
	MMSE	−0.228	0.244	28
Alzheimer's disease	Aβ 40 (pg/ml)	0.221	0.428	15
	Aβ 42 (pg/ml)	0.087	0.550	50
	TAU (pg/ml)	0.311	0.02[a]	50
	MMSE	0.040	0.791	46

Number of subjects is indicated as (n). Statistical analyses are performed by Spearman's correlations. (a) p value is not significant after correction with Benjamini-Hochberg multiple comparisons. Abbreviations: UPDRS = Unified Parkinson's Disease Rating Scale; MMSE = Mini-Mental State Examination; BDI = Beck Depression Inventory.

2003). In agreement with this, we failed to detect by WB a measurable DAAO protein amount in all CPu specimens tested. Conversely, SR was detected in all tested CPu samples of PD patients and non-demented controls (Fig. 4A,B). In this regard, we found a slight but non-significant increase of SR protein in the *post-mortem* CPu of PD patients versus non-demented control subjects ($p = 0.158$; Mann–Whitney test; Fig. 4B).

Last, we measured the tyrosine hydroxylase (TH) protein levels in the CPu of PD patients and control subjects. As expected, data showed a dramatic decrease of TH protein expression in all PD patients compared to non-demented controls (Ctrl *vs* PD Braak LB 3–4, $p < 0.0001$; Ctrl *vs* PD Braak LB 6, $p < 0.0001$; Fig. 4D).

3.6. Increased L-serine content in the caudate putamen and cerebrospinal fluid of PD patients

To clarify the origin of the D-Ser upregulation found in the *post-mortem* CPu and CSF of *de novo* living PD patients, we measured the levels of its precursor, L-Ser, in the same specimens. Similar to D-Ser results, we found increased L-Ser content in the CPu of PD patients regardless of their Braak LB stages (median [IQR] of nmol/mg protein, L-Ser: Ctrl = 7.14 [5.68–11.49] *vs* PD Braak LB 3–4 = 14.64 [9.53–20.35], $p = 0.009$; Ctrl = 7.14 [5.68–11.49] *vs* PD Braak LB 6 = 13.84 [8.80–18.43], $p = 0.041$; Mann-Whitney test; Fig. 5A). Conversely, no alterations in L-Ser levels were found in the *post-mortem* SFG of PD patients compared to non-demented controls ($p = 0.887$; Kruskal-Wallis test; Fig. 5B). ANCOVA analysis (considering the effect of age as a covariate) and Bonferroni *post-hoc* tests revealed significantly higher L-Ser levels in the CSF of *de novo* PD compared to OND, AD, and ALS subjects ($F_{(3, 154)} = 8.402$, $p < 0.0001$; OND = 52.22 [45.04–59.44] *vs* PD = 70.04 [54.62–76.07], $p < 0.0001$; AD = 55.22 [47.30–66.55] *vs* PD = 70.04 [54.62–76.07], $p = 0.0260$; ALS = 51.96 [43.64–58.05] *vs* PD = 70.04 [54.62–76.07], $p = 0.0003$; Fig. 5C). As reported for D-Ser (Table 4), L-Ser levels were not correlated with demographic or clinical features of PD patients (Table 5). Likewise, CSF L-Ser levels in *de novo* AD patients were not significantly associated with Aβ40, Aβ42 and tau levels or with MMSE (Table 5). To investigate the molecular determinants leading to the L-Ser levels alteration in the CPu of PD patients, we measured the striatal protein levels of 3-phosphoglycerate dehydrogenase (PHGDH), the astrocytic rate-limiting enzyme of the phosphorylated pathway regulating the *de novo* L-Ser synthesis (Murtas et al., 2021). Western blotting analysis revealed unchanged PHGDH protein levels in the *post-mortem* CPu of the whole cohort of PD patients compared to controls ($p = 0.9064$; Mann-Whitney test; Fig. 5D,E).

In addition, we measured the protein levels of the astrocytic alanine serine cysteine transporter 1 (ASCT1), also known as SLC1A4 (Hofmann et al., 1994), which is primarily involved in Ser enantiomers trafficking between neurons and astrocytes (Kaplan et al., 2018). Notably, we found a significant downregulation of ASCT1 expression in the *post-mortem* CPu of the entire cohort of PD patients compared to controls ($p = 0.0353$; Mann-Whitney test; Fig. 5D,F).

Overall, HPLC determinations indicated abnormally higher L-Ser availability in the *post-mortem* CPu and CSF of *de novo* living PD patients, compared to their respective controls. In addition, the neurochemical alterations of Ser enantiomers in the CPu of PD patients were accompanied by a remarkable ASCT1 reduction.

4. Discussion

Altered striatal NMDAR transmission following dopamine denervation contributes to the onset and progression of motor and non-motor PD symptoms (Campanelli et al., 2022; Cenci et al., 2022; Gardoni and Di Luca, 2015). Hence, identifying specific molecules mirroring the state of NMDAR activation during the disease progression may contribute to new approaches for PD diagnosis and therapy. To date, most studies

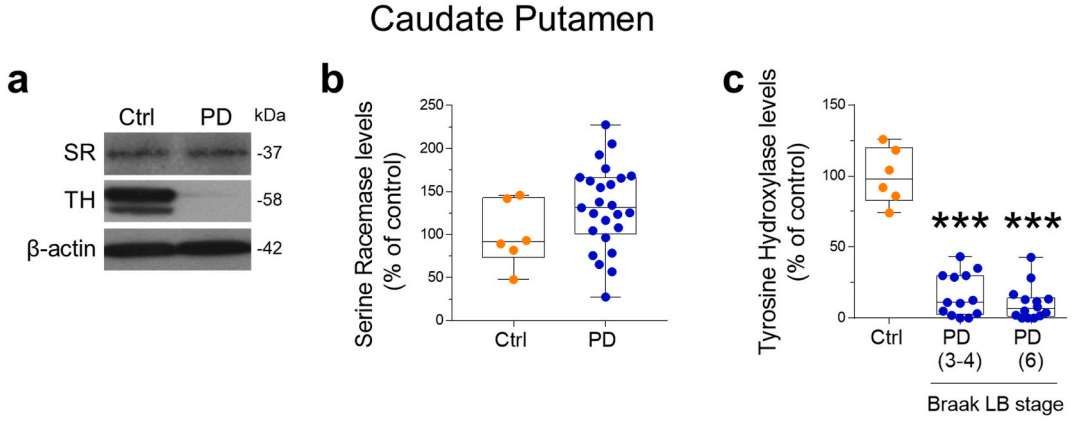

Fig. 4. Protein expression levels of serine racemase and tyrosine hydroxylase in the *post-mortem* caudate putamen of PD patients. (**a**) Representative blots of serine racemase (SR), tyrosine hydroxylase (TH), and β-actin immunodensity obtained in the *post-mortem* caudate putamen lysates by Western blotting. Quantification of (**b**) SR (Ctrl, $n = 6$; PD, $n = 25$) and (**c**) TH protein levels in the *post-mortem* caudate putamen of PD patients with Braak LB stage 3–4 (PD 3–4, $n = 13$) and Braak LB stage 6 (PD 6, $n = 14$), compared to non-demented controls (Ctrl, $n = 6$). Proteins variations are expressed as a percentage relative to the control group. β-actin was used to normalize variations in the loading and transfer procedures. Dots represent the single subjects' values while bars illustrate the median with interquartile range. ***$p < 0.0001$, compared to Ctrl (Mann–Whitney test).

