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D-amino acids: new clinical pathways for brain diseases

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Abstract

Free D-amino acids (D-AAs) are emerging as a novel and important class of signaling molecules in many organs including the brain and endocrine systems. There has been considerable progress in our understanding of the fundamental roles of these atypical messengers with increasingly recognized implications in a wide range of neuropathologies including schizophrenia, epilepsy, Alzheimer's disease, amyotrophic lateral sclerosis, substance abuse, chronic pain, among others. Research has enabled the discovery that D-serine, D-aspartate and more recently D-cysteine are essential for the healthy development and function of the central nervous system. We discuss recent progress that have profoundly transformed our vision of numerous physiological processes but also show how D-AAs are now offering therapeutic promise in clinical settings for several human diseases.

1 **Free D-amino acids: a novel class of brain messengers**

2 Amino acids (AAs) serve important functions in all biological processes.
3 Common AAs, excepted glycine, exhibit a chiral center resulting theoretically in the
4 occurrence of L- and D- amino acids (D-AAs) stereoisomers. However, D-AAs had been
5 long assumed to be 'unnatural' and to occur only in microorganisms or invertebrates[1].
6 Nevertheless, seminal observations in the 1990s have revealed the presence of
7 various D-AAs in body fluids and organs of mammals, including humans. Amongst
8 them D-serine (D-Ser), D-aspartate (D-Asp) and the newly incomer D-cysteine (D-Cys)
9 have now been found in multiple organs, especially in the nervous system and sensory
10 organs (Figure 1). There, they form a new class of neuromodulators[1], overturning
11 fundamental axioms of biology and gradually transforming biomedical sciences,
12 particularly the field of brain sciences.

13 Given the vast literature, this review will not attempt to address the many facets
14 of D-AAs in all organs but rather ambitions to give an overview on the interlaced roles
15 of three particular D-AAs: D-Ser, D-Asp and D-Cys in the healthy and diseased brain.
16 We will show how their metabolic pathways are dynamically regulated and cross other
17 biochemical processes; discuss how disturbances in their metabolism cause
18 developmental and homeostatic alterations; and illustrate that mutations in the genes
19 encoding their metabolic enzymes confer high risk for major brain disorders. Finally,
20 we outline promising directions for translating our current knowledge of D-AAs to the
21 clinic as pertinent prognostics biomarkers and drug targets.

22

23 **D-AAs in the healthy nervous system: where and how?**

24 Under physiological conditions, most D-AAs are found at trace levels in the brain, with
25 the exception of D-Ser, D-Asp and D-Cys[2], which are also present in sensory organs
26 including the retina and the cochlea[3]. D-AAs also populate body fluids (ie
27 cerebrospinal fluid (CSF), urine and blood), where their levels are 10-1000 times lower
28 than in the brain. Anomalous levels of D-AAs in body fluids and tissues have been
29 observed in many pathological conditions, making D-AAs promising biomarkers for
30 disease monitoring and treatment[1].

31 While brain D-AAs can partially derive from diet or from gut microbiota[4],
32 mammalian cells have acquired the capacity to synthesize some of them, namely D-
33 Ser, D-Cys and D-Asp (see Box 1). Several other D-AAs are found in the brain
34 parenchyma (ie D-Ala, D-Arg, D-Gln, D-Glu, D-allo-Ile, D-Leu, D-Lys, D-Met, D-Phe, and

1 D-Trp)[2] but have no specific biosynthetic pathways identified so far. These D-AAs
2 could be produced by intestinal bacteria[5] and/or derive from diet as recently
3 demonstrated for D-Ala[6]. Accordingly, these D-AAs absorbed by the intestine would
4 have to cross the blood brain barrier (BBB) to reach the brain's parenchyma likely
5 through solute carriers such as ASC, γ^+L , X^-c LAT1 or ATB^{0,+} that transport AAs with
6 poor stereoselectivity[7]. Thus, ASC, LAT1 and ATB^{0,+}, which are highly expressed by
7 brain cells along with other transporters, might play significant roles in the importation
8 and mobilization of peripheral D-AAs into the brain (Figure 2). Physiological metabolic
9 breakdown of D-AAs is insured by D-amino acid oxidase (DAAO) and D-aspartate
10 oxidase (Ddo), two enzymes that resides in peroxisomes[8,9] (see Box 2).

11 **D-AAs in the healthy nervous system: what are they good for?**

12 Although, the physiological functions of many D-AAs remain to be established, it has
13 become clear that D-Ser, D-Asp and D-Cys play vital roles in the formation and
14 dynamics of brain circuits and therefore are critical for higher cognitive functions[9,10].

15 *Roles of D-AAs in synaptic network development and homeostasis*

16 Increasing evidence indicate that D-Ser, D-Cys and D-Asp contribute to all stages of
17 neuronal circuit development and function by controlling neuronal proliferation,
18 corticogenesis and adult neurogenesis[9–11]. The exploration of the physiological
19 roles of D-Ser has gained a particular attention as a co-agonist of synaptic **NMDA**
20 **receptors** (NMDARs)[12]. By doing so, D-Ser controls dendritic integration[13],
21 synaptic plasticity at mature synapses in many brain areas (Key Figure, Figure 3)[14–
22 17], and sleep[18] apparently supplanting the classic co-agonist glycine. Still, the
23 identity of the co-agonist of NMDARs is developmentally regulated switching from
24 glycine to D-Ser[14], and is also dictated by the synaptic regime. Not surprisingly SR-
25 null mice display impaired oscillatory network activity and memory impairment[19,20].
26 In addition, D-Ser also binds the **GluD** receptors to regulate long term depression (LTD)
27 at cerebellar parallel fiber-Purkinje cell synapses and motor coordination in developing
28 mice[21] as well as the formation of cortical inhibitory synapses[22]. Activation of
29 CB1[23], cholinergic[16] or dopaminergic[17] receptors regulates the synaptic
30 availability of D-Ser. The gasotransmitters H₂S and polysulfides also promote the
31 synaptic availability of D-Ser through sulfhydration of SR, then potentiating NMDAR-
32 dependent LTP and memory formation[24]. As H₂S can be formed from D-Cys by the
33 DAAO-3-MPS pathway, D-Cys in the brain may regulate synaptic transmission

1 through its conversion into H₂S and partly by regulating D-Ser metabolism and
2 function.

3 Information on the physiological roles of D-Asp in the mature brain stands
4 behind. Ddo-null mice do not show the normal decline in D-Asp with increasing age
5 (Figure 1) and D-Asp accumulates in brain to levels 10–25 times higher than
6 normal[25]. Adult Ddo-null mice show altered grooming behavior and enhanced
7 NMDA receptor-mediated responses [25]. Conversely, knock-in mouse
8 overexpressing Ddo from the embryonic stage to remove D-Asp in early life reveals
9 that D-Asp regulates the generation of cortical interneurons, boosts memory
10 performance[9], and controls L-AAAs and lipid metabolism [26].

