D-aspartate (D-Asp) is an atypical amino acid, which is especially abundant in the developing mammalian brain, and can bind to and activate N-methyl-D-Aspartate receptors (NMDARs). In line with its pharmacological features, we find that mice chronically treated with D-Asp show enhanced NMDAR-mediated miniature excitatory postsynaptic currents and basal cerebral blood volume in fronto-hippocampal areas. In addition, we show that both chronic administration of D-Asp and deletion of the gene coding for the catalytic enzyme D-aspartate oxidase (DDO) trigger plastic modifications of neuronal cytoarchitecture in the prefrontal cortex and CA1 subfield of the hippocampus and promote a cytochalasin D-sensitive form of synaptic plasticity in adult mouse brains. To translate these findings in humans and consistent with the experiments using Ddo gene targeting in animals, we performed a hierarchical stepwise translational genetic approach. Specifically, we investigated the association of variation in the gene coding for DDO with complex human prefrontal phenotypes. We demonstrate that genetic variation predicting reduced expression of DDO in postmortem human prefrontal cortex is mapped on greater prefrontal gray matter and activity during working memory as measured with MRI. In conclusion our results identify novel NMDAR-dependent effects of D-Asp on plasticity and physiology in rodents, which also map to prefrontal phenotypes in humans.

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whether genetic variation of the DDO gene (6q21) affects DDO mRNA expression and, in turn, modulates prefrontal gray matter volume and activity during working memory (WM) processing in healthy subjects.

MATERIALS AND METHODS

Animals

C57BL/6J male mice were used to test the effects of 1-month-long oral administration of D-Asp. D-Asp was delivered in drinking water at the concentration of 20 mg/mL on mice the age of 4 months, when they were used for experiments. Mutant mice for the Ddo gene were generated and genotyped by PCR as described previously.23 All research involving animals was carried out in accordance with the European directive 86/609/EEC governing animal welfare and protection, which is acknowledged by the Italian Legislative Decree no. 116, 27 January 1992. Animal research protocols were also reviewed and consented to by a local animal care committee.

Electrophysiology

Coronal slices from mouse medial PFC (mPFC; 250 μm) were cut in ice-cold artificial cerebral spinal fluid using standard procedures. Visually guided whole-cell recordings were performed as previously described25 (see also Supplementary Methods) using 1.5 mm borosilicate glass electrodes (3–4 MΩ) filled with a solution containing (in mM) CsMeSO4 (130), HEPES (5.0), EGTA (0.5), MgCl2 (1.0), NaCl (1.0), CaCl2 (0.34), QX-314 (5.0), adjusted to pH 7.5 with CsOH. Miniature excitatory post synaptic currents (mEPSCs) were collected from layer II/III mPFC pyramidal neurons and NMDA currents were pharmacologically isolated according to a previously described procedure.23

For extracellular recordings, parasagittal hippocampal slices (thickness, 400 μm) were cut using a Vibratome (Leica VT1000 S, Leica Biosystems, Wetzlar, Germany). Slices were incubated for 1 h in a holding chamber and then transferred to a recording chamber, completely submerged in artificial cerebral spinal fluid (30–31 °C) of the following composition (in mM): NaCl (124), KCl (3.0), MgCl2 (1.0), CaCl2 (2.0), NaH2PO4 (1.25), NaHCO3 (26), glucose (10); saturated with 95% O2, 5% CO2. Bipolar stimulating electrodes placed in the stratum radiatum to activate the Schaffer collateral commissural fibres. Recordings of field excitatory post synaptic potentials were made in the middle of the stratum radiatum by using microelectrodes filled with artificial cerebral spinal fluid (resistance 3–5 MΩ). LTP was induced with 1 s, 100 Hz stimulation. For statistical analysis we used unpaired t-tests after LTP induction (on the average of the last 10 min of recording).

