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Research report

Behavioural alterations in male mice lacking the gene for D-aspartate oxidase

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Abstract

D-serine and D-aspartate are important regulators of mammalian physiology. D-aspartate is found in nervous and endocrine tissue, specifically in hypothalamic supraoptic and paraventricular nuclei, pituitary, and adrenal medullary cells. Endogenous D-aspartate is selectively degraded by D-aspartate oxidase. We previously reported that adult male mice lacking the gene for D-aspartate oxidase (*Ddo*$^{-/-}$ mice*)* display elevated concentrations of D-aspartate in several neuronal and neuroendocrine tissues as well as impaired sexual performance and altered autogrooming behaviour. In the present study, we analyzed behaviours relevant to affect, cognition, and motor control in *Ddo*$^{-/-}$ mice. *Ddo*$^{-/-}$ mice display deficits in sensorimotor gating and motor coordination as well as reduced immobility in the forced swim test. Basal corticosterone concentrations are elevated. The *Ddo*$^{-/-}$ mice have D-aspartate immunoreactive cells in the cerebellum and adrenal glands that are not observed in the wild-type mice. However, no differences in anxiety-like behaviour are detected in open field or light–dark preference tests. Also, *Ddo*$^{-/-}$ mice do not differ from wild-type mice in either passive avoidance or spontaneous alternation tasks. Although many of these behavioural deficits may be due to the lack of Ddo during development, our results are consistent with the widespread distribution of D-aspartate and the hypothesis that endogenous D-aspartate serves diverse behavioural functions.

Keywords: D-aspartate; D-aspartate oxidase; D-amino acids; Knockout mice; Behavioural phenotyping; Prepulse inhibition; RotoRod

1. Introduction

Amino acids come in two mirror-image forms (D and L) termed enantiomers. Traditionally, D-enantiomer amino acids were considered important only in bacterial physiology. In the first half of the 20th century, enzymes were identified for the degradation of D-amino acids in vertebrates [19,30]. However, because of the doctrine that D-amino acids do not exist in vertebrate tissues, these enzymes were generally considered anomalous or possibly involved in the degradation of dietary D-amino acids.

In recent years, however, two D-amino acids (D-serine and D-aspartate) have been identified as important regulators of mammalian physiology. D-serine is formed from L-serine by a serine racemase [34] and is likely metabolized by D-amino acid oxidase [14]. D-serine is expressed in glial cells and appears to be an endogenous ligand for the regulatory glycine site on the NMDA (*N*-methyl D-aspartate) receptor [22].

D-aspartate was originally localized in the brains and peripheral ganglia of marine cephalopods and gastropods [7,8]. Subsequently, D-aspartate was identified in nervous and endocrine tissue of rodents and humans [12]. Immunohistochemical analyses localize cellular D-aspartate in hypothalamic supraoptic and paraventricular nuclei, the pituitary, and adrenal medullary cells [29]. Endogenous D-aspartate is selectively degraded by D-aspartate oxidase as evidenced by the increased concentrations of the D-amino acid in mice lacking the gene for D-aspartate

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oxidase [16]. In addition, there is reciprocal expression of D-aspartate and D-aspartate oxidase in the rat brain; i.e., areas rich in D-aspartate or D-aspartate oxidase do not express high levels of the oxidative enzyme or the D-aspartate substrate, respectively [29]. D-aspartate regulates neuroendocrine function particularly at the level of hypothalamic–pituitary interface [9,32].

Recently, we reported that adult male mice lacking the gene for D-aspartate oxidase (*Ddo*$^{-/-}$ mice*)* show significantly elevated concentrations of D-aspartate in several neuronal and neuroendocrine tissues [16]. In assessing D-aspartate immunoreactivity in the *Ddo*$^{-/-}$ mice we have observed staining in several brain regions including the cerebellum and hippocampus as well as immunoreactivity in peripheral glands [16,29]. Ddo-deficient mice displayed impaired sexual performance and altered autogrooming behaviour as well as increased body weight, potentially reflecting altered pituitary melanocortin function [16]. D-Amino acids are now recognized as playing an important role in neural and neuroendocrine function [2]. In the present study, we analyzed behaviours relevant to affect, cognition, and motor control in the *Ddo*$^{-/-}$ mice to better elucidate the role of endogenous D-aspartate.

2. Methods

2.1. Animals

D-aspartate oxidase transgenic (*Ddo*$^{-/-}$) mice were produced on a C57BL/6 background as previously described [16]. Wild-type littermates served as controls, $n = 8$/genotype. All animals were group-housed in polycarbonate cages (27.8 cm × 7.5 cm × 13 cm) in colony rooms held under constant temperature (21 ± 4 °C) and relative humidity (50 ± 10%), and provided ad libitum access to food (Harlan Teklad 8640 Rodent Diet, Indianapolis, IN) and filtered tap water. The colony rooms were maintained on a 14:10 light:dark cycle; lights on at 0100 h Eastern Standard Time (EST). All behavioural testing was conducted during the early portion of the dark period. The Ohio State University Institutional Lab Animal Care and Use Committee approved all animal protocols in accordance with National Institutes of Health guidelines.