11 Alterations in the metabolism of D-AAAs cause cognitive decline during healthy
12 aging, further consolidating their role as homeostatic agents. Indeed, D-Ser levels drop
13 during aging in the forebrain of rodents and a negative association between serum D-
14 Ser levels and age of healthy subjects has been reported as well[27]. Chronic D-Ser
15 supplementation reverts the age-associated decrease in cognitive flexibility, functional
16 brain connectivity, and neuronal morphology in rats[28]. Conversely, persistent
17 increased levels of D-Asp as found in Ddo-null mice accelerates the age-related decline
18 in **synaptic plasticity** and worsen learning and memory in old mice[29].

19

20 **D-AAAs in the diseased brain**

21 Owing to their important functions in brain physiology, alterations in the metabolism,
22 levels and signaling of D-AAAs appear henceforth linked to many neurological,
23 neurodegenerative and psychiatric disorders (Figure 3)[1].

24

25 *Roles of D-AAAs in psychiatric disorders*

26 • **Schizophrenia (SCZ) and Autism Spectrum Disorder (ASD)**

27 Recent **genome wide association studies (GWAS)** and **exome studies** (see
28 Glossary) indicate that 30 % of risk genes for SCZ encode for proteins that cluster at
29 glutamatergic synapses, ultimately affecting NMDAR function and substantiating the
30 centrality of the glutamatergic hypothesis in the etiology of the disease[20].

1 Several **single nucleotide polymorphisms** (SNPs) mutations in both the DAAO
2 and G72 genes (that encodes a DAAO interactor) have been consistently linked to
3 SCZ patients in cohorts worldwide. Both the expression and the activity of DAAO are
4 increased in post-mortem cortical and cerebellar tissue from SCZ patients as well as
5 serum levels of DAAO and G72[30,31], which could partially explain the NMDAR
6 hypofunction observed in the disease. Likewise, SR-null mutant mice remarkably
7 phenocopy many SCZ features[20]. Significant interactions between SR and other
8 proteins were found in preclinical models. Some of them are key risk factors for SCZ,
9 such as C-kinase 1 (PICK1) and DISC-1[32], which polymorphisms and expression are
10 also associated with cognitive dysfunction in patients[33]. The prospect that some of
11 those faulty interactions facilitate degradation of SR may explain the low levels of SR
12 protein found in the plasma and post-mortem brain tissue of SCZ patients[31] .

13 A meta-analysis of 12 studies concludes that decreased D-Ser levels in both blood
14 and plasma is a clinical feature of SCZ, while brain or CSF levels are unaffected[34].
15 Additionally, lower serum D-Ser is correlated with impairment of executive function in
16 SCZ patients[35]. Likewise, higher serum D-Ser significantly aligned with
17 improvements in global cognition in SCZ patients after cognitive training[36]. These
18 data strengthen the proposition of serum D-Ser as a biomarker for disease severity[37].
19 Similarly, D-Asp is also reduced in post-mortem brain samples from drug-treated SCZ
20 patients[38]. This seems to relate to a decrease in the activity of Ddo rather than a
21 genetic alteration of the enzyme or alternative metabolic pathways[38]. Although larger
22 and drug-naïve cohorts are needed to confirm these findings, D-Asp might prove to be
23 a reliable disease biomarker in the future as well[1].

24 Consequently, treatment with D-AAAs is proposed for the disorder. Although D-Ser
25 displays a good safety profile in human even in high dosage (2 g/day) (Reviewed in
26 [39]), meta-analyses indicate a general, but small, benefit in the use of D-Ser as add-
27 on medication for non-clozapine users, in both positive, negative and cognitive
28 symptoms[34,40]. Finally, treatment for SCZ has seen exciting results for the food
29 preservative sodium benzoate, a DAAO inhibitor (see Box 3)[41]. In line with such
30 results, larger phase 2 and 3 clinical trials for both adolescent and adult treatment-
31 resistant SCZ patients have been launched (NCT01908192^I, NCT03094429^{II},
32 ACTRN12621000327886^{III}). Likewise, Neurocrine Biosciences and Takeda have
33 recently announced limited success for their DAAO inhibitor Luvadaxistat (NBI-

1 1065844 / TAK-831)^{IV}. Two phase 2 clinical trials for the drug are registered, but no
2 results are available as of August 2023 (NCT03359785^V and NCT03382639^{VI}).

3 ASD, another neurodevelopmental disorder, shares common risk factors with
4 SCZ. Despite the strong relationship between glutamatergic dysfunction, and
5 specifically NMDAR hypofunction, and ASD, recent molecular evaluation of four ASD
6 mice models reported no changes in D-Ser and D-Asp metabolism in cerebral tissue
7 and serum of Cntnap2-null, Shank3-null, and 16p11.2 heterozygous mice[42].
8 However, the black and tan, brachyury (BTBR) mice model of idiopathic ASD present
9 increased D-Asp content in the prefrontal cortex, hippocampus and serum along with
10 reduced mRNA levels of Ddo[42]. This argues in favor of altered D-Asp metabolism in
11 ASD but also sheds light in the limitations of genetic animal models of the disease.
12 Further studies in human cohorts are advised.

13 • **Major depressive disorder (MDD)**

14 Imbalance in the glutamatergic system of and the rapid and long-lasting antidepressant
15 effects of (R,S)-ketamine, a non-competitive NMDAR antagonist, have led to new
16 theories about the pathophysiology of MDD[43], where now D-Ser is placed front and
17 center. D-Ser levels in CSF are negatively correlated with severity scores in
18 patients[44]. Similarly, systemic D-Ser levels enhancement, through genetic
19 manipulation, dietary D-Ser supplementation or acute administration, displays
20 antidepressant-like effects in animal models[45]. Additionally, the DAAO inhibitor
21 sodium benzoate has beneficial effects in patients[46]. Accordingly, D-Ser metabolism
22 is affected in MDD: mRNA expression levels of SR are altered in MDD patients[47] and
23 SR-null mice show depression-like phenotype[48]. Since only contradictory data is
24 available regarding the connection between MDD and the G72/30 gene, additional
25 studies with larger cohorts are needed to shed a better light to the topic.

26

27 • **Bipolar disorder (BD)**

28 Altered plasma levels of AAs are associated with symptoms and severity of BD. Serum,
29 but not CSF, levels of D-Ser are significantly higher in clinically stable patients whereas
30 L-Ser levels are lower[49], suggesting a systemic aberration in AA metabolism related
31 to glutamate signaling. Indeed, some SNP risk haplotypes in the DAAO gene,
32 associated with decreased activity against D-Ser, are suspected to be involved in BD
33 pathogenesis. A recent meta-analysis of 741 genes with significant linkage or

1 association with BD indicates that a missense variant in the DAAO gene (DAO^{G130V} or
2 rs768676371) is the top scoring variant for high risk[50]. DAO^{G130V} harboring mice
3 display an enhanced susceptibility (or reduced resilience) to more severe forms of
4 stress caused by the inability to degrade D-Ser[50]. Furthermore, RNA sequencing of
5 cerebellar tissue reveals that DAO^{G130V} expression results in decreased expression of
6 genes encoding synaptic functions. Finally, in contrast to ambivalent data regarding
7 the DAAO gene, a body of evidence provides compelling evidence for an association
8 of G72/30 locus with BD. Different alleles and SNPs have been identified in multiple
9 cohorts[51].