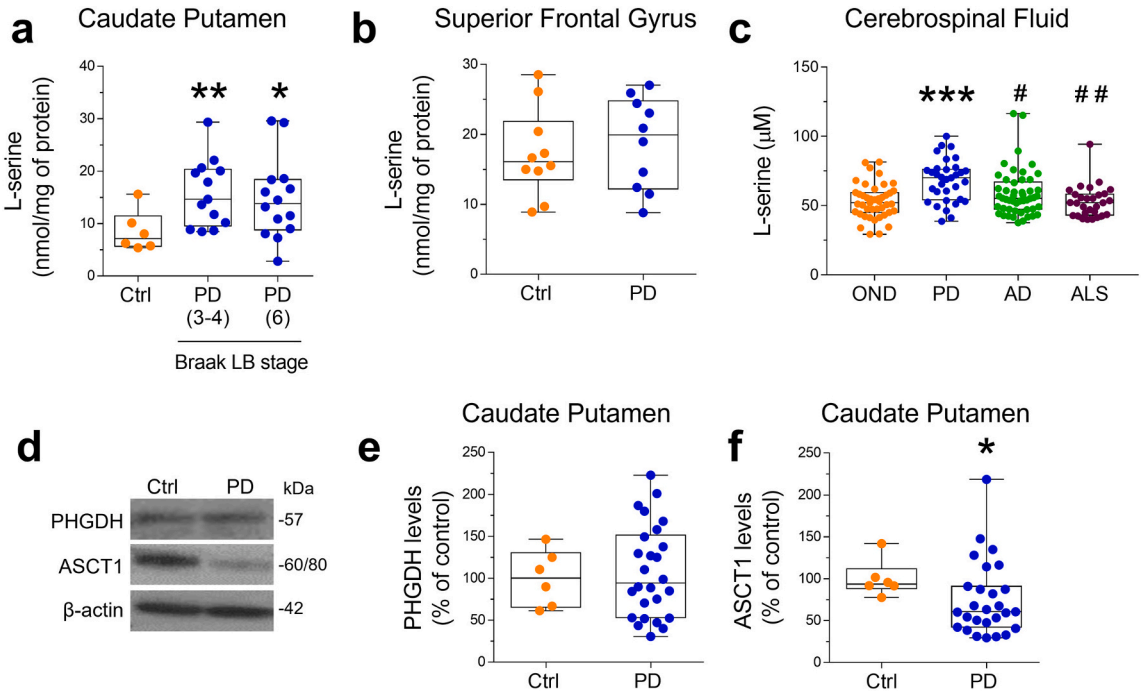

Fig. 5. Evaluation of L-serine levels in the *post-mortem* caudate putamen, superior frontal gyrus and cerebrospinal fluid of PD patients. (**a-c**) HPLC detection of L-serine in the *post-mortem* (**a**) caudate putamen of PD patients with Braak LB stage 3–4 (PD 3–4, $n = 13$), Braak LB stage 6 (PD 6, $n = 14$), compared to non-demented control subjects (Ctrl, $n = 6$), (**b**) superior frontal gyrus of PD patients ($n = 10$) and non-demented control subjects (Ctrl, $n = 10$), (**c**) cerebrospinal fluid of PD ($n = 33$), Alzheimer's disease (AD, $n = 50$), and Amyotrophic Lateral Sclerosis (ALS, $n = 29$) patients, compared to control subjects with other neurological disorders (OND, $n = 43$). In each sample, all free amino acids were detected in a single run. Dots represent the single subjects' values, expressed as nmol/mg protein for *post-mortem* tissues or µM concentration for cerebrospinal fluid, while bars illustrate the median with interquartile range. $*p < 0.05$, $**p < 0.01$, compared to Ctrl (Mann–Whitney test), $***p < 0.0001$, compared to OND; $\#p < 0.05$, $\#\#p < 0.01$, compared to PD (Bonferroni *post-hoc* multiple comparison). (**d**) Representative blots of 3-phosphoglycerate dehydrogenase (PHGDH), alanine serine cysteine transporter 1 (ASCT1) and β-actin immunodensity obtained in the *post-mortem* caudate putamen lysates by western blotting. (**e,f**) Quantification of (**e**) PHGDH and (**f**) ASCT1 protein levels in the *post-mortem* caudate putamen of PD patients (PHGDH, $n = 26$; ASCT1, $n = 27$), compared to non demented controls (Ctrl, $n = 6$). Variations are expressed as a percentage relative to the control group. β-actin was used to normalize variations in the loading and transfer procedures. Dots represent the single subjects' values while bars illustrate the median with interquartile range. $*p < 0.05$, compared to Ctrl (Mann–Whitney test).

Table 5
Correlation analysis between cerebrospinal fluid L-serine content and clinical characteristics of Parkinson's disease and Alzheimer's disease patients.

Diagnosis	Clinical parameter	L-Serine		
		N	r	*p value*
Parkinson's disease	Months of disease	32	0.047	0.796
	UPDRS-I	28	0.042	0.832
	UPDRS-II	28	−0.216	0.271
	UPDRS-III	33	−0.137	0.447
	Non-motor symptoms	25	−0.027	0.898
	BDI-II	29	−0.344	0.068
	MMSE	28	−0.188	0.339
Alzheimer's disease	Aβ 40 (pg/ml)	15	0.325	0.237
	Aβ 42 (pg/ml)	50	−0.107	0.461
	TAU (pg/ml)	50	0.093	0.519
	MMSE	46	0.184	0.221

Number of subjects is indicated as (n). Statistical analyses are performed by Spearman's correlations. Abbreviations: UPDRS = Unified Parkinson's Disease Rating Scale; BDI = Beck Depression Inventory; MMSE = Mini-Mental State Examination.

have focused on the modifications affecting the expression, structure, and function of NMDAR in PD. Surprisingly, little is known about the cerebral levels of the D- and L- amino acids that contribute to NMDAR activation.

Here we report for the first time a significant increase of the endogenous NMDAR co-agonist D-Ser in the *post-mortem* CPu of PD patients, compared to non-demented control subjects. In addition, we show a significant enhancement of the striatal levels of L-Asp and a trend towards the increase of its D-enantiomer, D-Asp, both acting as NMDAR agonists. This evidence corroborates previous data indicating abnormal aspartate content in the *post-mortem* CPu of PD patients (Rinne et al., 1988). Importantly, the concomitant increase of striatal L-Asp and D-Ser levels in the PD brain supports the notion that midbrain dopaminergic degeneration elicits enhanced NMDAR signaling (Campanelli et al., 2022), which may be due to a loss of dopaminergic regulation of cortico-striatal synaptic activity (Bamford et al., 2018; Wong et al., 2015). Interestingly, the present findings demonstrate that the increase of both NMDAR agonists similarly occur in patients with different Braak LB stages (3–4 *vs* 6). Further investigations are required to evaluate the striatal D-Ser and L-Asp levels in the very early stages of the disease (Braak LB 1–2). Similarly, it will be important to assess whether striatal levels of these NMDAR stimulating amino acids are influenced by PD medication.