2.2. Acoustic startle/prepulse inhibition

The acoustic startle reflex to a strong stimulus can be inhibited by the presentation of a weaker stimulus immediately preceding it [3]. Startle reactivity was measured using a single startle chamber (SR-Lab, San Diego Instruments, San Diego, CA). The chamber consisted of a clear nonrestrictive Plexiglas cylinder resting on a platform inside a ventilated chamber. A high-frequency loudspeaker inside the chamber produced both a continuous background noise of 65 dB and the various acoustic stimuli. Vibrations of the Plexiglas cylinder caused by the whole-body startle response of the animal were transduced into analogue signals by a piezoelectric unit attached to the platform. The signals were then digitized and stored by the computer. Sixty-five readings were taken at 1 ms intervals, starting at stimulus onset, and the average amplitude was used to determine the acoustic startle response. The mice were placed into the startle chambers immediately upon entering the behaviour room, and a 65 dB background noise level was presented for a 10 min acclimation period and continued throughout the test session. All pre-pulse inhibition (PPI) test sessions consisted of startle trials (pulse alone), prepulse trials (prepulse + pulse), and no-stimulus trials (no-stim). The pulse alone trial consisted of a 40 ms 120 dB pulse of broad-band noise. PPI was measured by prepulse + pulse trials that consisted of a 20 ms noise prepulse, 100 ms delay, then a 40 ms 120 dB startle pulse. The acoustic prepulse intensities were 69, 73, and 81 dB. The no-stimulus trial consisted of background noise only. The test session began and ended with five presentations of the pulse alone trial; in between, each trial type was presented 10 times in pseudorandom order. There was an average of 15 s (range 12–30 s) between trials. Percent of PPI was determined by dividing the startle response from each prepulse trial by the mean startle from pulse only trials.

2.3. Porsolt swim test

The Porsolt test is a standard assay of depressive-like behaviours. The principal dependent measure is the time spent swimming versus the time spent floating in a cylinder full of water [5,25,26]. Increased time floating is taken as indicative of a 'depressive-like' state. Mice were transferred to a darkened procedure room under photographic red light. After a short acclimation to the room each mouse was placed into a bucket (white, 35.5 cm in diameter) filled 15 cm with 26 °C water. All sessions were videotaped and scored offline by an observer unaware of the genotype of the mouse. Tapes were scored for time spent immobile and time spent swimming. Immobility was operationally defined as floating in the water without struggling or making small movements necessary to keep the head above water. Swimming was defined as active movements by the limbs and tail to an extent greater than necessary to keep the head above water.

2.4. Light–dark preference test

The light–dark preference test like the open-field test measure the conflict between the tendency mice have to explore a novel environment versus the aversive qualities of an open lit space [4,5]. Greater amounts of time spent in the dark side of the apparatus are indicative of increases in 'anxiety-like' behaviour. All mice were examined for their light–dark preference in a light–dark apparatus that was a 90 cm × 48 cm × 14 cm enclosure made of white plastic. Half of the box was lined with black cardboard and covered externally with thick black plastic to block light, whereas the other half of the box was illuminated with both room light and two 60 W bulbs. Mice were placed in the centre of the open side facing the entrance to the covered side. Sessions were 5 min, and behaviour was scored live by an experimenter blind to the genotype of the experimental animals. Latency to enter the enclosed half, time spent in the dark, and total number of transitions between the two sides served as the dependent measures. The apparatus was cleaned with 70% ethanol between trials.

2.5. Open field

The open-field task also measures the outcome of a conflict based on the tendency to explore a novel space versus remaining in a safe environment. In this task greater amount of time spent in close apposition to the sides of the arena is associated with greater 'anxiety-like' behaviour. The open-field test chamber was enclosed in a sound and light attenuating cabinet that consisted of a 60 cm^3 clear Plexiglas arena with corncob bedding at the bottom. The arena was surrounded by a series of infrared lights that tracked the movement of the mouse in three-dimensions. Each mouse was tested for 30 min during the dark cycle. The test chamber was rinsed thoroughly with a 70% ethanol solution and the bedding changed between each test. The results were generated online by the PAS software package (San Diego Instruments, San Diego, CA). The total locomotor activity (number of beam breaks), percentage of time spent in the periphery versus the centre of the arena, and the total number of rears served as the dependent measures.

2.6. Hot plate

Pain reactivity was assessed in a glass cylinder (16 cm high, 16 cm diameter) that was used to keep the mice on the heated surface of a plate (Cold/Hotplate Analgesia Meter; Columbus Instruments, Columbus, OH, USA), kept at a temperature of 55 °C using a thermoregulated water circulating pump. The latency to jump from the hotplate was evaluated, and a cut-off of 240 s was used to prevent tissue damage.