10

11 • ***Substance use disorder (SUD)***

12 Substance abuse in individuals with SCZ is remarkably higher than in the general
13 population. As D-Asp and D-Ser are both linked to the pathogenesis of SCZ, and
14 NMDAR have been consistently proved to be involved in SUD, studies have explored
15 possible associations of substance abuse phenotypes with D-AAs aberrations. Passive
16 cocaine consumption in rodents alters the metabolism of D-Ser in the
17 mesocorticolimbic areas, involved in neuroplastic adaptations underlying addictive
18 behaviors, which contributes to the development of locomotor sensitization. SR-null
19 mice have blunted hedonic responses to cocaine and also exhibit attenuated locomotor
20 sensitization[52]. Treatment of SR-null mice with 300 mg/kg/day of D-Ser, a dose that
21 normalizes cortical D-Ser levels[52], restores the preference for cocaine during
22 extinction as normally observed in wild-type mice. Paternal cocaine use causes
23 epigenetic remodeling at the DAAO gene leading to its overexpression in the
24 hippocampus of cocaine-sired male progeny. Accordingly, progeny has decreased
25 levels of hippocampal D-Ser, impaired synaptic plasticity and spatial working
26 memory[53]. Administration of D-Ser to the hippocampus reversed all deficits[53].
27 Collectively, these observations further confirm that drugs modulating NMDARs, such
28 as D-Ser itself, may offer promising pharmacotherapies for the extinction of cocaine-
29 primed relapse[54].

30 Likewise, converging lines of evidence indicate that NMDAR hypofunction within
31 particular brain regions reflects alcohol-related behaviors[55]. D-Ser, either
32 administered systemically or directly in the NAc, selectively reduces compulsion-like
33 drinking in a manner similar to the NMDAR blocker D-AP5[56]. Co-administration of the

1 DAAO inhibitor sodium benzoate with subthreshold D-Ser, suppresses compulsive
2 alcohol drinking, providing a novel intervention for alcohol use disorder[57]. The
3 alcohol abuse in some individuals may result from lower hedonic responses to the
4 substance and seems to be caused by compromised metabolism of D-AAs from
5 chronic use, as demonstrated for cocaine. This might be true as well for other
6 substances of abuse such as nicotine, which have high impact in public health.

7

8 *Roles of D-AAs in neurological disorders*

9 • **Chronic pain**

10 Although the pathological mechanisms are multiple, preclinical studies indicate that D-
11 AAs play a significant role in neuropathic pain. High levels of D-Asp in Ddo-null mice
12 exacerbates nociceptive responses in mechanical and thermal-induced pain
13 conditions[58] in a sex specific manner. Nociceptive response are worsened in the late,
14 inflammatory phase of the formalin test in Ddo-null mice of both sexes when compared
15 to controls[58] in association with altered spinal neuronal activity. Paradoxically,
16 treatment with D-Asp (20 mM) in drinking water for 1 month following spared nerve
17 injury (SNI) surgery reverts mechanical allodynia, motor and cognitive impairments[59]
18 further illustrating the relevance but opposing roles of D-Asp in different pain
19 mechanisms. Endogenous D-Ser regulates gliogenic LTP[60] in nociceptive pathways
20 and not surprisingly is also implicated in several pain conditions. Pathological
21 activation of spinal sigma-1 receptor during chronic neuropathic conditions increase
22 SR expression in spinal glia[61], and spinal D-Ser contributes to mechanical allodynia
23 and hyperalgesia[62] by activating neuronal nitric oxide synthase (nNOS) activity
24 leading ultimately to increases in PKC-dependent GluN1
25 phosphorylation[63,64]. Inhibition of CP-450 with aminoglutethimide[65] or boosting
26 GABAergic transmission with pregabalin[66] attenuates spinal D-Ser content and
27 NMDAR-mediated synaptic transmission in the superficial dorsal horn thus alleviating
28 mechanical allodynia. Both SR and DAAO are now considered targets for chronic
29 pain[67]. SR inhibitors have repetitively shown to reduce allodynia in rodents[63,64].
30 Preclinical studies indicates that several DAAO inhibitors reverse formalin-induced
31 tonic pain in a strain-dependent manner in mice[68]. Interestingly, subcutaneous
32 injections of the DAAO inhibitor CBIO prevents the morphine-induced thermal

1 hyperalgesia in mice[69]. Further studies to comprehend central and spinal
2 mechanisms as well as the role of less studied D-AAs, such as D-Cys and D-Ala, have
3 potential to transform the field in the next decades.

4

5 • **Epilepsy/migraine**

6 Mounting preclinical evidence indicates that D-Ser is involved in the pathogenesis and
7 progression of epilepsy. In the pilocarpine temporal lobe epilepsy (pTLE) model,
8 pharmacological inhibition of astroglial connexin43 hemichannels protects against
9 seizures by lowering extracellular D-Ser levels that normally accompanies
10 convulsions[70]. Consistently, chemically-induced epileptic seizures are decreased
11 upon pharmacological inhibition of SR[71]. Such implications of D-Ser were recently
12 confirmed in a genetic study involving TLE patients: three SR tag SNPs were
13 associated with decreased susceptibility to and lower severity of TLE[72]. Although the
14 exact role that D-Ser may play in epilepsy is still illusive, administration of D-Ser is
15 effective in preventing epileptogenesis in an animal model of TLE by attenuating
16 neuron loss and gliosis[73], offering new hope as a therapeutic agent for refractory
17 TLE. Other D-AAs could be involved in epilepsy such as D-Leu, which accounts for 16–
18 20% of total leucine in the brain[2]. D-Leu inhibits acute kainic acid-induced seizures
19 and elevates seizure threshold during the dark cycle, even when administered after
20 seizure onset[74].

21 Several inborn errors of Ser biosynthesis can cause neurological disorders
22 which symptomatology includes from seizures to intractable epilepsy. Mutations in
23 ASCT1, the main serine transporter in the CNS, have been recently described in
24 children with neurological manifestations similar to those observed in Neu-Laxova
25 Syndrome[75]. Finally, nonketotic hyperglycinemia (NKH) is accompanied by a
26 decrease in CSF L-Ser and D-Ser, more pronounced in the latter, likely reflecting a
27 perturbation of D-Ser metabolism[76]. Glycine is the most robust regulator of SR and
28 pathological glycine levels may exert persistent inhibition on SR activity thus blunting
29 the production of D-Ser in NKH patients[77]. Interestingly, ketogenic diet that regulate
30 Ser metabolism and most likely D-Ser has been successful in treating those metabolic
31 conditions in varying degrees[78].

1 Lastly, subjects with epilepsy have increased risk for migraine, which shares
2 clinical features and pathophysiological mechanisms with the former. A study
3 examining migraine life-time prevalence in epileptic patients revealed that SR
4 polymorphism rs4523957, which affects transcription of the enzyme, is significantly
5 associated with reduced migraine risk[79]. Although waiting for replication, these
6 observations suggest that aberrant NMDAR activation by D-Ser would represent an
7 important pathophysiological mechanism in migraine.