Studies conducted in rodents have previously shown that the cerebral concentration of various amino acids, including D-Ser, can be influenced by the PMI (Hashimoto et al., 2003; Kumashiro et al., 1995). However, in the present work, this is not a confounding factor as there are comparable PMI values with control and PD *post-mortem* samples (Tables 1–2).

HPLC analysis did not reveal abnormal D-Ser and L-Asp amounts in the SFG of PD patients, suggesting that the selective alteration observed in the CPu is a specific consequence of the dysfunctional dopaminergic nigrostriatal pathway in the PD brain.

As an independent confirmation of alteration in homeostatic D-Ser levels in PD pathology, we document a significant and selective increase

in the levels of this NMDAR co-agonist in the CSF of *de novo* living PD patients, compared to OND controls, whose concentrations were in the range of *de novo* living AD and ALS patients. Interestingly, we found that CSF D-Ser concentrations were not significantly correlated with disease duration and clinical severity scales of patients, including UPDRS, NMSS, BDI, and MMSE, suggesting that variations in the central levels of this atypical amino acid in PD may represent an early primary manifestation of PD pathophysiology. Nonetheless, it remains unclear whether the increased CSF levels of D-Ser may represent *per se* a reliable neurochemical signature of PD. In our previous study involving a very limited cohort of nine PD patients, we observed a slight decrease in the CSF levels of D-Ser (Nuzzo et al., 2019). To reconcile these apparent divergent observations, it is essential to recognize the complexity of D-Ser measurement in the CSF, which involves factors such as precursor availability, SR and DAAO expression/ activity, and transport dynamics between the CNS and CSF. Furthermore, discrepancies between the two clinical investigations may arise from variations in age, gender, disease duration, sample sizes, medication, nutrition, and enrolment criteria for subjects with OND used as controls. Consequently, well-controlled studies involving larger cohorts of PD patients and controls are warranted.

Importantly, our findings highlight the presence of higher L-Ser levels in the *post-mortem* CPu of PD patients compared to their respective controls. This observation, along with the unaltered SR protein levels found in the CPu of PD patients, strongly suggests that the elevation in D-Ser amount observed in the same *post-mortem* cerebral specimens may directly reflect the abnormally higher occurrence of its L-precursor. However, striatal protein levels of PHGDH, the astrocytic rate-limiting enzyme of the *de novo* L-Ser biosynthesis (Grant, 2018; Murtas et al., 2021, 2020), did not differ between PD and control subjects, thus indicating that the overall increased amount of Ser enantiomers likely mirror an upstream perturbation in the glycolytic flux of astrocytes. In keeping with this, previous *in vitro* and *in vivo* studies showed the existence of a cross-talk between the aerobic glycolytic flux in astrocytes and the production of L-Ser (Le Douce et al., 2020) and D-Ser (Suzuki et al., 2015).

Additionally, the pronounced astrogliosis reported in the CPu of both animal models and patients with PD (Charron et al., 2014), may offer insight into the cellular origin of increased Ser enantiomers levels, considering that a net shift in SR expression and D-Ser production from neurons to astrocytes have been previously reported under pathological conditions (Coyle et al., 2020; Wolosker et al., 2016).

In addition to the abnormalities in D-Ser and L-Ser concentrations in the PD brain, we documented a significant reduction of ASCT1 expression in the CPu of patients compared to controls, which could ultimately reflect a negative-feedback response caused by abnormally greater concentration of Ser enantiomers. Previous findings demonstrated that ASCT1, expressed in astrocytes, modulates NMDAR-dependent transmission, by controlling D-Ser availability in the synaptic clefts (Kaplan et al., 2018; Krishnan and Billups, 2023); thus, we cannot rule out that the downregulation of this transporter found in the *post-mortem* CPu of PD patients could impact on extracellular D-Ser availability, influencing NMDAR signalling within the basal ganglia circuitry.

However, our HPLC analysis in homogenized *post-mortem* brain samples does not allow us to distinguish intracellular from extracellular compartments or to identify a specific cellular population responsible for Ser changes. Therefore, future neurochemical (e.g., microdialysis) and neuroanatomical examinations in animal models will be required to assess the effect of decreased ASCT1 expression on striatal D-Ser availability at extracellular site. However, irrespectively of the precise mechanism, our results indicate a significant disruption in Ser enantiomers homeostasis along with a substantial downregulation of ASCT1 in the *post-mortem* CPu of patients and envisage a direct implication of astrocytes, reported highly activated in PD (Booth et al., 2017; Charron et al., 2014).

We argue that higher D-Ser occurrence found in the *post-mortem* CPu and in the CSF of *de novo* living PD patients represents a compensatory mechanism aimed at enhancing GluN1 NMDAR subunit activation, previously reported to elicit neuroprotective and antiparkinsonian effects in rodent and macaque models of PD (Frouni et al., 2022, 2021; Schmitz et al., 2013).

The strengths of our study include the following points: i) CSF samples of controls and those of *de novo* living PD, AD, and ALS patients were collected at the same Medical Center (Neuromed Hospital) under standardized experimental conditions, minimizing pre-analytical errors unrelated to the patient's diagnosis; ii) access to a comprehensive set of clinical and biochemical measurements for all diagnosed PD and AD patients; iii) this is the first HPLC analysis evaluating all the main neuroactive amino acids, in the D- and L-configuration, acting on NMDAR neurotransmission in *post-mortem* CPu samples from PD brain at different stages of disease severity. Limitations of the study include a limited number of *post-mortem* CPu controls and the fact that the *post-mortem* CPu and SFG human tissues were obtained from two independent cohorts of PD patients and age-matched non-demented controls.

5. Conclusion

Our findings suggest that the concomitant augmentation of both D-Ser and L-Ser observed in the CPu and CSF of PD patients may represent an adaptive biochemical event aimed at contrasting the ongoing degeneration of midbrain dopaminergic neurons. We propose that central changes in Ser enantiomers metabolism and transport found in PD patients may provide a disease-specific signature associated with midbrain dopaminergic degeneration. Further investigations are warranted to clarify the underlying mechanisms contributing to D-Ser changes and the complex interactions between midbrain dopaminergic degeneration and NMDAR mediated neurotransmission.

Author contributions

LG, FB, MSB and DC collected CSF samples and provided clinical data. ADM, TN and ADR performed HPLC and WB experiments, acquired and analyzed data, and prepared figs. AU conceived the study and supervised experiments. AU and MS wrote the manuscript. AU, MS, FE, MM, MC, JS, DS and DC revised the manuscript. All authors read and approved the final version of the paper. ADM, TN and LG contributed equally to this work.

Declaration of Competing Interest

F.B. acted as Advisory Board members of Teva and Roche and received honoraria for speaking or consultation fees from Merck Serono, Teva, Bio-gen Idec, Sanofi, and Novartis and non-financial support from Merck Serono, Teva, Biogen Idec, and Sanofi. DC is an Advisory Board member of Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novar-tis, Roche, Sanofi19 Genzyme, and Teva and received honoraria for speaking or consultation fees from Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novartis, Roche, Sanofi-Genzyme, and Teva. He is also the principal investigator in clinical trials for Bayer Schering, Biogen, Merck Serono, Mitsubishi, Novartis, Roche, Sanofi-Genzyme, and Teva. His preclinical and clinical research was supported by grants from Bayer Schering, Biogen Idec, Celgene, Merck Serono, Novartis, Roche, Sanofi-Genzyme, and Teva. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. All the other authors declare no competing non-financial or financial interests to disclose.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Andersen, J.V., Markussen, K.H., Jakobsen, E., Schousboe, A., Waagepetersen, H.S., Rosenberg, P.A., Aldana, B.I., 2021. Glutamate metabolism and recycling at the excitatory synapse in health and neurodegeneration. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2021.108719.