2.7. Paw preference

To assess paw preference individual mice were placed inside a clear plastic cylinder (8 cm internal diameter, 12 cm height) for 5 min and videotaped. The experimenter recorded each time the mouse placed its paw or paws on the side

of the cylinder, noting whether the left or right paw touched first. Right paw preference was determined using the formula [right/(left + right + simultaneous)] × 100.

2.8. Passive avoidance and spontaneous alternation

Mice were tested for cued and contextual conditioning memory using passive avoidance. On day 1 of testing, mice were placed in one chamber of a dark two-chambered box separated by a door (San Diego Instruments). After 30 s of acclimation, a house light in the chamber in which the mouse resided was illuminated, and the door was opened. To escape the light, mice entered the other chamber (which remained dark) where they received a 0.06 mA electrical shock (1 s duration) from the grid floor. Mice were then returned to their home cage. On day 2, the same procedure was followed, except the shock was removed. Animals that did not enter the other dark chamber were assigned a latency of 300 ms.

Spontaneous alternation behaviour was recorded to assess working memory performance with a spatial component during the dark phase. The maze was made of black Plexiglas. Each arm was 35 cm and converged at an equal angle. Mice were placed in one arm and allowed to move freely through the maze during a 3 min session. The series of arm entries was recorded. An alternation was defined as entries into all three arms on consecutive occasions. The percentage of alternation was calculated using the following formula: (actual alternations/maximum possible alternations) × 100. The maximum possible number of alternations was calculated as the total number of arm entries −2.

2.9. Motor testing

Motor skills were assessed on a RotoRod mouse treadmill (San Diego Instruments, San Diego, CA). Mice were habituated to the RotoRod for 60 s the night before testing began. Three trials at each of three successively increasing speeds (20, 28, and 32 rpm) were conducted, and the latency to fall from the rotating treadmill was recorded.

2.10. HPA activity

To assess HPA function, mice were lightly anesthetized with isoflurane vapours, and blood (150 μl) was drawn from the retroorbital sinus. Samples were drawn within 2 min of touching the cage. Two days later, all animals were restrained in adequately ventilated clear polypropylene restrainers (50-ml conical tubes measuring 9.7 cm in length and internal diameter of 2.8 cm) for 2 h. Immediately following the restraint stressor, animals were anesthetized, and a second sample was taken. All samples were taken between 0700 and 0900 h EST. Samples were allowed to clot for at least 30 min, the clot was removed, and the blood was spun at 3000 rpm for 30 min at 4 °C. Corticosterone concentrations were determined by radioimmunoassay The RIA was conducted in a single assay following the guidelines in the MP Biomedicals (Costa Mesa, CA) ^{125}I double-antibody kit instructions. The intra-assay coefficient of variance was <10%. This kit is highly specific and cross-reacts with other steroids less than 1% and the minimum detection thresholds was 5 ng/ml.

2.11. Immunohistochemistry

D-aspartate immunohistochemistry was conducted as previously described [16,29]. Mice were perfused 5% glutaraldehyde/0.5% paraformaldehyde at 37 °C. Brains and adrenal glands were post-fixed for 2 h and cryoprotected. Free-floating sections (45-μm) were quenched with 0.1% H_2O_2 in methanol/PBS, reduced with 0.5% $NaBH_4$, blocked (4% NGS and 0.2% Triton X-100 in TBS), and incubated with a D-asp antibody (1:750) overnight (4 °C) in the presence of L-aspartate-glutaraldehyde conjugate (200 μM). Labeling was visualized with the Vectastain Elite kit (Vector Laboratories, Burlingame, CA). Specificity was determined by abolishing signal with D-aspartate-glutaraldehyde conjugate (200 μM).

2.12. Statistical analyses

Porsolt forced swim, paw preference, open field, and hot plate data were analyzed with a one way analysis of variance (ANOVA) for genotype. Prepulse inhibition data were analyzed with a two factor repeated measures ANOVA with genotype as a between subject variable and prepulse amplitude as a within subject variable. Similarly, RotoRod latencies were log transformed and analyzed with a repeated measures ANOVA with genotype as a between subjects variable and rotation speed as a within subjects variable. We previously reported genotype differences in body weight as such we included body weight as a covariate in initial statistical analyses. As no significant effects of body weight on the behavioural measures were detected it was excluded from final the reported analyses. All mean differences were considered statistically significant if $p < 0.05$.