Functional magnetic resonance imaging measurement of basal cerebral blood volume

Animal preparation for functional magnetic resonance imaging (fMRI) was adapted from previous studies26–28 and optimized for physiological stability. Briefly, mice were anesthetized with halothane, intubated and artificially ventilated. A femoral artery was cannulated for contrast agent administration, blood pressure monitoring and sampling, and infusion of paralyzing agent. Experiments were carried out at a maintenance anesthesia level of 0.7%. Ventilation parameters were adjusted to maintain physiological levels of arterial blood gases (pCO2 and pO2). Image acquisition parameters have been recently described.29 High-resolution anatomic images were acquired at 7 Tesla with a fast spin echo sequence. Co-centered cerebral blood volume (CBV) weighted fMRI times series were acquired using a fast low-angle shot sequence with a TR = 394.7 ms, TE = 3.1 ms, α = 30°; FOV 2 × 2 cm², 156 × 156 × 500 μm resolution, dt = 60 s. Images were sensitized to reflect alterations in CBV20–31 by injecting 5 μL g−1 of superparamagnetic iron oxide (Molday Ion, Biopal Inc., Worcester, MA, USA) intravascularly after five baseline images. The procedure used to calculate basal CBV (bCBV) has been recently described in greater detail.23,31 bCBV time-series were calculated over a 5-min time-window starting 15 min after contrast agent injection and spatially normalized to a study-based anatomical template. Voxel-wise group statistics was carried out using FSL using multilevel Bayesian inference and a Z-threshold > 1.7, a corrected cluster significance threshold of P = 0.001.34 Age, weight and terminal arterial gas levels (pCO2) were used as nuisance regressors in the design matrix.

Golgi-Cox staining and dendritic spine measurements

Golgi-Cox staining was performed on brains of naive animals, according to a previous protocol.35 Fully impregnated pyramidal neurons laying in the PFC and in the CA1 region of the dorsal hippocampus were visualized at ×100 (oil-immersion) using a microscope (DMLB, Leica Biosystems) equipped with a camera (resolution = 2600 × 2600, AxioCam, Zeiss AG, Oberkochen, Germany), and the KS500 3.0 system (Zeiss). A computer-based neuron tracing system (Neurolucida, Microbrightfield, MBB Bioscience, Williston, VT, USA) was used to trace single neurons. Total dendritic length and spine density were calculated according to D’Amelio et al.35 and analyzed using Student’s t-test. Dendritic complexity was calculated according to Balu et al.36 and analyzed using two-way analysis of variance with repeated measures, followed by Fisher’s post hoc comparison when required.

Candidate DDO SNPs screening and mRNA expression

Data from 268 brains of nonschizophrenic individuals (176 males; mean age ± s.d. 27.89 ± 22.15 years; pH 6.53 ± 0.3; postmortem interval: 26.37 ± 17.11) were analyzed (Supplementary Table 1). These data were obtained from the largest postmortem collection publicly available (BRAINCLOUD, courtesy of the Lieber Institute for Brain Development, Baltimore, USA39 (http://braincloud.jhmi.edu/)). Details of tissue acquisition, handling, processing, dissection, clinical characterization, diagnoses, neuropathological examinations, RNA extraction and quality control measures were described previously.35–36 From each subject in the brain collection, RNA from prefrontal gray matter was analyzed using spotted oligonucleotide microarrays yielding data from 30,176 gene expression probes and allowing us to focus on DDO mRNA expression (see Supplementary Information). After normalization, log2 intensity ratios were further adjusted to reduce the impact of known and unknown sources of systematic noise on gene expression measures using surrogate variable analysis.38 DNA from cerebellar tissue was studied with Illumina (Illumina Inc., San Diego, CA, USA) BeadChips producing 625,439 SNP genotypes called using the BeadExpress software for each subject as described elsewhere35 (see also Supplementary Methods). For the purpose of this study, we selected DDO mRNA expression in prefronal cortex and all SNPs (n = 92) within DDO, Two of these SNPs (rs2057149 and rs3757351) were significantly associated with DDO mRNA expression (see sections results) and were thus used for in vivo analysis with imaging tools (see below).