Antonini, A., Abbruzzese, G., Ferini-Strambi, L., Tilley, B., Huang, J., Stebbins, G.T., Goetz, C.G., Barone, P., Bandettini Di Poggio, M., Fabbrini, G., Di Stasio, F., Tinazzi, M., Bovi, T., Ramat, S., Meoni, S., Pezzoli, G., Canesi, M., Martinelli, P., Maria Scaglione, C.L., Rossi, A., Tambasco, N., Santangelo, G., Picillo, M., Morgante, L., Morgante, F., Quatrale, R., Sensi, M., Pilleri, M., Biundo, R., Nordera, G., Caria, A., Pacchetti, C., Zangaglia, R., Lopiano, L., Zibetti, M., Zappia, M., Nicoletti, A., Quattrone, A., Salsone, M., Cossu, G., Murgia, D., Albanese, A., Del Sorbo, F., 2013. Validation of the Italian version of the movement disorder society - unified Parkinson's disease rating scale. Neurol. Sci. 34 https://doi.org/10.1007/s10072-012-1112-z.

Bamford, N.S., Wightman, R.M., Sulzer, D., 2018. Dopamine's effects on corticostriatal synapses during reward-based behaviors. Neuron. https://doi.org/10.1016/j.neuron.2018.01.006.

Biemans, E.A.L.M., Verhoeven-Duif, N.M., Gerrits, J., Claassen, J.A.H.R., Kuiperij, H.B., Verbeek, M.M., 2016. CSF d-serine concentrations are similar in Alzheimer's disease, other dementias, and elderly controls. Neurobiol. Aging 42. https://doi.org/10.1016/j.neurobiolaging.2016.03.017.

Booth, H.D.E., Hirst, W.D., Wade-Martins, R., 2017. The role of astrocyte dysfunction in Parkinson's disease pathogenesis. Trends Neurosci. https://doi.org/10.1016/j.tins.2017.04.001.

Braak, H., Braak, E., 1991. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. https://doi.org/10.1007/BF00308809.

Braak, H., Braak, E., 1995. Staging of alzheimer's disease-related neurofibrillary changes. Neurobiol. Aging 16. https://doi.org/10.1016/0197-4580(95)00021-6.

Bradley, W.G., Miller, R.X., Levine, T.D., Stommel, E.W., Cox, P.A., 2018. Studies of environmental risk factors in amyotrophic lateral sclerosis (ALS) and a phase I clinical trial of l-serine. Neurotox. Res. 33, 192–198. https://doi.org/10.1007/s12640-017-9741-x.

Brooks, B.R., Miller, R.G., Swash, M., Munsat, T.L., 2000. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. 1 https://doi.org/10.1080/146608200300079536.

Campanelli, F., Natale, G., Marino, G., Ghiglieri, V., Calabresi, P., 2022. Striatal glutamatergic hyperactivity in Parkinson's disease. Neurobiol. Dis. https://doi.org/10.1016/j.nbd.2022.105697.

Cenci, M.A., Skovgård, K., Odin, P., 2022. Non-dopaminergic approaches to the treatment of motor complications in Parkinson's disease. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2022.109027.

Charron, G., Doudnikoff, E., Canron, M.H., Li, Q., Vega, C., Marais, S., Baufreton, J., Vital, A., Oliet, S.H.R., Bezard, E., 2014. Astrocytosis in parkinsonism: Considering tripartite striatal synapses in physiopathology? Front. Aging Neurosci. 6 https://doi.org/10.3389/fnagi.2014.00258.

Chaudhuri, K.R., Martinez-Martin, P., Brown, R.G., Sethi, K., Stocchi, F., Odin, P., Ondo, W., Abe, K., MacPhee, G., MacMahon, D., Barone, P., Rabey, M., Forbes, A., Breen, K., Tluk, S., Naidu, Y., Olanow, W., Williams, A.J., Thomas, S., Rye, D., Tsuboi, Y., Hand, A., Schapira, A.H.V., 2007. The metric properties of a novel non-motor symptoms scale for Parkinson's disease: results from an international pilot study. Mov. Disord. 22 https://doi.org/10.1002/mds.21596.

Coenen, V.A., Schumacher, L.V., Kaller, C., Schlaepfer, T.E., Reinacher, P.C., Egger, K., Urbach, H., Reisert, M., 2018. The anatomy of the human medial forebrain bundle: ventral tegmental area connections to reward-associated subcortical and frontal lobe regions. NeuroImage Clin. 18 https://doi.org/10.1016/j.nicl.2018.03.019.

Coyle, J.T., Balu, D., Wolosker, H., 2020. D-serine, the shape-shifting NMDA receptor co-agonist. Neurochem. Res. 45, 1344–1353. https://doi.org/10.1007/S11064-020-03014-1.

Cuomo, M., Keller, S., Punzo, D., Nuzzo, T., Affinito, O., Coretti, L., Carella, M., De Rosa, V., Florio, E., Boscia, F., Avvedimento, V.E., Cocozza, S., Errico, F., Usiello, A., Chiariotti, L., 2019. Selective demethylation of two CpG sites causes postnatal activation of the Dao gene and consequent removal of d-serine within the mouse cerebellum. Clin. Epigenetics 11. https://doi.org/10.1186/s13148-019-0732-z.

Curtis, D.R., Watkins, J.C., 1960. The excitation and depression of spinal neurones by structurally related amino acids. J. Neurochem. 6 https://doi.org/10.1111/j.1471-4159.1960.tb13458.x.

Damseh, N., Simonin, A., Jalas, C., Picoraro, J.A., Shaag, A., Cho, M.T., Yaacov, B., Neidich, J., Al-Ashhab, M., Juusola, J., Bale, S., Telegrafi, A., Retterer, K., Pappas, J. G., Moran, E., Cappell, J., Yeboa, K.A., Abu-Libdeh, B., Hediger, M.A., Chung, W.K., Elpeleg, O., Edvardson, S., 2015. Mutations in SLC1A4, encoding the brain serine transporter, are associated with developmental delay, microcephaly and hypomyelination. J. Med. Genet. 52 https://doi.org/10.1136/jmedgenet-2015-103104.

De Koning, T.J., Snell, K., Duran, M., Berger, R., Poll-The, B.T., Surtees, R., 2003. L-serine in disease and development. Biochem. J. https://doi.org/10.1042/BJ20021785.

Del Campo, M., Mollenhauer, B., Bertolotto, A., Engelborghs, S., Hampel, H., Simonsen, A.H., Kapaki, E., Kruse, N., Le Bastard, N., Lehmann, S., Molinuevo, J.L., Parnetti, L., Perret-Liaudet, A., Sáez-Valero, J., Saka, E., Urbani, A., Vanmechelen, E., Verbeek, M., Visser, P.J., Teunissen, C., 2012. Recommendations to standardize preanalytical confounding factors in Alzheimers and Parkinsons disease cerebrospinal fluid biomarkers: An update. Biomark. Med. https://doi.org/10.2217/bmm.12.46.