3. Results

3.1. Acoustic startle

Ddo$^{-/-}$ mice displayed significant deficits in prepulse inhibition, although startle responsivity did not differ between genotypes ($p > 0.05$, data not shown). There was a significant main effect of tone intensity ($F_{2,15} = 155.67$, $p < 0.001$) (Fig. 1) and an interaction between genotype and prepulse intensity ($F_{2,15} = 18.079$, $p < 0.001$). *Ddo*$^{-/-}$ mice had significantly less prepulse inhibition at each of the two high prepulse intensities.

3.2. Porsolt forced swim test

There were no genotype differences in the latency to the first bout of floating or in the total numbers of floating bouts. However, wild-type mice spent significantly more time floating than the *Ddo*$^{-/-}$ mice ($F_{1,15} = 6.896$, $p < 0.05$, see Fig. 2).

3.3. Light–dark, open field, hot-plate, paw preference, passive avoidance and spontaneous alternation

Light–dark preference behaviours did not differ between genotypes. *Ddo*$^{-/-}$ and wild-type animals had similar latencies to enter the dark half, time spent in the dark, and number of light–dark transitions ($p > 0.05$, see Table 1). Similarly open field behaviour including total activity rears, or central tendency did not differ between the two genotypes. There were no differences in thermal hyperalgesia as indicated by the hot plate test. The two genotypes did not differ in the direction or magnitude of paw preference. No differences were evident in the

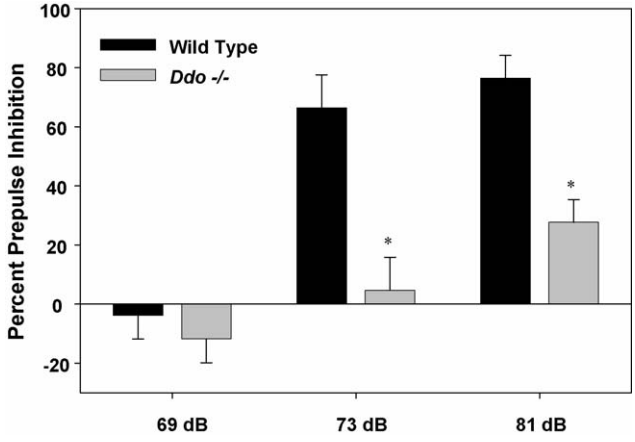

Fig. 1. *Ddo*$^{-/-}$ mice display significant deficits in prepulse inhibition of the acoustic startle reflex. Data are presented as mean ±S.E.M.; $^{*}p < 0.05$.

Table 1
Behavioural phenotypic comparison between wild-type and *Ddo*$^{-/-}$ mice

Test	Behaviour	Wild-type	*Ddo*$^{-/-}$
Light/dark preference	Latency to enter dark (s)	45.625 ± 16.10	69.375 ± 19.75
	Light/dark transitions	8.625 ± 1.91	8.625 ± 1.22
	Time in dark (s)	208.75 ± 17.38	191.25 ± 19.65
Open field	Total activity (beam breaks)	3944.25 ± 329.56	3442.125 ± 194.89
	Rears	181.625 ± 24.83	142.125 ± 17.99
	Percentage of beam breaks in the centre	14.20 ± 1.57	15.15 ± 1.00
Hot plate	Latency to respond (s)	8.41 ± 1.10	9.93 ± 1.19
Paw preference	Total rears	21.13 ± 1.44	21.13 ± 2.86
	Percent right paw use	42.83 ± 6.04	49.58 ± 10.48
Passive avoidance	Day 1 latency (s)	7.03 ± 1.58	6.89 ± 0.79
	Day 2 latency (s)	94 ± 46.79	178.66 ± 49.95
Spontaneous alternation	Total alternations	13.5 ± 2.04	15.63 ± 2.56
	Total correct	7.5 ± 1.31	9.38 ± 1.56
	Percent correct	53.6 ± 5.30	61.7 ± 4.02

passive avoidance or spontaneous alternation tests of learning and memory.

3.4. RotoRod motor testing

Ddo$^{-/-}$ mice were significantly impaired in their RotoRod performance. There was a significant effect of increasing rotation speed on the latency to fall ($F_{2,15} = 11.523$, $p < 0.001$, see Fig. 3) and an interaction between rotation speed and genotype ($F_{2,15} = 5.543$, $p < 0.05$). Post hoc analyses indicated that the *Ddo*$^{-/-}$ mice displayed shorter latencies to fall at each of the two higher speeds.