Imaging

All subjects participating in the imaging protocols were Caucasian healthy volunteers matched for age, gender or IQ across DDO genotype groups (Supplementary Table 2 and Table 3). All SNP associations were tested in Statistical Parametric Mapping 8.0 (http://www.fil.ion.ucl.ac.uk/spm) with random effects analyses. For gray matter volumes (n = 152), we performed voxel-based morphometry (VBM) following standardized methods (new segmentation and diffeomorphic registration algorithms) with appropriate covariates as described previously35 (Supplementary Methods). During 3T BOLD fMRI scanning, subjects (n = 143) performed a WM task (1- and 2-back) to robustly engage the dorsolateral PFC as in previous reports40 (Supplementary Methods). Random-effects analyses were performed on a priori defined regions of interest with genotype as predictor and load as the repeated measures factor. Statistical correction for multiple comparisons within the regions of interest was performed as appropriate, respectively, for VBM and fMRI data (Supplementary Methods).

RESULTS

Oral administration of D-Asp affects NMDAR-mediated miniature excitatory postsynaptic currents and basal metabolic function in fronto-hippocampal areas of adult mice

We first measured the ability of exogenous application of D-Asp to modulate isolated NMDAR currents from mPFC layer II/III pyramidal neurons, a cortical layer implicated in schizophrenia-like phenotypes in mice.41 Data obtained in C57BL/6J mice show that perfusion of D-Asp (300 μM) on mPFC slices did not affect the cumulative distribution of NMDAR components of miniature excitatory postsynaptic currents (mEPSCNMDA) amplitude (P > 0.05; Figure 1a, left panel), but reduced the inter-event
interval of mEPSC\textsuperscript{NMDA}, as observed by the leftward shift of the cumulative mEPSC\textsuperscript{NMDA} amplitude (left panel, $n=8$ for vehicle, $n=8$ for D-Asp perfused; bin size 10 pA; K-S test $P<0.001$) and inter-event interval (right panel, $n=8$ for vehicle, $n=8$ for D-Asp perfused; bin size 50 ms; K-S test $P<0.001$) recorded in mPFC layer II/III pyramidal neurons in the absence or presence of perfused D-Asp (300 μM). Representative traces of NMDAR current detected in the mPFC layer II/III pyramidal neurons before and after exogenous perfusion of D-Asp (top). Scale: vertical bar, 10 pA; horizontal bar, 1s. (b) Pooled (mean) cumulative distributions of mEPSC\textsuperscript{NMDA} amplitude (left panel, $n=9$ for H\textsubscript{2}O, $n=9$ for D-Asp; bin size 50 ms; K-S test $P>0.05$) and inter-event interval (right panel, $n=9$ for H\textsubscript{2}O, $n=9$ for D-Asp; bin size 50 ms; K-S test $P>0.001$) recorded in the mPFC layer II/III pyramidal neurons from C57BL/6J mice untreated or treated for 1 month with a 20 mM D-Asp solution. (c) Anatomical distribution of brain areas exhibiting a significant increase in bCBV in mice exposed to D-Asp with respect to vehicle controls ($P<1.7$, corrected cluster significance $P=0.001$). Activation maps (red/orange) are superimposed onto contiguous 0.75 mm MRI coronal images. a.u., arbitrary unit; DhHC, dorsal hippocampus; dPFC, dorsal prefrontal cortex; mPFC, medial prefrontal cortex; Sp, septum; vDB, ventral diagonal band.

Interestingly, voxel-wise bCBV mapping revealed region-specific foci of significantly greater bCBV in D-Asp-treated animals as compared with controls ($Z>1.6$, corrected cluster significance, $P<0.001$) that encompassed several fronto-septo-hippocampal regions, including the dorsal and mPFC, septum and dorsal hippocampus (Figure 1c). The effect was statistically significant also when expressed in terms of mean activation over predefined anatomical volume of interests ($P<0.05$, all regions, Student’s t-test; Figure 1c). No brain regions exhibited significant reductions of bCBV in D-Asp-treated animals. No significant differences in arterial p\textsubscript{CO}$_2$ (or p\textsubscript{O}$_2$) levels were observed between groups at the end of the fMRI sessions ($P>0.88$, Student’s t-test), thus allowing to rule out a contribution of unspecific vasoactive events to the differences mapped.
Oral administration of D-Asp in adult mice influences neuronal spine density, dendritic length and converts E-LTP into L-LTP in the hippocampus.