Ding, S.L., Royall, J.J., Sunkin, S.M., Ng, L., Facer, B.A.C., Lesnar, P., Guillozet-Bongaarts, A., McMurray, B., Szafer, A., Dolbeare, T.A., Stevens, A., Tirrell, L., Benner, T., Caldejon, S., Dalley, R.A., Dee, N., Lau, C., Nyhus, J., Reding, M., Riley, Z.L., Sandman, D., Shen, E., van der Kouwe, A., Varjabedian, A., Write, M., Zollei, L., Dang, C., Knowles, J.A., Koch, C., Phillips, J.W., Sestan, N., Wohnoutka, P., Zielke, H.R., Hohmann, J.G., Jones, A.R., Bernard, A., Hawrylycz, M.J., Hof, P.R., Fischl, B., Lein, E.S., 2016. Comprehensive cellular-resolution atlas of the adult human brain. J. Comp. Neurol. 524 https://doi.org/10.1002/cne.24080.

Dubois, B., Feldman, H.H., Jacova, C., Hampel, H., Molinuevo, J.L., Blennow, K., Dekosky, S.T., Gauthier, S., Selkoe, D., Bateman, R., Cappa, S., Crutch, S., Engelborghs, S., Frisoni, G.B., Fox, N.C., Galasko, D., Habert, M.O., Jicha, G.A., Nordberg, A., Pasquier, F., Rabinovici, G., Robert, P., Rowe, C., Salloway, S., Sarazin, M., Epelbaum, S., de Souza, L.C., Vellas, B., Visser, P.J., Schneider, L., Stern, Y., Scheltens, P., Cummings, J.L., 2014. Advancing research diagnostic criteria for Alzheimer's disease: The IWG-2 criteria. Lancet Neurol. https://doi.org/10.1016/S1474-4422(14)70090-0.

El-Hattab, A.W., 2016. Serine biosynthesis and transport defects. Mol. Genet. Metab. https://doi.org/10.1016/j.ymgme.2016.04.010.

Errico, F., Nisticò, R., Napolitano, F., Mazzola, C., Astone, D., Pisapia, T., Giustizieri, M., D'Aniello, A., Mercuri, N.B., Usiello, A., 2011. Increased d-aspartate brain content rescues hippocampal age-related synaptic plasticity deterioration of mice. Neurobiol. Aging 32. https://doi.org/10.1016/j.neurobiolaging.2010.01.002.

Fridman, V., Suriyanarayanan, S., Novak, P., David, W., Macklin, E.A., McKenna-Yasek, D., Walsh, K., Aziz-Bose, R., Oaklander, A.L., Brown, R., Hornemann, T., Eichler, F., 2019. Randomized trial of l -serine in patients with hereditary sensory and autonomic neuropathy type 1. Neurology 92. https://doi.org/10.1212/WNL.0000000000006811.

Frouni, I., Belliveau, S., Maddaford, S., Nuara, S.G., Gourdon, J.C., Huot, P., 2021. Effect of the glycine transporter 1 inhibitor ALX-5407 on dyskinesia, psychosis-like behaviours and parkinsonism in the MPTP-lesioned marmoset. Eur. J. Pharmacol. 910 https://doi.org/10.1016/j.ejphar.2021.174452.

Frouni, I., Kang, W., Bédard, D., Belliveau, S., Kwan, C., Hadj-Youssef, S., Bourgeois-Cayer, É., Ohlund, L., Sleno, L., Hamadjida, A., Huot, P., 2022. Effect of glycine transporter 1 inhibition with bitopertin on parkinsonism and L-DOPA induced dyskinesia in the 6-OHDA-lesioned rat. Eur. J. Pharmacol. 929 https://doi.org/10.1016/j.ejphar.2022.175090.

Gardoni, F., Di Luca, M., 2015. Targeting glutamatergic synapses in Parkinson's disease. Curr. Opin. Pharmacol. https://doi.org/10.1016/j.coph.2014.10.011.

Gardoni, F., Picconi, B., Ghiglieri, V., Polli, F., Bagetta, V., Bernardi, G., Cattabeni, F., Di Luca, M., Calabresi, P., 2006. A critical interaction between NR2B and MAGUK in L-DOPA induced dyskinesia. J. Neurosci. 26 https://doi.org/10.1523/JNEUROSCI.5326-05.2006.

Gatto, E.M., Aldinio, V., 2019. Impulse control disorders in Parkinson's disease. a brief and comprehensive review. Front. Neurol. 10 https://doi.org/10.3389/fneur.2019.00351.

Gelfin, E., Kaufman, Y., Korn-Lubetzki, I., Bloch, B., Kremer, I., Javitt, D.C., Heresco-Levy, U., 2012. D-serine adjuvant treatment alleviates behavioural and motor symptoms in Parkinson's disease. Int. J. Neuropsychopharmacol. 15, 543–549. https://doi.org/10.1017/S1461145711001015.

Gerlach, M., Gsell, W., Kornhuber, J., Jellinger, K., Krieger, V., Pantucek, F., Vock, R., Riederer, P., 1996. A post mortem study on neurochemical markers of dopaminergic, GABA-ergic and glutamatergic neurons in basal ganglia-thalamocortical circuits in Parkinson syndrome. Brain Res. 741 https://doi.org/10.1016/S0006-8993(96)00915-8.

Gonda, Y., Ishii, C., Mita, M., Nishizaki, N., Ohtomo, Y., Hamase, K., Shimizu, T., Sasabe, J., 2022. Astrocytic d-amino acid oxidase degrades d-serine in the hindbrain. FEBS Lett. 596, 2889–2897. https://doi.org/10.1002/1873-3468.14417.

Grant, G.A., 2018. D-3-phosphoglycerate dehydrogenase. Front. Mol. Biosci. https://doi.org/10.3389/fmolb.2018.00110.

Guimarães, R.P., Santos, M.C.A., Dagher, A., Campos, L.S., Azevedo, P., Piovesana, L.G., De Campos, B.M., Larcher, K., Zeighami, Y., Amato-Filho, A.C.S., Cendes, F., Frota D'Abreu, A.C., 2017. Pattern of reduced functional connectivity and structural abnormalities in Parkinson's disease: An exploratory study. Front. Neurol. 7 https://doi.org/10.3389/fneur.2016.00243.

Hallett, P.J., Dunah, A.W., Ravenscroft, P., Zhou, S., Bezard, E., Crossman, A.R., Brotchie, J.M., Standaert, D.G., 2005. Alterations of striatal NMDA receptor subunits associated with the development of dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. Neuropharmacology 48. https://doi.org/10.1016/j.neuropharm.2004.11.008.

Handzlik, M.K., Gengatharan, J.M., Frizzi, K.E., Mcgregor, G.H., Martino, C., Rahman, G., Gonzalez, A., Moreno, A.M., Green, C.R., Lin, T., Tseng, P., Ideguchi, Y., Fallon, R.J., Chaix, A., Panda, S., Wallace, M., 2022. Insulin-Regulated Serine and Lipid Metabolism Drive Peripheral Neuropathy. https://doi.org/10.1038/s41586-022-05637-6.

Hashimoto, K., Fukushima, T., Shimizu, E., Komatsu, N., Watanabe, H., Shinoda, N., Nakazato, M., Kumakiri, C., Okada, S.I., Hasegawa, H., Imai, K., Iyo, M., 2003. Decreased serum levels of D-serine in patients with schizophrenia: evidence in support of the N-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia. Arch. Gen. Psychiatry 60, 572–576. https://doi.org/10.1001/ARCHPSYC.60.6.572.