3.5. Basal and restraint-evoked corticosterone concentrations

Basal corticosterone concentrations were elevated in *Ddo*$^{-/-}$ as compared to wild-type mice ($F_{1,14} = 6.580$, $p < 0.05$, Fig. 4). One hour of restraint increased circulating corticosterone in both groups. However, there were no differences in the restraint-

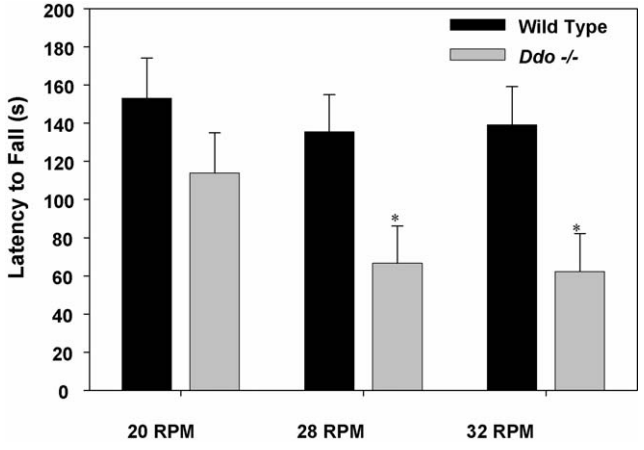

Fig. 3. *Ddo*$^{-/-}$ mice have motor deficits as indicated by shorter latency to fall from the RotoRod. Data are presented as mean ±S.E.M.; $^{*}p < 0.05$. RPM: revolutions per minute.

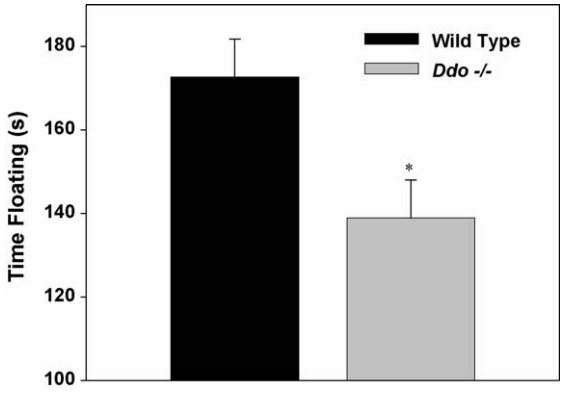

Fig. 2. Transgenic mice lacking the gene for D-aspartate oxidase spend significantly less time immobile in the Porsolt forced swim test. Data are presented as mean ±S.E.M.; $^{*}p < 0.05$.

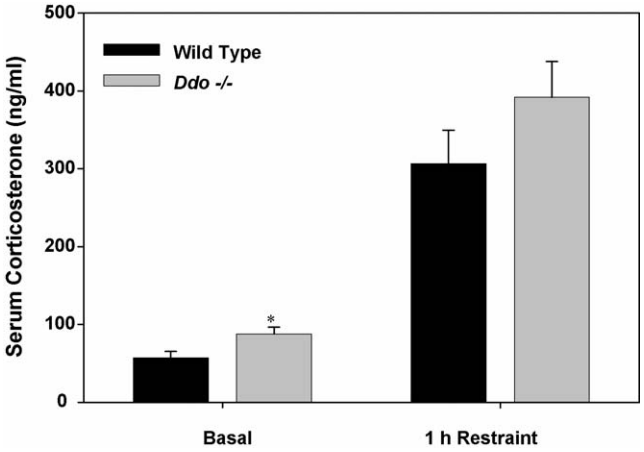

Fig. 4. *Ddo*$^{-/-}$ mice have higher basal, but not restraint-induced, corticosterone concentrations. Data are presented as mean ±S.E.M.; $^{*}p < 0.05$.