To investigate the possible structural changes associated with increased NMDAR-dependent transmission, a Golgi-Cox analysis was performed in adult D-Asp-treated mice in the same brain regions where we observed increased bCBV. Consistent with neuroimaging, pyramidal neurons of the PFC exhibited significantly increased dendritic length in D-Asp-treated mice, compared with the untreated group (H2O vs D-Asp (mean ± s.e.m.): 1564.33 ± 62.16 μm vs 2068.94 ± 49.13 μm, P < 0.01; Figure 2a). Moreover, count of dendritic intersections by Sholl analysis revealed different arborization of basal dendritic segments between D-Asp-treated mice and controls (treatment: F(1,56) = 55.438, P < 0.0001; distance from soma x treatment: F(7,56) = 6.019, P < 0.0001; Figure 2b). More in detail, the complexity of dendritic tree in D-Asp-treated group is greater between 25 and 125 μm (P < 0.01 at 25, 50, 75 and 100 μm; P < 0.05 at 125 μm). The apical dendritic complexity is also different between treatments (distance from soma x genotype: F(9,72) = 5.111, P < 0.0001; Figure 2b). In particular, increased number of dendritic segments appears between 200 and 250 μm in animals drinking D-Asp (P < 0.05). Finally, oral administration of D-Asp is also associated with increased spine density in prefrontal cortical neurons (H2O vs D-Asp (mean ± s.e.m.): 3.03 ± 0.25 spines per 10 μm vs 4.01 ± 0.25 spines per 10 μm, P < 0.05; Figure 2c).

Next, we examined dendritic architecture of pyramidal neurons in the CA1 area of the hippocampus. Morphological analysis revealed a similar trophic effect for D-Asp in this area. More specifically, we found a significant extension of dendrites in animals treated with D-Asp, compared with controls (H2O vs D-Asp (mean ± s.e.m.): 1925.80 ± 98.97 μm vs 2352.93 ± 126.97 μm, F(1,56) = 55.438, P < 0.0001; Figure 2c).
Figure 3. Dendritic morphology and late-phase LTP in Ddo+/− mice. Analysis of dendritic morphology was performed on Ddo+/+ and Ddo−/− mice in pyramidal neurons of the (a–c) PFC (n = 5 mice per genotype, 3 neurons per mouse) and (d–f) CA1 subfield of the hippocampus (n = 5 mice per genotype, 4 neurons per mouse) after Golgi-Cox staining. (a) Total dendritic length (in μm) for PFC and (d) CA1 subfield of the hippocampus. (b and e) Number of intersections between basal or apical dendrites and Sholl concentric circle lines at different distances from soma center in both (b) PFC and (e) CA1 area. Concentric circles increase in diameter by 25 μm increments. (c and f) Input–output relation shows comparable EPSPs slopes for both Ddo+/+ (n = 6) and Ddo−/− (n = 6) littermates (P > 0.05, Student’s t-test). (h) Time plot of hippocampal EPSP responses showing that (left panel) an E-LTP stimulation paradigm elicited E-LTP in Ddo+/+ mice and L-LTP in Ddo−/− mice (n = 7 for Ddo+/+, n = 7 for Ddo−/−; left panel). Hippocampal L-LTP in Ddo−/− slices was insensitive to the effects of rapamycin (transiently bath-applied for 40 min; n = 7 vehicle-treated Ddo−/− slices, n = 5 rapamycin-treated Ddo−/− slices; middle panel) but was fully prevented by cytochalasin D (continuously bath-applied; n = 7 for vehicle-treated Ddo−/− slices, n = 6 for cytochalasin D-treated Ddo−/− slices; right panel). Insets show field EPSPs from representative experiments during a baseline interval and following LTP induction (1 s, 100 Hz tetanus). Scale: vertical bar, 0.5 mV; horizontal bar, 10 ms. All data are expressed as mean ± s.e.m. LTP, long-term potentiation; PFC, prefrontal cortex.