9 • ***Ischemic conditions***

10 The ischemic penumbra results from over-activation of NMDARs that triggers cell-
11 death pathways. SR and D-Ser are found to play key roles in the progress of stroke.
12 The deletion of SR was reported to reduce by ~50% ischemic damage in mouse middle
13 cerebral artery occlusion (MCAO) model of stroke[80] which is comparable to
14 protection offered by pharmacologic blockade of NMDARs. Likewise, traumatic brain
15 injury (TBI) can lead to tissue loss and neurological dysfunction as a result of
16 excitotoxic-induced neurodegeneration. Recent studies show that mild TBI in mice
17 results in a switch in the cellular source of D-Ser from neurons to glia in the
18 hippocampus[81]. Glia-derived D-Ser promotes excitotoxic damage and synaptic
19 dysfunction after TBI through the activation of extrasynaptic GluN2B-containing
20 NMDAR in the injured hippocampus[81]. Nonetheless, enhancement of brain D-Ser
21 through the inhibition of DAAO could also offer neuroprotection and mediates recovery
22 of cognitive function after TBI[82]. Whether these beneficial effects of D-Ser are caused
23 by reestablishing its 'neuronal' functions are not known.

25 **Roles of D-AAAs in neurodegenerative disorders**

26 • ***Amyotrophic Lateral Sclerosis (ALS)***

27 ALS, the most common adult-onset neuromuscular disease, is a highly debilitating,
28 rapidly progressing condition leading to fatal paralysis 2-5 years after onset. ALS is by
29 far the most documented neurodegenerative disease where D-Ser has important
30 pathogenic roles. D-Ser levels are increased in the spinal cord of patients with
31 sporadic/familial ALS and in the G93A-SOD1 mice model of ALS leading to
32 motoneuron degeneration through over activation of NMDARs[83]. ddY/DAAO-

1 transgenic mice, harboring the naturally occurring mutation DAAO^{G181R}[83], show
2 abnormal reflexes, as observed in the SOD1^{G93A} mouse model. Identification of the
3 the missense DAAO^{R199W} substitution in familial (f-ALS) cases definitively incriminates
4 DAO in ALS[84]. Transgenic mice expressing DAAO^{R199W} mutation show axonal
5 degeneration with muscle atrophy together with increased ubiquitination in the spinal
6 cord, as well as progressive rigid thoracolumbar kyphosis, caused by a loss of muscle
7 tone, secondary to motor neuron degeneration[84], similar to other mouse models of
8 motor neuron disease. Overexpression of wild-type DAAO significantly enhances the
9 survival of transgenic mice expressing the SOD1^{G93A} mutation. Several other ALS-
10 associated DAAO mutations are now reported[85,86]. A large exome sequencing study
11 with sporadic ALS patients found that only polymorphisms in the DAAO gene were
12 associated with clinical outcome, which resulted in decreased rates of survival[87]. The
13 contribution of D-Ser/DAAO system in ALS is further indicated in studies on the targets
14 of RNA binding proteins (RBPs), which are associated with the control of RNA
15 processing. Loss of the RBP hnRNP A2/B1 results in a highly unstable DAAO isoform
16 with ~6-fold reduction in enzymatic activity [88]. How this pre-clinical and clinical
17 knowledge will translate to therapeutics is yet to come.

18 • **Brain ataxias**

19 Spinocerebellar ataxias constitute a heterogeneous group of rare hereditary
20 progressive disorders. Striking sensory ataxia, cardiac hypertrophy and mitochondrial
21 dysfunction similarities with Friedreich's ataxia have been identified in chemogenetic
22 transgenic mice expressing DAAO under control of endothelial-specific Cdh5
23 promoter[89]. A recent exome sequencing study conducted in patients revealed a
24 homozygous c.2128C > T, p.(Arg710Trp) missense variant in the GRID2 gene
25 encoding the glutamate receptor GluD2 subunit[90]. The resulting AA substitution
26 alters a highly conserved residue in the extracellular D-Ser binding domain of GluD2,
27 thereby altering, but not abolishing, D-Ser binding to the receptor. It supports the notion
28 that D-Ser, by binding to GluD2, is critical for normal cerebellum development and
29 functions[21]. Remarkably, these observations also echo the general cortical atrophy
30 found in SR-null mice[20] and the recent evidence that Luvadaxistat, a highly selective
31 and potent inhibitor of DAAO, by increasing D-Ser levels, could be clinically relevant
32 [91].

33

1 • ***Alzheimer's disease (AD) and dementias***

2 The link between D-AAs and AD pathogenesis has been difficult to establish as
3 conflicting data has been reported in patients and across disease models. Analysis of
4 a large cohort of age-matched healthy subjects and AD patients indicates that serum
5 D-Ser is positively correlated with AD while D-Asp levels do not change[92], data which
6 were corroborated by a recent meta-analysis of 7 studies[93]. In addition to D-Ser and
7 D-Asp, serum levels of other D-AAs including D-alanine, D-leucine, D-glutamate, D-
8 phenylalanine and D-proline may be altered in AD[94] and most likely play different
9 roles in the progression of the disease[95]. These modifications may well reflect
10 alterations in the microbiomes of patients. Not surprisingly, alterations in D-Ser
11 metabolism are implicated at different nodes of AD pathogenesis[96]. For instance,
12 hyperglycemia is a risk factor for the onset of AD and impaired brain insulin signaling
13 is known to occur in the disease. Furthermore, low insulin response in AD is linked to
14 D-Ser metabolism[97,98] and D-Ser may as well control systemic glucose metabolism
15 *via* NMDARs activation by blunting insulin secretion from pancreatic beta cells[99].
16 Inhibition of pancreatic NMDARs, as well as loss of SR, decreases insulin secretion, a
17 mechanistic association supported by SNPs analysis[99].

18 D-Ser may be involved in the pathology through two other scenarios. Preclinical
19 studies favor an excitotoxic scenario as A β oligomers promote SR expression[100] and
20 thereby contributes to neuropathological outcomes by boosting the production of D-
21 Ser. Accordingly, deletion of SR in the APPKi AD mice model protect against A β
22 oligomers-induced cell loss[101]. Levels of DAAO are increased in the serum of AD
23 patients as well as those with mild cognitive impairment and correlate positively with
24 the severity of cognitive deficits[102,103]. Accordingly, increase in DAAO might
25 represent an adaptive response to the increase of circulating D-Ser. Likewise, G72/30
26 gene variants modify the age of onset in AD[104], although displaying a non-linear
27 association with the severity of cognitive decline[105]. Another scenario implies the
28 contrary: a failure in brain aerobic glycolysis would result in impaired L-Ser production
29 and in turn hampered D-Ser synthesis, thereby impacting synaptic plasticity and
30 cognitive performance[15]. Interestingly, all symptoms observed in the 3xTg-AD mice
31 could be fully rescued by L-Ser or D-Ser supplementation in diet or drinking water[15],
32 opening new avenues for future treatment based on nutrient supplementation. These
33 up and down regulations of D-Ser metabolism and levels may be differently engaged
34 at various stages along the pathogenic trajectories and could also be gender

1 specific[97]. Further studies are needed to establish whether dysfunction of D-
2 Ser/NMDAR system is a triggering event for AD or a consequence of the on-going
3 neurodegenerative process.