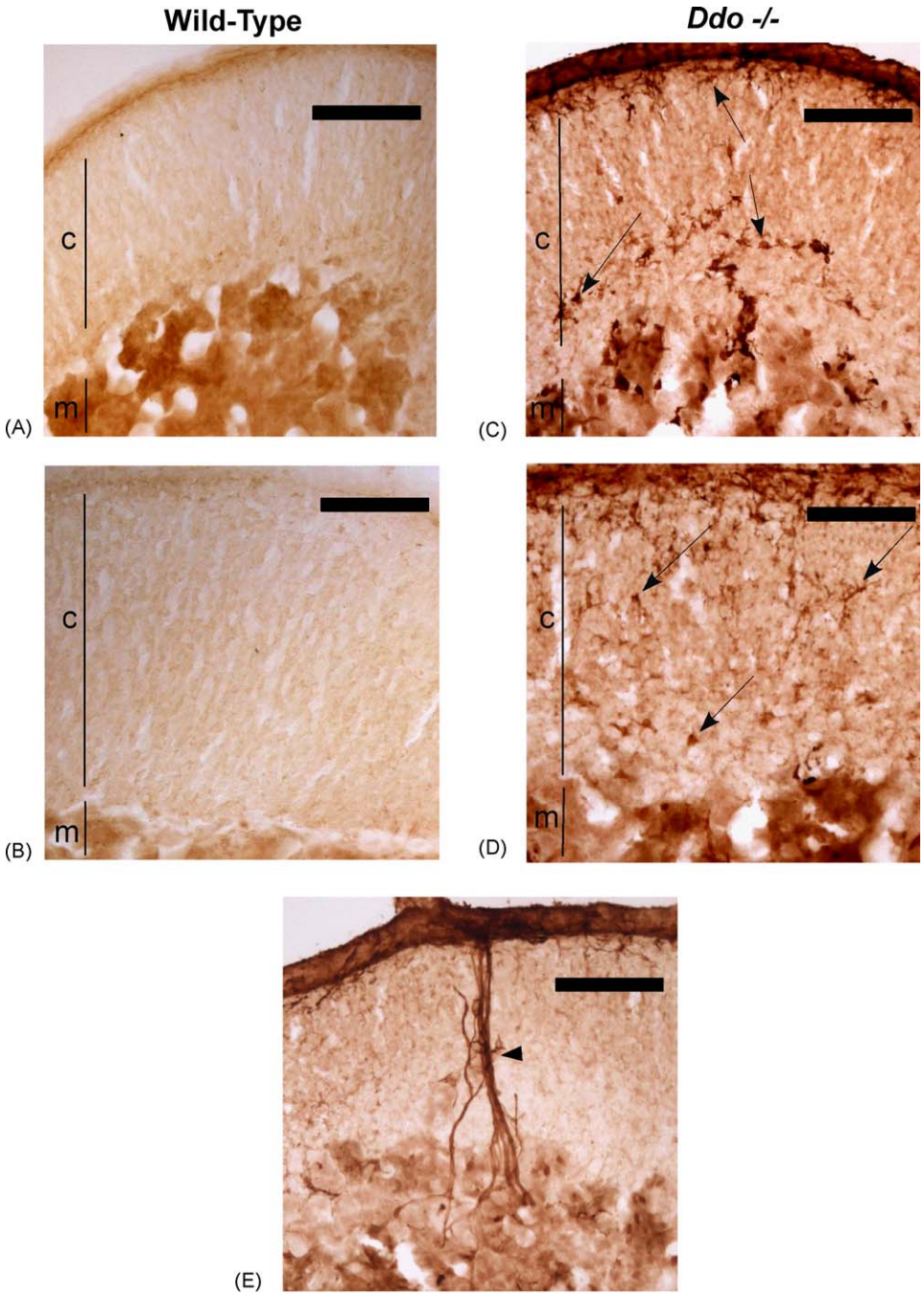

Fig. 5. D-aspartate immunohistochemistry in wild-type and *Ddo*$^{-/-}$ adrenal glands. In WT mice, there is minimal D-aspartate immunoreactivity in adrenal cortex (A and B). In *Ddo*$^{-/-}$ mice, there are immunoreactive cells throughout the cortex (C and D, arrows) and also reactivity in vasculature traversing the cortex (E, arrowhead). Scale bars = 100 μm; c: cortex; m: medulla.

evoked concentrations of corticosterone between genotypes as measured by either absolute or relative values.

3.6. Immunohistochemistry

The adrenal cortex of WT mice was free of D-aspartate immunoreactivity. However, the *Ddo*$^{-/-}$ mice exhibited cellular staining in the cortex as well as reactivity in vasculature traversing the adrenal cortex (Fig. 5). In the cerebellum, Bergmann glia surrounding Purkinje cells and extending into the molecular layer were positive for D-aspartate in the *Ddo*$^{-/-}$ mice but not the wild-types (Fig. 6A and B). In both WT and *Ddo*$^{-/-}$ mice, large deep cerebellar nuclear cells were D-asp positive. However, a population of small D-asp positive cells (Fig. 6B and D) also appeared throughout just the *Ddo*$^{-/-}$ cerebellum which may be GABAergic interneurons.

4. Discussion

Ddo$^{-/-}$ mice exhibited reduced immobility in the forced swim test, deficits in sensorimotor gating, motor coordination,