$P < 0.05$; Figure 2d). Complexity of basal and apical dendrites is similar between treatments (Figure 2e). Density of spines is also increased in D-Asp-treated mice, compared with untreated animals (H2O vs D-Asp (mean±s.e.m.): 4.74 ± 0.25 spines per 10μm vs 5.78 ± 0.33 spines per 10μm, $P < 0.05$; Figure 2f). For further morphological analyses, see Supplementary Results and Supplementary Figure 2.

Dendritic spine density and morphology are strictly correlated with functional synaptic plasticity. Based on dendritic structural modifications associated with oral D-Asp administration, we investigated baseline synaptic transmission and LTP at hippocampal CA1 synapses of D-Asp-treated animals. No significant differences in stimulus-response curves were observed between D-Asp-treated mice and control mice ($P > 0.05$; Figure 2g). Next, we used an early-LTP (E-LTP) induction paradigm (100 Hz, 1 s) and recorded responses for 3 h. As expected, this paradigm caused decaying LTP in wild-type slices after 1 h but, strikingly, it was sufficient to induce stable L-LTP in D-Asp-treated mice (LTP at 160 min, H2O = 16 ± 7%; D-Asp = 57 ± 7%; t-test, last 10 min of recording, $P < 0.001$; Figure 2h, left panel). As this long-lasting form of LTP has been reported to be sensitive to rapamycin, we tested whether the lowered threshold for the induction of L-LTP following D-Asp oral administration could be influenced by this compound. Notably, when rapamycin was administered before the conditioning train, L-LTP still persisted (LTP at 160 min, vehicle = 57 ± 7%; rapamycin = 53 ± 4%; t-test, last 10 min of recording, $P > 0.05$; Figure 2h, middle panel). On the other hand, also rearrangements of cytoskeleton have been recently found to be crucial in L-LTP. Therefore, we tested the effect of the actin polymerization inhibitor, cytochalasin D. Remarkably, L-LTP was fully prevented in D-Asp-treated mice in the presence of this drug (LTP at 160 min, vehicle = 57 ± 7%; rapamycin = 53 ± 4%).
cytochalasin D = 4 ± 5%; t-test, last 10 min of recording, P < 0.001; Figure 2h, right panel).

Genetic inactivation of Ddo gene in mice affects spine density, dendritic length and converts E-LTP into L-LTP in the adult hippocampus.

To further evaluate the influence of deregulated high levels of D-Asp on structural and functional synaptic plasticity, we also used knockout mice for Ddo gene, that show a 10–20-fold increase in the cerebral content of D-Asp.41 In the PFC, total dendritic length of pyramidal neurons was significantly increased in Ddo−/− mice, compared with controls (Ddo+/+ vs Ddo−/− (mean ± s.e.m.): 1189.37 ± 28.63 μm vs 1537.47 ± 138.10 μm, P < 0.05; Figure 3a). Sholl analysis revealed no difference in the complexity of basal and apical dendrites between Ddo+/+ and Ddo−/− mice (Figure 3b). Conversely, dendrites from Ddo−/− pyramidal neurons have greater spine density compared with control neurons (Ddo+/+ vs Ddo−/− (mean ± s.e.m.): 2.12 ± 0.09 spines per 10 μm vs 2.76 ± 0.24 spines per 10 μm, P < 0.05; Figure 3c). We then examined dendritic architecture of pyramidal neurons in the CA1 area of the hippocampus. Dendritic length was significantly increased in Ddo−/− mice, compared with Ddo+/+ animals (Ddo+/+ vs Ddo−/− (mean ± s.e.m.): 1765.25 ± 117.58 μm vs 2219.51 ± 43.86 μm, P < 0.01; Figure 3d). Sholl analysis revealed a significantly different morphological organization of basal dendrites between genotypes (genotype: F(1,40) = 8.131, P = 0.0214; distance from soma × genotype: F(5,40) = 4.152, P = 0.0039; Figure 3e), as revealed by a greater number of intersections in Ddo−/− mice between 50 and 125 μm, compared with controls (P < 0.01 at 50 μm; P < 0.05 at 75, 100 and 125 μm). Apical intersections are not different between genotypes (Figure 3e).