4

5 • ***Parkinson's Disease (PD) and dyskinesias***

6 The roles of D-AAAs in PD pathogenesis have recently been uncovered. Non
7 physiological high levels of D-Asp in Ddo-null mice accelerate loss of corticostriatal
8 synaptic depotentiation which normally develops in animals only after dopamine
9 denervation and chronic long-term levodopa (L-DOPA) administration. This results in
10 precocious expression of L-DOPA induced dyskinesia (LID)[106]. 1-methyl-4-phenyl-
11 1,2,3,6-tetrahydropyridine hydrochloride (MPTP) treatment of Rhesus macaques
12 increases D-Asp and D-Ser in the putamen while L-DOPA restores both D-AAAs levels
13 [107]. Conversely, dopaminergic denervation is associated with selective D-Ser
14 reduction in the substantia nigra, confirming previous preclinical observations that D-
15 Ser levels are altered in the brain of MPTP- and 6-OHDA-lesioned rodents[108].
16 Furthermore, D-Ser levels are also lower in the CSF of L-DOPA-free PD patients[107]
17 and SR is a possible susceptibility factor within the PD genetic background
18 complementing LRRK2-G2019S pathogenicity in patients[109].

19

20 **D-AAAs: Are we ready for clinic?**

21 Several clinical trials for the use of D-AAAs in brain disorders are recently finished or on-
22 going, both in the academic setting and pharmaceutical companies worldwide. Dietary
23 D-Ser supplementation is already employed in the treatment of many psychiatric
24 disorders including SCZ and mood disorders, encephalopathies and SUD. D-Asp has
25 also been approved for use in humans as a dietary supplement and its oral
26 administration is a promising approach in multiple sclerosis[110]. However, oral
27 treatments with either D-AAAs require high dosage of several g/day/kg, which could pose
28 potential safety concerns, especially to patients with renal or hepatic co-
29 morbidities[39]. In consequence, intense drug discovery programs are in place to
30 develop compounds for the treatment of diseases to which D-Ser and D-Asp are
31 associated (See Box 1). Although limited so far, development of novel D-AAAs-based
32 pharmacotherapy is expected to reshape treatment for brain disorders in the near
33 future.

1

2 **Concluding Remarks**

3

4 This review has discussed several examples of D-AAs that form an enigmatic class of
5 signaling molecules populating the brain, body fluids but also other organs. We have
6 highlighted the current advances in the field and used them to illustrate how our
7 understanding of the roles of D-AAs in physiology and pathophysiology has
8 revolutionized the field of basic brain sciences in recent years, as well as the way we
9 now consider to treat patients. Though we recognize their importance as their
10 misregulation has been increasingly implicated in a wide range of major brain and
11 metabolic disorders, too much else remains shrouded in mystery (see Outstanding
12 Questions). With the rapid adoption of innovative technologies for detecting and
13 controlling them precisely and the introduction of novel model organisms, we anticipate
14 that many new findings will emerge on the physiopathological roles of D-AAs and
15 notably on their roles in inter-organ communication (see Clinician's Corner).

1 **Glossary**

2 **Exome studies:** Exome studies use the molecular biology method of exome
3 sequencing to identify only the genes that code for proteins, in a given genome. Since
4 only a minute portion of the human genome code for proteins, it is a faster and cheaper
5 technique compared to whole-genome sequencing.

6 **Genome wide association studies:** Genome wide association studies (GWAS) are
7 hypothesis free methods to identify associations between genetic regions (loci) and
8 traits (including diseases) in populations.

9 **GluD (or GluR δ) receptors:** A sub-class of ionotropic glutamate-gated receptors
10 (iGluR) massively present in the cerebellum but also, albeit at lower levels, in multiple
11 mouse brain regions including the cerebral cortex, hippocampus, striatum, thalamus,
12 mesencephalon and in the retina. These receptors regulate synapse maintenance and
13 synaptic plasticity.

14

15 **NMDA receptors (NMDARs):** A sub-class of glutamate-gated ionotropic receptors
16 (iGluR) that is crucial for the formation of synapses and the induction of diverse forms
17 of synaptic plasticity. These receptors form heterotetramers through the association of
18 homologous subunits. 7 subunits have been identified falling in three subfamilies:
19 GluN1, GluN2A-D, and GluN3A-B. Native NMDAR are either diheteromers containing
20 two GluN1 with two identical GluN2 (or GluN3) or triheteromers containing two GluN1
21 and two different GluN2 (or a combination of GluN2 and GluN3). Diheteromeric (except
22 for GluN1/GluN3 receptors) or triheteromeric NMDAR require for their activation the
23 binding of glutamate on GluN2 subunit and the binding of a co-agonist, D-Ser or
24 glycine, on GluN1 subunit.

25 **Single nucleotide polymorphism:** A single nucleotide polymorphism (SNP) is a
26 single base-pair variation in genome among individuals of a species. SNPs are often
27 associated to differences in drug metabolism and disease susceptibility between
28 individuals and populations.

29 **Synaptic plasticity:** Synaptic plasticity is the ability of chemical synapses to
30 strengthen or weaken their activity and adapt their structure and biochemistry over

- 1 time, following specific patterns. Synaptic plasticity is thought to contribute to learning
- 2 and memory. Both pre- and post-synaptic mechanisms are engaged in these changes.
- 3

1 **Clinicians Corner**

- 2 • Within the realm of D-AAs, D-Ser levels are closely associated with the severity of
3 cognitive and negative symptoms in SCZ. Even though oral D-Ser supplementation
4 has demonstrated therapeutic effects, DAAO inhibition has received major traction in
5 the past years. The first-in-class DAAO inhibitor Luvadaxistat, a joint development
6 of Neurocrine Biosciences and Takeda, is currently in phase 2 clinical trials.
- 7 • DAAO loss-of-function mutations are strongly implicated in the etiology of ALS. Not
8 surprisingly, overexpression of wildtype DAAO preserved motor neurons and
9 increased survival in a classic mice model of the disease. Given the numerous clinical
10 trials involving gene therapy strategies for ALS, DAAO recovery strategy must be
11 evaluated in future trials.
- 12 • Alterations in the serum or urine levels of several D-AAs including D-Asp and D-Ser
13 are closely associated with many pathological conditions and predict the therapeutic
14 response of patients. Therefore, sampling the levels of D-AAs in serum or urine of
15 patients should be included in clinical settings and could be then used to guide
16 individualized precision treatment
- 17 • Errors in serine biosynthesis and degradation cause diverse syndromes which
18 symptomatology converge in a higher risk for seizure development and epilepsy.
19 Ketogenic diet is already advised for certain infantile epilepsies with various degrees
20 of success. Adjunctive dietary supplementation with D-serine could be of value for
21 many of those.
- 22 • While specific mutations of SR, DAAO and Ddo may be shared by several
23 pathological conditions, we need to have a better stratification of patients cohorts in
24 order to better precise the pathogenic trajectories of those diseases.
- 25 • There's no current specific compounds targeting SR. Further investigations are
26 needed to treat SR-related brain disorders like stroke, AD or PD and improves the
27 lives of patients living with those diseases.