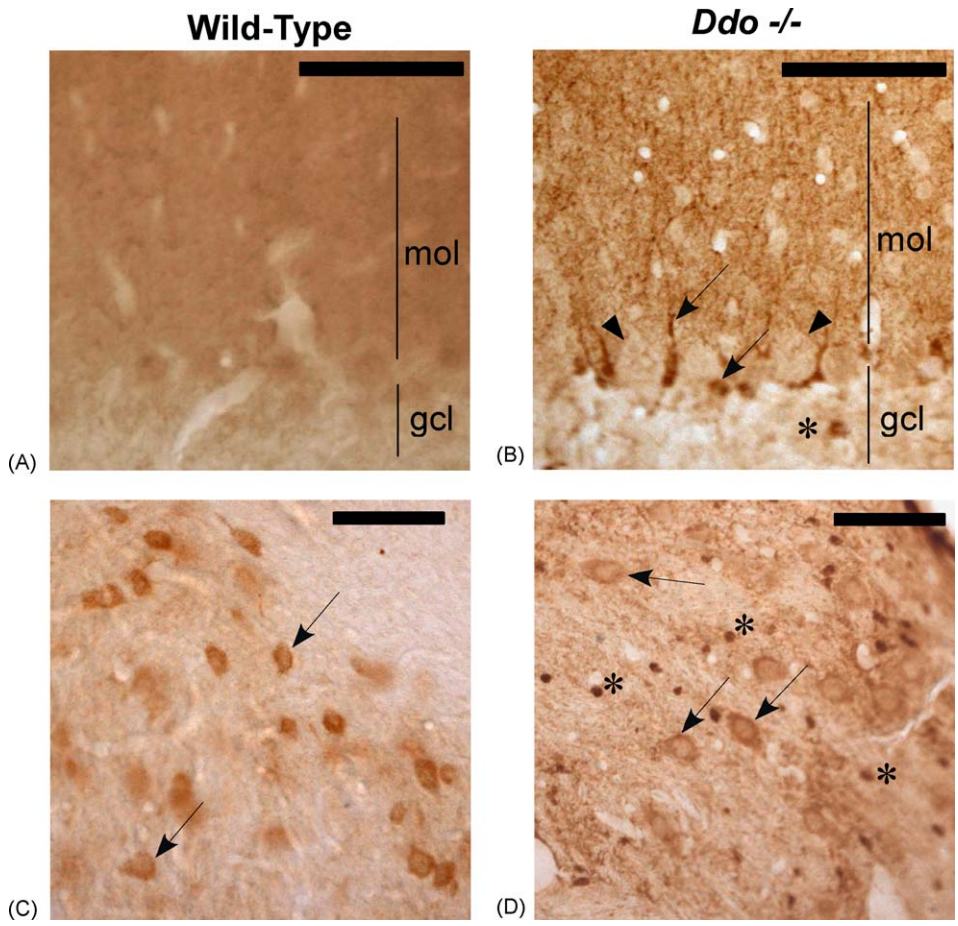

Fig. 6. D-aspartate immunohistochemistry in wild-type and *Ddo*$^{-/-}$ cerebellum. In WT mice cerebellum, D-aspartate appears diffusely throughout the molecular layer (A). In *Ddo*$^{-/-}$ mice cerebellum, immunoreactive Bergmann glia (B; arrows) surround Purkinje cell bodies (arrowheads). The Purkinje cells do not stain for D-aspartate. In both genotypes, deep cerebellar nuclear neurons are positive for D-aspartate (C and D; arrows). Throughout the *Ddo*$^{-/-}$ cerebellum, small cells immunoreactive for D-aspartate appear (B and D; asterisks) which may be GABAergic interneurons. Scale bars = 100 μm; ml: molecular layer; gcl: granule cell layer.

as. The knockout mice also displayed elevated basal corticosterone concentrations. However, no differences in anxiety-like behaviour were detected in *Ddo*$^{-/-}$ mice in the open field or light–dark preference tests in the present study. In addition, *Ddo*$^{-/-}$ mice did not differ from wild-type in either the passive avoidance or spontaneous alternation tasks.

In terms of affective behaviours, *Ddo*$^{-/-}$ mice did not exhibit increased anxiety-like behaviours in the open field or light–dark preference tests. These results are consistent with our previous data indicating no differences in the elevated plus maze [16]. However, *Ddo*$^{-/-}$ mice reduced immobility time in the Porsolt forced-swim test. The increased swimming behaviour is unlikely to reflect a secondary elevation in locomotor activity because no differences in total locomotor activity or total arm entries in the plus maze were observed. Considered together, these results indicate that the genetic ablation of *Ddo* had a specific anti-depressant action, but no effect on anxiety-like behaviours. Further work is needed to confirm this finding with other tests of depressive-like behaviours and to identify the underlying neural substrates although altered NMDA receptor activity is certainly a candidate.

Ddo$^{-/-}$ deficient mice displayed significant deficits in prepulse inhibition. Prepulse inhibition (PPI) is a test of sensorimotor gating wherein the unconditioned response to an auditory startle is reduced by the presentation of a weaker stimulus immediately preceding it [15]. PPI is impaired in schizophrenics and is used as a model for the attentional processing deficits in that disorder [31]. One potential mechanism for this effect is the interaction of endogenous D-aspartate with NMDA receptors. D-aspartate can activate NMDA receptors, albeit with significantly less affinity than glutamate [23]. However, in wild-type animals endogenous Ddo appears to degrade D-aspartate in areas with rich NMDA receptor density such as the brain and adrenals [29]. D-aspartate can also be methylated to form *N*-methyl-D-aspartate (NMDA) and then activate NMDA receptors with nearly 100-fold greater affinity [6]. Most of the work on NMDA-mediation of prepulse inhibition has focused on the deleterious effects of NMDA blockade (e.g. [18,21]). However, NMDA-activation of the ventral hippocampus also reduces prepulse inhibition [1]. Taken together, this would suggest that the excess D-aspartate in the *Ddo*$^{-/-}$ mice may inappropriately activate NMDA receptors and thus interfere with sensorimotor gating. Other types of hippocampal dysfunction are certainly possible in these mice as evidenced by the pattern of D-aspartate staining in the CA1/CA2 regions [12]. However, no deficits were observed in the spontaneous alternation or passive avoidance tasks. Additional assess-

ments of hippocampal-dependent behaviours in *Ddo*$^{-/-}$ mice may be warranted.