Also, spine density is significantly increased in Ddo−/− mice, compared with controls (Ddo+/+ vs Ddo−/− (mean ± s.e.m.):
3.99 ± 0.16 spines per 10 μm vs 4.53 ± 0.13 spines per 10 μm, P < 0.05; Figure 3f). For further morphological analyses, see Supplementary Results and Supplementary Figure 3.

Next, we examined whether altered dendritic spine density and length might be associated with changes in synaptic function in Ddo<sup>+/−</sup> mice. Similar to D-Asp treated mice, no differences were found between the input-output curves of both genotypes (P > 0.05; Figure 3g). On the contrary, a single tetanus elicited L-LTP in wild-type slices, whereas it induced a stable L-LTP in Ddo<sup>−/−</sup> slices (LTP at 160 min, Ddo<sup>+/−</sup> = 14 ± 5%, Ddo<sup>−/−</sup> = 36 ± 5%; t-test, last 10 min of recording, P < 0.001; Figure 3h, left panel). This form of L-LTP still persisted in the presence of rapamycin (LTP at 160 min, vehicle = 36 ± 5%, rapamycin = 28 ± 5%; t-test, last 10 min of recording, P > 0.05; Figure 3h, middle panel), but not of cytochalasin D (LTP at 160 min, vehicle = 36 ± 5%, cytochalasin D = 5 ± 8%; t-test, last 10 min of recording, P < 0.001; Figure 3h, right panel).

Association of DDO genetic variants with human prefrontal DDO mRNA expression, prefrontal gray matter volume and physiology during working memory

To investigate the possible translational relevance of these results to humans, we examined the association of variation in the gene coding for the catalytic enzyme DDO with a series of hierarchically more complex prefrontal phenotypes. The phenotype most proximal to genetic variation is mRNA expression. Therefore, we investigated the association of SNPs in the DDO gene with mRNA expression in postmortem PFC of healthy human subjects (n = 268). Separate analyses of variance on mRNA expression as the dependent variable, and 92 SNPs genotypes as predictors indicate that two intronic SNPs within DDO are significantly associated with DDO mRNA expression, that is, rs2057149 (A/G) and rs3757351 (C/T) (respectively, F<sub>2,265</sub> = 9.6, P = 0.01 and F<sub>2,265</sub> = 11.3, P = 0.003, after Bonferroni correction for the number of independent comparisons). In particular, reduced DDO mRNA expression is predicted by rs2057149 AA (Fisher’s post hoc vs AG P = 0.00005; vs GG P = 0.02) and rs3757351 CC (Fisher’s post hoc vs CT P = 0.06; vs TT P = 0.00003; Figure 4a) genotypes.

We further investigated the in vivo association of rs2057149 and rs3757351 with prefrontal gray matter volume. Results in healthy humans indicate a main effect of rs3757351 on prefrontal gray matter volume (Montreal Neurological Institute, MNI: x = 44, y = 41, z = 33, k = 39, Z = 3.04, P = 0.03, after nonstationary cluster extent correction; Figure 4b). In particular, CC and CT genotypes predicted greater prefrontal gray matter volume compared with the TT genotype (Fisher’s post hoc, all P < 0.005). rs2057149 was not significantly associated with prefrontal gray matter volume.