28

1 **Box 1. Serine racemase, the multiple D-AA synthesizing enzyme**

2 Serine racemase (SR), expressed by various cell-types in the brain (ie neurons, glia,
3 endothelial cells)[111], catalyzes the synthesis of D-Ser and D-Cys, and presumably D-
4 Asp, from their respective L-enantiomers, though with different efficiencies[10,112].
5 The enzyme is also capable of catalyzing D- and L-Ser α,β -elimination to produce
6 pyruvate and ammonia[111]. However, the physiological relevance of this process *in*
7 *vivo* remains enigmatic as it would only occur when the levels of D-Ser exceed L-Ser
8 levels. The catalytic efficiency of racemization by SR is very low, likely due to the low
9 metabolic requirement of D-AAAs. For this reason, SR activity is tightly regulated by
10 several ligands, interactions with proteins and post-translational modifications that
11 dynamically contribute to the homeostasis of these D-AAAs (extensively reviewed in
12 [111]). SR seats at the interface of several other metabolic hubs. On one hand,
13 synthesis of D-Ser highly relies on the phosphorylated pathway by astrocytes for the
14 production of L-Ser[15,111]. On the other hand, SR produces pyruvate that may serve
15 to fuel mitochondria in neurons for ATP production or may participate in the production
16 of lactate in astrocytes for signaling purposes. SR catalyzes also the α,β elimination of
17 water from L-threonine generating α -ketobutyrate and ammonia[111] and could then
18 constitute an alternative pathway for threonine degradation. SR-null mutant mice show
19 near complete absence of D-Ser and D-Cys (~80-90%) and profound reduction in the
20 levels of D-Asp in the brain[10,17]. The origin of the residual D -AAAs portion of SR-null
21 mice is not known.

22

23 **Box 2. Metabolic D-AAAs breakdown**

24 Ddo degrades D-Asp but can also metabolize other acidic D-AAAs such as D-Glu. Ddo-
25 null mutant mice show elevations of D-Asp in all areas of the brain[25]. As for DAAO,
26 the enzyme is highly enriched in the hindbrain, where it degrades D-Ser[113], but better
27 accommodates other non-polar and hydrophobic D-AAAs (order of preference: D-Cys >
28 D-Ala > D-Pro > D-Ser). The primary function of DAAO, however, still requires
29 clarification as DAAO-null mice show no elevation in D-Ser in the forebrain[113]. In
30 addition, DAAO appears to be a metabolic hub for other neuromodulators as well.
31 Indeed, during the oxidative catabolism of D-Cys, DAAO produces 3-mercaptopyruvate
32 (3MP) which is subsequently used by the mitochondrial 3-MP sulfurtransferase (3-

1 MPS) for the production of the neuromodulator H₂S[24]. DAAO may be also linked to
2 the kynurenine pathway of tryptophan (TRP) degradation by converting D-TRP
3 produced by microbiota into L-TRP and thereafter for the biosynthesis of KYNA[114].
4 In conclusion, D-AAAs are found at the crossroad of different metabolic and signaling
5 hubs and therefore the levels of D-AAAs are expected to fluctuate according to the global
6 metabolic fate of the cell. Conversely, any disruption of this cooperation may well
7 explain the contribution of D-AAAs to the initiation or progression of several brain
8 disorders and their use as biomarkers[1].

9

10 **Box 3. D-AAAs metabolism and signaling pathways in medicinal chemistry**

11 Monotherapy with dietary D-Ser is effective in patients with SCZ[115] and for cognitive
12 dysfunction after TBI[82] but only at high doses. Therefore, DAAO inhibition may
13 represent the best alternative. Use of sodium benzoate shows promising results in the
14 treatment of SCZ[116] in phase 1 clinical trials, in patients at early-phase Alzheimer's
15 disease[117] or with major depression[46], but off-target effects limit its clinical
16 translation[116]. Therefore, new and more selective DAAO inhibitors were developed.
17 First generated DAAO inhibitors had suboptimal clinical features. Therefore, the search
18 for second generation DAAO inhibitors with nanomolar activity, advantageous
19 physicochemical properties and ADME profile is justified[118]. Luvadaxistat (also
20 known as TAK-831), a potential first-in-class DAAO inhibitor developed for the
21 treatment of negative symptoms and cognitive impairment in SCZ, has completed
22 multiple phase I and phase II studies[119] (See SCZ section).

23 SR is another druggable target in diseases like stroke, AD, ALS, and
24 neuropathic pain. Several attempts were made to develop SR inhibitors but only few
25 compounds have been identified, exhibiting weak inhibitory activity with inhibition
26 constants in the high micromolar to millimolar range. The only exception, malonate,
27 also inhibits DAAO and Ddo, with a K_i of 19 μM[8]. Despite the identification of amide
28 inhibitors and halogen-substituted derivatives that display improved inhibitory activity
29 and affinity, they exhibit limited potency and unfavorable ADME[71]. Drug-discovery of
30 future SR modulators are guided on structure-based and combinatorial
31 approaches[120,121].

32 D-Asp is central to the onset of various mental disorders, including SCZ.
33 Interestingly, antipsychotic medication itself can increase D-Asp by inhibiting Ddo[122],

1 which could be a significant mechanism for its clinical efficacy. Several low molecular
2 weight compounds such as 5-aminonicotinic acid (K_i 3.80 μ M) were identified using
3 ligand-docking method for their ability to inhibit human Ddo with high affinity and
4 specificity[123], opening the development of clinically useful Ddo inhibitors.

5 Finally, transporters for D-Ser are also promising druggable targets. Several
6 small molecules have been characterized that inhibit the Na⁺-independent alanine-
7 serine-cysteine antiporter 1 (ASC-1/SLC7A10) with high affinity. Both competitive
8 inhibitors and selective non-transportable ones have been developed[124]. However,
9 these compounds do not pass the BBB, occluding their use in clinic. The neutral AA
10 transporters alanine serine cysteine transporter 1 & 2 (ASCT-1 and -2/SLC1)
11 transports D-Ser or others neutral AAs, such as alanine and glutamine, into the cell.
12 ASCT2 inhibitors including L-hydroxyhomoserine, and sulfonamides/sulfonic ester
13 derivatives with low micromolar K_i values were recently identified by *in silico* screening
14 methods, which could be exploited for future therapeutical opportunities[125].

15

16 †This review is dedicated to our friend and colleague Professor Jacqueline de
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18 diseases and whose contribution inspired this article.

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23 **Resources:**

24 I This study is registered at clinicaltrials.gov/study/NCT01908192

25 II This study is registered at clinicaltrials.gov/study/NCT03094429

26 III This study is registered at

27 anzctr.org.au/Trial/Registration/TrialReview.aspx?id=381442.