Heimer, G., Marek-Yagel, D., Eyal, E., Barel, O., Oz Levi, D., Hoffmann, C., Ruzzo, E.K., Ganelin-Cohen, E., Lancet, D., Pras, E., Rechavi, G., Nissenkorn, A., Anikster, Y., Goldstein, D.B., Ben Zeev, B., 2015. SLC1A4 mutations cause a novel disorder of intellectual disability, progressive microcephaly, spasticity and thin corpus callosum. Clin. Genet. 88 https://doi.org/10.1111/cge.12637.

Heresco-Levy, U., Shoham, S., Javitt, D.C., 2013. Glycine site agonists of the N-methyl-d-aspartate receptor and Parkinson's disease: A hypothesis. Mov. Disord. 28 https://doi.org/10.1002/mds.25306.

Hoehn, M.M., Yahr, M.D., 1967. Parkinsonism: Onset, progression, and mortality. Neurology 17. https://doi.org/10.1212/wnl.17.5.427.

Hofmann, K., Düker, M., Fink, T., Lichter, P., Stoffel, W., 1994. Human neutral amino acid transporter ASCT1: structure of the gene (SLC1A4) and localization to chromosome 2p13-p15. Genomics 24. https://doi.org/10.1006/geno.1994.1577.

Holm, L.J., Buschard, K., 2019. L-serine: a neglected amino acid with a potential therapeutic role in diabetes. APMIS. https://doi.org/10.1111/apm.12987.

Hughes, A.J., Daniel, S.E., Kilford, L., Lees, A.J., 1992. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J. Neurol. Neurosurg. Psychiatry. 55 (3), 181–184.

Jiménez-Jiménez, F.J., Alonso-Navarro, H., García-Martín, E., Agúndez, J.A.G., 2020. Cerebrospinal and blood levels of amino acids as potential biomarkers for Parkinson's disease: review and meta-analysis. Eur. J. Neurol. https://doi.org/10.1111/ene.14470.

Kaplan, E., Zubedat, S., Radzishevsky, I., Valenta, A.C., Rechnitz, O., Sason, H., Sajrawi, C., Bodner, O., Konno, K., Esaki, K., Derdikman, D., Yoshikawa, T., Watanabe, M., Kennedy, R.T., Billard, J.M., Avital, A., Wolosker, H., 2018. ASCT1 (Slc1a4) transporter is a physiologic regulator of brain D-serine and neurodevelopment. Proc. Natl. Acad. Sci. U. S. A. 115 https://doi.org/10.1073/pnas.1722677115.

Katane, M., Kuwabara, H., Nakayama, K., Saitoh, Y., Miyamoto, T., Sekine, M., Homma, H., 2018. Rat D-aspartate oxidase is more similar to the human enzyme than the mouse enzyme. Biochim. Biophys. Acta, Proteins Proteomics 1866. https://doi.org/10.1016/j.bbapap.2017.12.009.

Keller, S., Punzo, D., Cuomo, M., Affinito, O., Coretti, L., Sacchi, S., Florio, E., Lembo, F., Carella, M., Copetti, M., Cocozza, S., Balu, D.T., Errico, F., Usiello, A., Chiariotti, L., 2018. DNA methylation landscape of the genes regulating D-serine and D-aspartate metabolism in post-mortem brain from controls and subjects with schizophrenia. Sci. Rep. 8 https://doi.org/10.1038/s41598-018-28332-x.

Kondori, N.R., Paul, P., Robbins, J.P., Liu, K., Hildyard, J.C.W., Wells, D.J., De Belleroche, J.S., 2017. Characterisation of the pathogenic effects of the in vivo expression of an ALS-linked mutation in D-amino acid oxidase: Phenotype and loss of spinal cord motor neurons. PLoS One 12. https://doi.org/10.1371/journal.pone.0188912.

Krashia, P., Ledonne, A., Nobili, A., Cordella, A., Errico, F., Usiello, A., D'Amelio, M., Mercuri, N.B., Guatteo, E., Carunchio, I., 2016. Persistent elevation of D-Aspartate enhances NMDA receptor-mediated responses in mouse substantia nigra pars compacta dopamine neurons. Neuropharmacology 103. https://doi.org/10.1016/j.neuropharm.2015.12.013.

Krishnan, K.S., Billups, B., 2023. ASC Transporters Mediate D-Serine Transport into Astrocytes Adjacent to Synapses in the Mouse Brain.

Kuiper, M.A., Teerlink, T., Visser, J.J., Bergmans, P.L.M., Scheltens, P., Wolters, E.C., 2000. L-glutamate, L-arginine and L-citrulline levels in cerebrospinal fluid of Parkinson's disease, multiple system atrophy, and Alzheimer's disease patients. J. Neural Transm. 107 https://doi.org/10.1007/s007020050016.

Kumashiro, S., Hashimoto, A., Nishikawa, T., 1995. Free d-serine in post-mortem brains and spinal cords of individuals with and without neuropsychiatric diseases. Brain Res. 681, 117–125. https://doi.org/10.1016/0006-8993(95)00307-C.

Le Douce, J., Maugard, M., Veran, J., Matos, M., Jégo, P., Vigneron, P.A., Faivre, E., Toussay, X., Vandenberghe, M., Balbastre, Y., Piquet, J., Guiot, E., Tran, N.T., Taverna, M., Marinesco, S., Koyanagi, A., Furuya, S., Gaudin-Guérif, M., Goutal, S., Ghettas, A., Pruvost, A., Bemelmans, A.P., Gaillard, M.C., Cambon, K., Stimmer, L., Sazdovitch, V., Duyckaerts, C., Knott, G., Hérard, A.S., Delzescaux, T., Hantraye, P., Brouillet, E., Cauli, B., Oliet, S.H.R., Panatier, A., Bonvento, G., 2020. Impairment of glycolysis-derived l-serine production in astrocytes contributes to cognitive deficits in Alzheimer's disease. Cell Metab. 31, 503–517.e8. https://doi.org/10.1016/J.CMET.2020.02.004.

Levine, T.D., Miller, R.G., Bradley, W.G., Moore, D.H., Saperstein, D.S., Flynn, L.E., Katz, J.S., Forshew, D.A., Metcalf, J.S., Banack, S.A., Cox, P.A., 2017. Phase I clinical trial of safety of L-serine for ALS patients. Amyotroph. Lateral Scler. Front. Degener. 18 https://doi.org/10.1080/21678421.2016.1221971.

Li, L., Ji, B., Zhao, T., Cui, X., Chen, J., Wang, Z., 2022. The structural changes of gray matter in Parkinson disease patients with mild cognitive impairments. PLoS One 17, 1–17. https://doi.org/10.1371/journal.pone.0269787.

Madeira, C., Lourenco, M.V., Vargas-Lopes, C., Suemoto, C.K., Brandão, C.O., Reis, T., Leite, R.E.P., Laks, J., Jacob-Filho, W., Pasqualucci, C.A., Grinberg, L.T., Ferreira, S. T., Panizzutti, R., 2015. d-serine levels in Alzheimer's disease: implications for novel biomarker development. Transl. Psychiatry 5. https://doi.org/10.1038/TP.2015.52.