Then, we examined the association of rs2057149 and rs3757351 with BOLD fMRI prefrontal activity of healthy humans during performance of the 1- and 2-Back WM task. There was no effect of genotype or genotype by load interaction on behavioral performance (all P > 0.2). Thus, the effect of genotype on brain responses during WM processing in this sample reflects how the brain processed WM and not how individuals scored on the task. Imaging results revealed a main effect of rs3757351 on prefrontal activity (MNI: x = 36, y = 46, z = 24, k = 80, Z = 3.35, P = 0.04, after family wise error correction for multiple comparisons, Figure 4c). Post hoc analysis on BOLD response extracted from this cluster indicated that subjects with the CC and CT genotypes have greater prefrontal activity when compared with TT individuals (Fisher’s post hoc P = 0.02 and P = 0.05, respectively; Figure 4c). No rs3757351 by load interaction was present. Furthermore, no significant association of rs2057149 with prefrontal activity was found.

DISCUSSION

In the present work, we demonstrate that short-term administration of D-Asp, an amino acid enriched in the embryo brain, increases the frequency of NMDA-mediated mEPSCs in adulthood. The enhancement of NMDAR-dependent transmission in D-Asp-treated mice is mirrored by greater basal metabolic activity, increased dendritic arborization and spine density, and facilitated induction of late-phase LTP. NMDAR signaling is implicated in changes of dendritic spine morphology and in induction of persistent forms of plasticity. Given the agonistic property of D-Asp on NMDARs, the effects we demonstrate on structural and functional synaptic dynamics in Ddo<sup>+/−</sup> and D-Asp-treated mice are likely to occur through the activation of NMDARs.

Increased dendritic length and arborization in mice with higher levels of D-Asp is consistent with the severe structural defects in dendritic length and branch number found in the brain of a mouse model with local depletion of D-Asp. Remarkably, in serine racemase-deficient mice, reduction in D-Ser levels that results in NMDAR hypofunction, causes similar morphological defects, as well as alterations in mEPSCs and synaptic plasticity, paralleled by reduced mTOR signaling. Based on the evidence that chronic administration of rapamycin produces a comparable reduction of dendritic length and complexity in both Ddo<sup>+/−</sup> and Ddo<sup>−/−</sup> mice (see Supplementary Results and Supplementary Figure 4), we cannot rule out that the plastic changes associated to higher D-Asp levels may occur through the activation of mTOR pathway. Previous evidence indicates that storage of long-term memories is likely dependent on enhancement of long-term synaptic plasticity. Thus, facilitation of L-LTP induced by higher levels of D-Asp is coherent with the improvement of cognitive abilities previously found in D-Asp-treated and Ddo<sup>−/−</sup> mice.

Our results in humans provide further translational validity to these animal models. Variation in the DDO gene predicts mRNA levels in prefrontal postmortem tissue, prefrontal gray matter and prefrontal activity during WM processing. More in detail, our findings indicate that the A allele of rs2057149 and the C allele of rs3757351 predicts lower DDO mRNA expression relative to rs2057149 G and rs3757351 T alleles, respectively. This association suggests a functional role of these SNPs on modulation of prefrontal DDO levels, which may reflect on D-Asp tone (that is, differential catalobolism of D-Asp as a function of DDO genetic variation). Consistent with this contention, we found that DDO rs3757351 is also associated with in vivo prefrontal phenotypes in healthy humans. In particular, our results indicate that the C allele of rs3757351 also predicts greater prefrontal gray matter volume and activity during WM processing relative to the T allele. Thus, genetically mediated reduced expression of DDO is mapped on prefrontal phenotypes suggestive of greater prefrontal neuronal plasticity and greater activation of prefrontal neuronal networks during WM.

Previous results have indicated lower levels of D-Asp in the PFC of patients with schizophrenia. Furthermore, converging evidence suggests the possible involvement of NMDAR-dependent signaling in the pathophysiology of this brain disorder, in which prefrontal dysfunction is crucially implicated. This knowledge and the findings of the present study call for further investigation of the relationship between schizophrenia and D-Asp as well as of the potential relevance of this D-amino acid as a target for treatment of schizophrenia.

CONFLICT OF INTEREST

All is a full-time employee of Hoffman-La Roche, Ltd. The remaining authors declare no conflict of interest.
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