28 IV “Neurocrine Biosciences Announces Top-Line Results from Phase II INTERACT
29 Study Evaluating Luvadaxistat (NBI-1065844) for the Treatment of Negative

1 Symptoms and Cognitive Impairment Associated with Schizophrenia (CIAS).” *PR*
2 *Newswire*, 2021. Available at [prnewswire.com/news-releases/neurocrine-biosciences-](https://prnewswire.com/news-releases/neurocrine-biosciences-announces-top-line-results-from-phase-ii-interact-study-evaluating-luvadaxistat-nbi-1065844-for-the-treatment-of-negative-symptoms-and-cognitive-impairment-associated-with-schizophrenia-cias-)
3 [announces-top-line-results-from-phase-ii-interact-study-evaluating-luvadaxistat-nbi-](https://prnewswire.com/news-releases/neurocrine-biosciences-announces-top-line-results-from-phase-ii-interact-study-evaluating-luvadaxistat-nbi-1065844-for-the-treatment-of-negative-symptoms-and-cognitive-impairment-associated-with-schizophrenia-cias-)
4 [1065844-for-the-treatment-of-negative-symptoms-and-cognitive-impairment-](https://prnewswire.com/news-releases/neurocrine-biosciences-announces-top-line-results-from-phase-ii-interact-study-evaluating-luvadaxistat-nbi-1065844-for-the-treatment-of-negative-symptoms-and-cognitive-impairment-associated-with-schizophrenia-cias-)
5 [associated-with-schizophrenia-cias-](https://prnewswire.com/news-releases/neurocrine-biosciences-announces-top-line-results-from-phase-ii-interact-study-evaluating-luvadaxistat-nbi-1065844-for-the-treatment-of-negative-symptoms-and-cognitive-impairment-associated-with-schizophrenia-cias-)

6 V This study is registered at clinicaltrials.gov/study/NCT03359785

7 VI This study is registered at clinicaltrials.gov/study/NCT03382639

8

9

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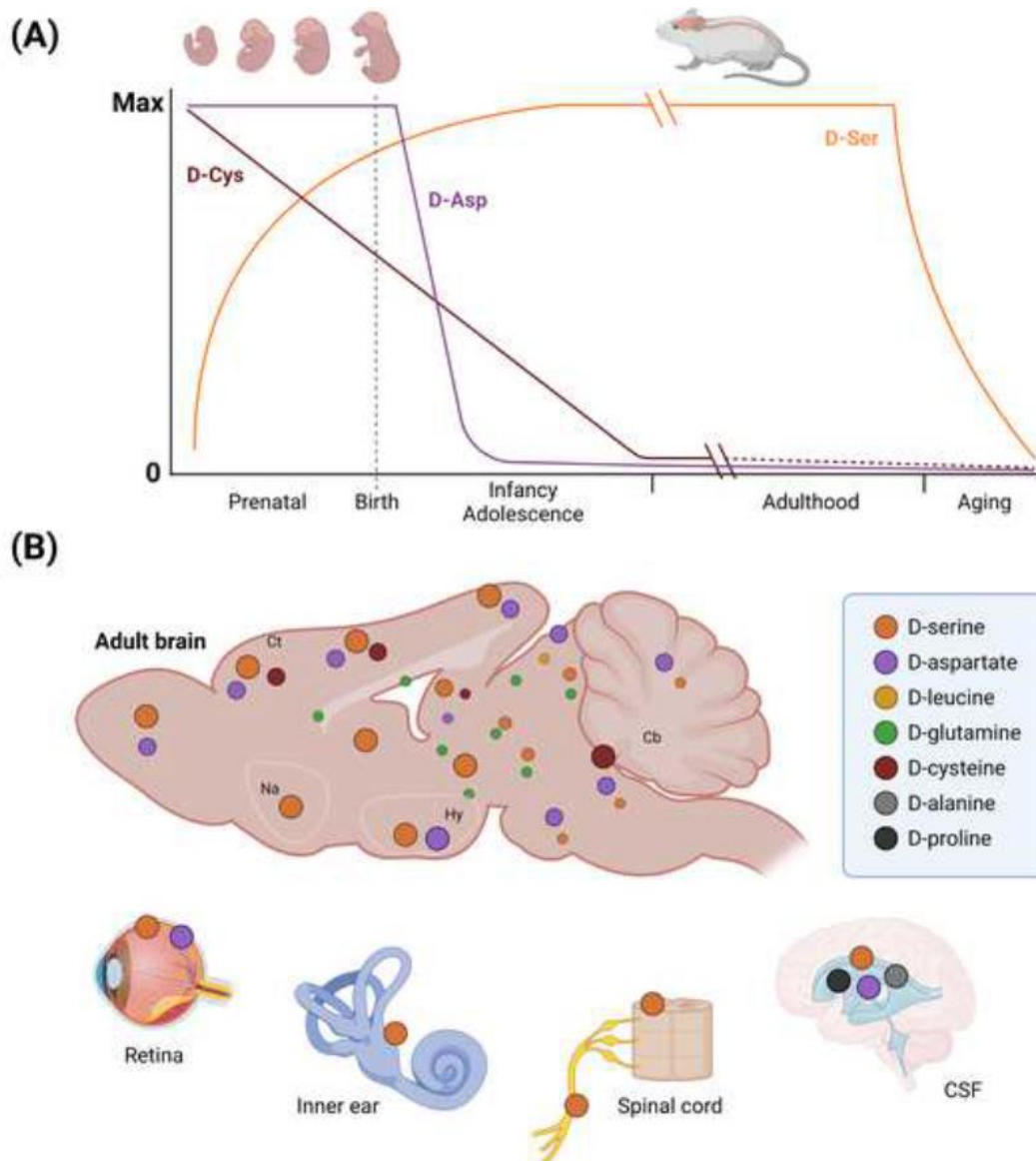
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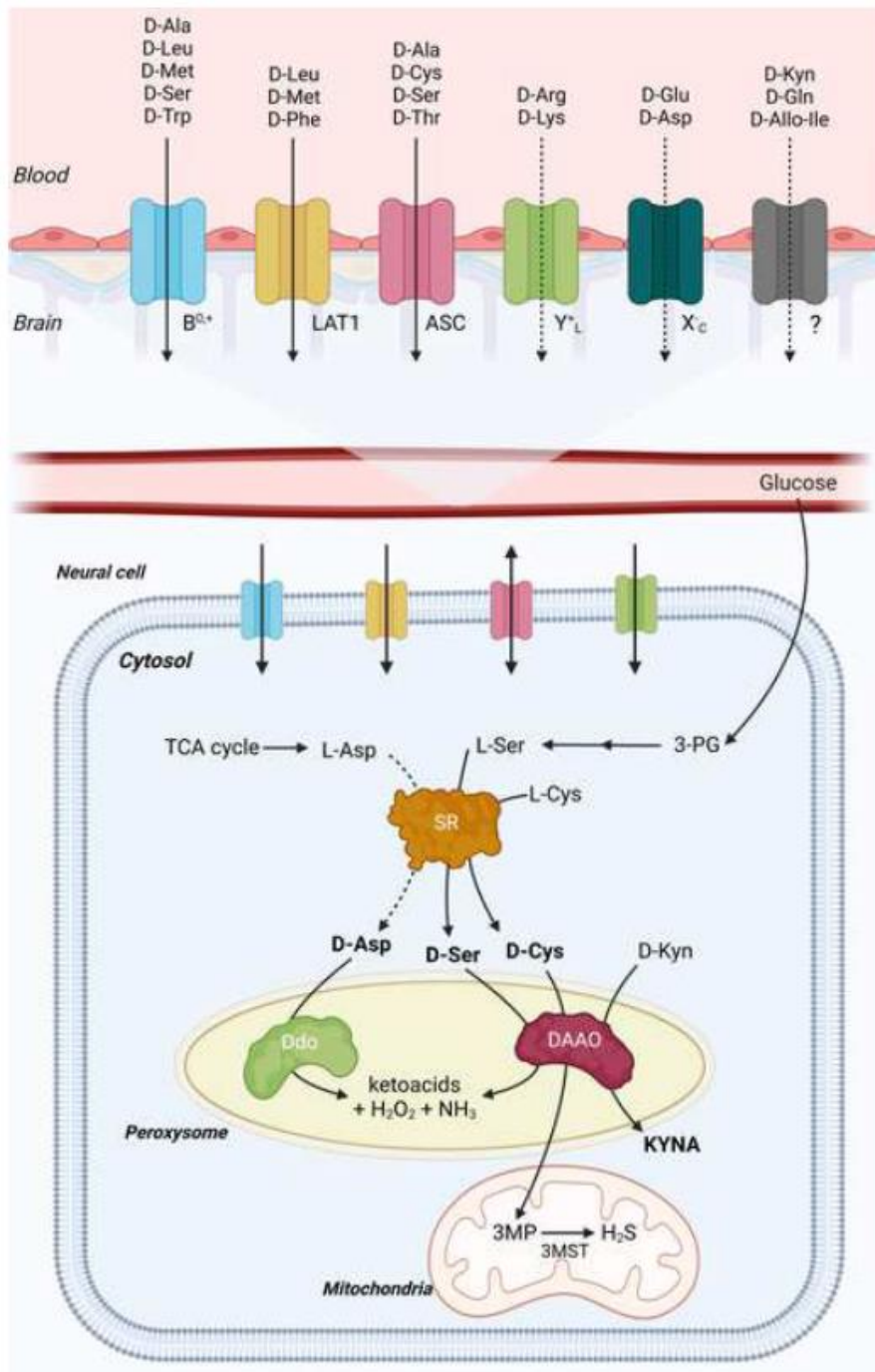
1 **Figures**