Maffioli, E., Murtas, G., Rabattoni, V., Badone, B., Tripodi, F., Iannuzzi, F., Licastro, D., Nonnis, S., Rinaldi, A.M., Motta, Z., Sacchi, S., Canu, N., Tedeschi, G., Coccetti, P., Pollegioni, L., 2022. Insulin and serine metabolism as sex-specific hallmarks of Alzheimer's disease in the human hippocampus. Cell Rep. 40, 111271 https://doi.org/10.1016/J.CELREP.2022.111271.

Mally, J., Szalai, G., Stone, T.W., 1997. Changes in the concentration of amino acids in serum and cerebrospinal fluid of patients with Parkinson's disease. J. Neurol. Sci. 151 https://doi.org/10.1016/S0022-510X(97)00119-6.

Martineau, M., Baux, G., Mothet, J.P., 2006. d-Serine signalling in the brain: friend and foe. Trends Neurosci. https://doi.org/10.1016/j.tins.2006.06.008.

Maugard, M., Vigneron, P.A., Bolaños, J.P., Bonvento, G., 2021. L-Serine links metabolism with neurotransmission. Prog. Neurobiol. https://doi.org/10.1016/j.pneurobio.2020.101896.

Measso, G., Cavarzeran, F., Zappala, G., Lebowitz, B.D., Crook, T.H., Pirozzolo, F.J., Amaducci, L.A., Massari, D., Grigoletto, F., 1993. The mini-mental-state-examination - normative study of an Italian random sample. Dev. Neuropsychol. 9.

Metcalf, J.S., Dunlop, R.A., Powell, J.T., Banack, S.A., Cox, P.A., 2018. L-Serine: a naturally-occurring amino acid with therapeutic potential. Neurotox. Res. https://doi.org/10.1007/s12640-017-9814-x.

Mitchell, J., Paul, P., Chen, H.J., Morris, A., Payling, M., Falchi, M., Habgood, J., Panoutsou, S., Winkler, S., Tisato, V., Hajitou, A., Smith, B., Vance, C., Shaw, C., Mazarakis, N.D., De Belleroche, J., 2010. Familial amyotrophic lateral sclerosis is associated with a mutation in D-amino acid oxidase. Proc. Natl. Acad. Sci. U. S. A. 107 https://doi.org/10.1073/pnas.0914128107.

Murtas, G., Marcone, G.L., Sacchi, S., Pollegioni, L., 2020. L-serine synthesis via the phosphorylated pathway in humans. Cell. Mol. Life Sci. https://doi.org/10.1007/s00018-020-03574-z.

Murtas, G., Marcone, G.L., Peracchi, A., Zangelmi, E., Pollegioni, L., 2021. Biochemical and biophysical characterization of recombinant human 3-phosphoglycerate dehydrogenase. Int. J. Mol. Sci. 22 https://doi.org/10.3390/ijms22084231.

Nuzzo, T., Punzo, D., Devoto, P., Rosini, E., Paciotti, S., Sacchi, S., Li, Q., Thiolat, M.L., Véga, C., Carella, M., Carta, M., Gardoni, F., Calabresi, P., Pollegioni, L., Bezard, E., Parnetti, L., Errico, F., Usiello, A., 2019. The levels of the NMDA receptor co-agonist D-serine are reduced in the substantia nigra of MPTP-lesioned macaques and in the cerebrospinal fluid of Parkinson's disease patients. Sci. Report. 91 (9), 1–15. https://doi.org/10.1038/s41598-019-45419-1.

Nuzzo, T., Miroballo, M., Casamassa, A., Mancini, A., Gaetani, L., Nisticò, R., Eusebi, P., Katane, M., Homma, H., Calabresi, P., Errico, F., Parnetti, L., Usiello, A., 2020. Cerebrospinal fluid and serum D-serine concentrations are unaltered across the whole clinical spectrum of Alzheimer's disease. Biochim. Biophys. Acta, Proteins Proteomics 1868. https://doi.org/10.1016/j.bbapap.2020.140537.

Orzylowski, M., Fujiwara, E., Mousseau, D.D., Baker, G.B., 2021. An overview of the involvement of d-serine in cognitive impairment in normal aging and dementia. Front. Psychiatry. https://doi.org/10.3389/fpsyt.2021.754032.

Palese, F., Bonomi, E., Nuzzo, T., Benussi, A., Mellone, M., Zianni, E., Cisani, F., Casamassa, A., Alberici, A., Scheggia, D., Padovani, A., Marcello, E., Di Luca, M., Pittaluga, A., Usiello, A., Borroni, B., Gardoni, F., 2020. Anti-GluA3 antibodies in frontotemporal dementia: effects on glutamatergic neurotransmission and synaptic failure. Neurobiol. Aging 86. https://doi.org/10.1016/j.neurobiolaging.2019.10.015.

Piubelli, L., Murtas, G., Rabattoni, V., Pollegioni, L., 2021. The role of D-amino acids in Alzheimer's disease. J. Alzheimers Dis. https://doi.org/10.3233/JAD-201217.

Pollegioni, L., Sacchi, S., 2010. Metabolism of the neuromodulator D-serine. Cell. Mol. Life Sci. https://doi.org/10.1007/s00018-010-0307-9.

Pollegioni, L., Sacchi, S., Murtas, G., 2018. Human D-amino acid oxidase: structure, function, and regulation. Front. Mol. Biosci. https://doi.org/10.3389/fmolb.2018.00107.

Postuma, R.B., Berg, D., Stern, M., Poewe, W., Olanow, C.W., Oertel, W., Obeso, J., Marek, K., Litvan, I., Lang, A.E., Halliday, G., Goetz, C.G., Gasser, T., Dubois, B., Chan, P., Bloem, B.R., Adler, C.H., Deuschl, G., 2015. MDS clinical diagnostic criteria for Parkinson's disease. Mov. Disord. https://doi.org/10.1002/mds.26424.

Rinne, J.O., Halonen, T., Riekkinen, P.J., Rinne, U.K., 1988. Free amino acids in the brain of patients with Parkinson's disease. Neurosci. Lett. 94 https://doi.org/10.1016/0304-3940(88)90292-3.

Sacchi, S., Caldinelli, L., Cappelletti, P., Pollegioni, L., Molla, G., 2012. Structure-function relationships in human D-amino acid oxidase. Amino Acids. https://doi.org/10.1007/s00726-012-1345-4.

Sasabe, J., Suzuki, M., 2019. Distinctive roles of d-Amino acids in the homochiral world: Chirality of amino acids modulates mammalian physiology and pathology. Keio J. Med. https://doi.org/10.2302/kjm.2018-0001-IR.

Sasabe, J., Chiba, T., Yamada, M., Okamoto, K., Nishimoto, I., Matsuoka, M., Aiso, S., 2007. D-Serine is a key determinant of glutamate toxicity in amyotrophic lateral sclerosis. EMBO J. 26 https://doi.org/10.1038/sj.emboj.7601840.

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 https://doi.org/10.1073/pnas.1114639109.

Schmitz, Y., Castagna, C., Mrejeru, A., Lizardi-Ortiz, J.E., Klein, Z., Lindsley, C.W., Sulzer, D., 2013. Glycine transporter-1 inhibition promotes striatal axon sprouting via NMDA receptors in dopamine neurons. J. Neurosci. 33, 16778–16789. https://doi.org/10.1523/JNEUROSCI.3041-12.2013.