The *Ddo*$^{-/-}$ mice were significantly impaired in RotoRod performance. The cerebellum is an important neural structure for RotoRod performance; rats cerebellectomized early in life display deficits in motor coordination in adulthood [35], and "lurcher" mice that lack Purkinje cells and granule cells in the cerebellar cortex also display motor learning deficits [20]. D-aspartate and Ddo are expressed reciprocally in the rat cerebellum with D-aspartate immunoreactivity in the molecular layer and Ddo expression in the granule cell layer [29]. In cerebella of both wild-type and *Ddo*$^{-/-}$ mice D-aspartate immunoreactivity is observed in the deep cerebellar nuclear cells. In addition, *Ddo*$^{-/-}$ mice have small cells which may be interneurons that are also D-aspartate positive. In the cerebellar cortex, *Ddo*$^{-/-}$ mice also have D-aspartate staining in Bergmann glia that does not occur in WT animals. Bergmann glia are specialized cerebellar astroglial cells that wrap their processes around the somata, dendrites and synaptic connections of Purkinje cells [13,24]. The Bergmann glia are important in maintaining the synaptic microenvironment and also serve to regulate glutamatergic and GABAergic transmission within the cerebellum [17,28] Taken together this suggests that motor deficits observed in the *Ddo*$^{-/-}$ mice are due to alterations in cerebellar physiology.

As reported previously, *Ddo*$^{-/-}$ mice exhibited significant alterations in mating behaviour as well as an increase in body weight when compared to wild-type [16]. These behavioural changes and the dense D-aspartate staining in the intermediate lobe of the pituitary suggested alterations in the melanocortin system. *Ddo*$^{-/-}$ mouse pituitary expresses less proopiomelanocortin (POMC) and produce less alpha-melanocyte stimulating hormone (α-MSH) than wild-type animals, although POMC neurons in the arcuate nucleus appear unaltered [16]. Another potential physiological mediator of the alterations in energy balance and mating behaviour is the HPA axis. Glucocorticoids can alter energy balance and also interact with the central melanocortin system [11] and may be involved in mediating the negative effects of chronic stress on sexual behaviour [27]. The *Ddo*$^{-/-}$ mice in this experiment displayed elevated basal corticosterone concentrations, but corticosterone concentrations after one hour of restraint (a mild stressor) did not differ between the genotypes. The effects of D-aspartate on the HPA axis are likely to be indirect. The transgenic animals have increased D-aspartate immunoreactivity in the paraventricular nucleus, but the cells appear to be part of the magnocellular system, rather than parvocellular corticotrophin releasing hormone cells. D-aspartate staining is present in the adrenal glands of rats but is generally restricted to the epinephrine-producing cells in the medulla [16,29]. In WT mice we observed little staining in any part of the adrenal, however the *Ddo*$^{-/-}$ mice show cellular and vascular staining in both the medullary and cortical regions. It is unlikely that the reductions in pituitary α-MSH underlie the increase in basal corticosterone because melanocortins tend to have positive effects on the HPA axis [10].

Although precise mechanisms underlying D-aspartate mode of action remain lacking, it is possible that D-aspartate plays a role in normal development. Concentrations of D-aspartate are highest in embryonic animals and decline steadily with age [33]. In particular, dense staining occurs at the level of the cortical plate and the subventricular zones [33] in embryonic neural tissue. Mice lacking Ddo fail to show the normal decline in D-aspartate concentrations with increasing age [16]. This would suggest that D-aspartate has a role in mediating normal development and that disruption of D-aspartate metabolism throughout life may be responsible for some of the behavioural results reported in our studies.

In conclusion, we report that the behavioural phenotype of the *Ddo*$^{-/-}$ mice is characterized by deficits in prepulse inhibition of the acoustic startle reflex and in motor coordination on the RotoRod. In addition, these mice reduce floating behaviour in the forced swim test and display elevated basal corticosterone concentrations. However, in tests of working memory and anxiety *Ddo*$^{-/-}$ mice do not differ from wild-type mice. Mice deficient in D-aspartate oxidase have dramatic increases in D-aspartate concentrations both during development and in adulthood. Taken together, this suggests that dysregulation of D-aspartate metabolism in these mice have important neurobehavioral consequences. Future studies should examine the precise mechanism and developmental timing underlying the effects of D-aspartate on behaviour.

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