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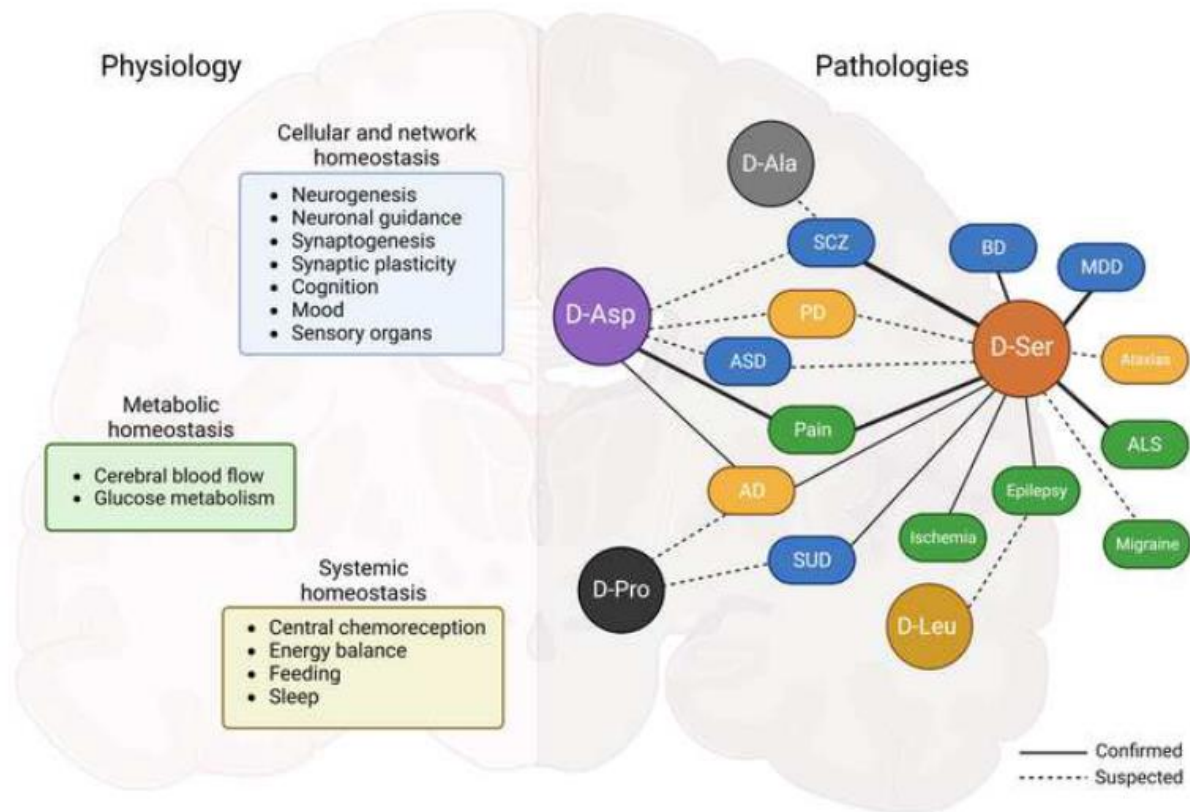
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4 **Figure 1. Temporal and spatial mapping of brain D-AAs.** D-AAs, initially relegated as
5 unnatural to the animal kingdom, are now recognized as crucial molecules for the
6 development and homeostasis of the human brain. (A) Brain levels of D-Ser (orange),
7 D-Asp (purple) and D-Cys (brown) are differentially altered during lifespan due to
8 changes in their metabolism. (B) Various D-AAs populate different adult brain areas,
9 retina, cochlea, spinal cord and CSF with varying amounts. Abbreviations: CSF,
10 cerebrospinal fluid ; ct, cortex ; cb, cerebellum ; hy, hypothalamus ; Na, accumbens
11 nucleus.



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Figure 2. Transport and metabolism of brain D-AAAs. (Top) Circulating D-AAAs originating from diet or microbiota can penetrate the brain parenchyma thanks to the presence of several AA transporter systems that are not stereoselective. (Bottom) D-Ser, D-Cys and D-Asp are formed from their respective L-antipode by serine racemase and then degraded by two peroxysomal enzymes DAAO and Ddo. Metabolism of D-Cys by DAAO results in the production of 3MP that can be converted in mitochondria into H₂S by 3MST. DAAO can use D-Kyn to produce kynurenic acid (KYNA). KYNA, H₂S together with D-Cys, D-Asp and D-Ser support cell-cell communications. Abbreviations : DAAO, D-amino acid oxidase ; Ddo, D-aspartate oxidase ; KYNA, kynurenic acid ; 3MP, 3-mercaptopyruvate ; 3MST, 3-mercaptopyruvate sulfurtransferase ; 3-PG, 3-Phosphoglyceric acid ; SR, serine racemase ; TCA, tricarboxylic acid ;



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2 **Figure 3. The physiological and pathological landscapes of brain D-AAs.** (Top) D-Ser,
3 D-Asp and D-Cys are implicated in various physiological functions in the developing
4 and mature nervous system. (Bottom) Alterations in the metabolism and levels of D-
5 AAs are associated to the onset or progression of several neurological (green),
6 psychiatric (blue) or neurodegenerative (orange) diseases. Broken lines, suspected
7 link with disease. Plain lines, consolidated link with disease. Abbreviations: AD,
8 Alzheimer’s disease ; ALS, amyotrophic lateral sclerosis ; ASD, Autism spectrum
9 disorders ; BD, bipolar disorder ; MDD, major depressive disorders ; PD, Parkinson’s
10 disease ; SCZ, schizophrenia ; SUD, Substance use disorder.

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