Seckler, J.M., Lewis, S.J., 2020. Advances in D-Amino acids in neurological research. Int. J. Mol. Sci. 21, 7325. https://doi.org/10.3390/IJMS21197325.

Sharp, D.J., Bonnelle, V., De Boissezon, X., Beckmann, C.F., James, S.G., Patel, M.C., Mehta, M.A., 2010. Distinct frontal systems for response inhibition, attentional capture, and error processing. Proc. Natl. Acad. Sci. U. S. A. 107 https://doi.org/10.1073/pnas.1000175107.

Shen, Y.T., Yuan, Y.S., Wang, M., Zhi, Y., Wang, J.W., Wang, L.N., Ma, K.W., Si, Q.Q., Zhang, K.Z., 2020. Dysfunction in superior frontal gyrus associated with diphasic dyskinesia in Parkinson's disease. npj Park. Dis. 6 https://doi.org/10.1038/s41531-020-00133-y.

Sica, C., Ghisi, M., 2007. The Italian versions of the Beck Anxiety Inventory and the Beck Depression Inventory-II: Psychometric properties and discriminant power. Leading-edge Psychol. tests Test. Res. 01, 27–50.

Singh, S.P., Singh, V., 2011. Meta-analysis of the efficacy of adjunctive NMDA receptor modulators in chronic schizophrenia. CNS Drugs 25. https://doi.org/10.2165/11586650-000000000-00000.

Srour, M., Hamdan, F.F., Gan-Or, Z., Labuda, D., Nassif, C., Oskoui, M., Gana-Weisz, M., Orr-Urtreger, A., Rouleau, G.A., Michaud, J.L., 2015. A homozygous mutation in SLC1A4 in siblings with severe intellectual disability and microcephaly. Clin. Genet. 88 https://doi.org/10.1111/cge.12605.

Suzuki, M., Sasabe, J., Miyoshi, Y., Kuwasako, K., Muto, Y., Hamase, K., Matsuoka, M., Imanishi, N., Aiso, S., 2015. Glycolytic flux controls D-serine synthesis through glyceraldehyde-3-phosphate dehydrogenase in astrocytes. Proc. Natl. Acad. Sci. U. S. A. 112 https://doi.org/10.1073/pnas.1416117112.

Suzuki, M., Imanishi, N., Mita, M., Hamase, K., Aiso, S., Sasabe, J., 2017. Heterogeneity of D-serine distribution in the human central nervous system. ASN Neuro. 9 https://doi.org/10.1177/1759091417713905.

Teunissen, C.E., Petzold, A., Bennett, J.L., Berven, F.S., Brundin, L., Comabella, M., Franciotta, D., Frederiksen, J.L., Fleming, J.O., Furlan, R., Hintzen, R.Q., Hughes, S. G., Johnson, M.H., Krasulova, E., Kuhle, J., Magnone, M.C., Rajda, C., Rejdak, K., Schmidt, H.K., Van Pesch, V., Waubant, E., Wolf, C., Giovannoni, G., Hemmer, B., Tumani, H., Deisenhammer, F., 2009. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. Neurology. https://doi.org/10.1212/WNL.0b013e3181c47cc2.

Thompson, M., Marecki, J.C., Marinesco, S., Labrie, V., Roder, J.C., Barger, S.W., Crow, J.P., 2012. Paradoxical roles of serine racemase and D-serine in the G93A mSOD1 mouse model of amyotrophic lateral sclerosis. J. Neurochem. 120, 598–610. https://doi.org/10.1111/J.1471-4159.2011.07601.X.

Tohgi, H., Abe, T., Hashiguchi, K., Takahashi, S., Nozaki, Y., Kikuchi, T., 1991. A significant reduction of putative transmitter amino acids in cerebrospinal fluid of patients with Parkinson's disease and spinocerebellar degeneration. Neurosci. Lett. 126 https://doi.org/10.1016/0304-3940(91)90542-2.

Usiello, A., Di Fiore, M.M., De Rosa, A., Falvo, S., Errico, F., Santillo, A., Nuzzo, T., Baccari, G.C., 2020. New evidence on the role of d-aspartate metabolism in regulating brain and endocrine system physiology: From preclinical observations to clinical applications. Int. J. Mol. Sci. https://doi.org/10.3390/ijms21228718.

Vanderstichele, H., Bibl, M., Engelborghs, S., Le Bastard, N., Lewczuk, P., Molinuevo, J. L., Parnetti, L., Perret-Liaudet, A., Shaw, L.M., Teunissen, C., Wouters, D., Blennow, K., 2012. Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: A consensus paper from the Alzheimer's Biomarkers Standardization Initiative. Alzheimers Dement. https://doi.org/10.1016/j.jalz.2011.07.004.

Wang, L.Z., Zhu, X.Z., 2003. Spatiotemporal relationships among D-serine, serine racemase, and D-amino acid oxidase during mouse postnatal development. Acta Pharmacol. Sin. 24.

Wolosker, H., Balu, D.T., 2020. d-Serine as the gatekeeper of NMDA receptor activity: implications for the pharmacologic management of anxiety disorders. Transl. Psychiatry. https://doi.org/10.1038/s41398-020-00870-x.

Wolosker, H., Balu, D.T., Coyle, J.T., 2016. The rise and fall of the D-serine-mediated gliotransmission hypothesis. Trends Neurosci. https://doi.org/10.1016/j.tins.2016.09.007.

Wong, M.Y., Borgkvist, A., Choi, S.J., Mosharov, E.V., Bamford, N.S., Sulzer, D., 2015. Dopamine-dependent corticostriatal synaptic filtering regulates sensorimotor behavior. Neuroscience 290. https://doi.org/10.1016/j.neuroscience.2015.01.022.

Wu, J., Wuolikainen, A., Trupp, M., Jonsson, P., Marklund, S.L., Andersen, P.M., Forsgren, L., Öhman, A., 2016. NMR analysis of the CSF and plasma metabolome of rigorously matched amyotrophic lateral sclerosis, Parkinson's disease and control subjects. Metabolomics 12. https://doi.org/10.1007/s11306-016-1041-6.

Ye, L., Sun, Y., Jiang, Z., Wang, G., 2021. L-Serine, an endogenous amino acid, is a potential neuroprotective agent for neurological disease and injury. Front. Mol. Neurosci. https://doi.org/10.3389/fnmol.2021.726665.

Yelamanchi, S.D., Jayaram, S., Thomas, J.K., Gundimeda, S., Khan, A.A., Singhal, A., Keshava Prasad, T.S., Pandey, A., Somani, B.L., Gowda, H., 2016. A pathway map of glutamate metabolism. J. Cell Commun. Signal. 10 https://doi.org/10.1007/s12079-015-0315-5.

Zhou, Y., Danbolt, N.C., 2014. Glutamate as a neurotransmitter in the healthy brain. J. Neural Transm. https://doi.org/10.1007/s00702-014-1180-8.

Zhou, X., He, L., Wu, C., Zhang, Y., Wu, X., Yin, Y., 2017. Serine alleviates oxidative stress via supporting glutathione synthesis and methionine cycle in mice. Mol. Nutr. Food Res. 61 https://doi.org/10.1002/mnfr.201700262.