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Behavioral and biochemical characterization of a mutant mouse strain lacking D-amino acid oxidase activity and its implications for schizophrenia

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D-Amino acid oxidase (DAO) degrades D-serine, a co-agonist at the NMDA receptor (NMDAR). Hypofunction of the NMDAR has been suggested to contribute to the pathophysiology of schizophrenia. Intriguingly, DAO has been recently identified as a risk factor for schizophrenia through genetic association studies. A naturally occurring mouse strain (ddY/DAO$^-$) has been identified which lacks DAO activity. We have characterized this strain both behaviorally and biochemically to evaluate DAO as a target for schizophrenia. We have confirmed that this strain lacks DAO activity and shown for the first time it has increased occupancy of the NMDAR glycine site due to elevated extracellular D-serine levels and has enhanced NMDAR function in vivo. Furthermore, the ddY/DAO$^-$ strain displays behaviors which suggest that it will be a useful tool for evaluation of the clinical benefit of DAO inhibition in schizophrenia.

Introduction

N-methyl-D-aspartate receptors (NMDAR) are expressed at excitatory synapses throughout the central nervous system and mediate a wide range of brain processes, including synaptic plasticity associated with memory formation and learning (Bliss and Collingridge, 1993). NMDAR activation requires binding of the excitatory amino acid L-glutamate to its NR2 subunit and a co-agonist (D-serine or glycine) at the glycine site on the NR1 subunit. D-Serine is abundant in the forebrain, where its expression is correlated with that of NMDARs (Hashimoto and Oka, 1997; Matsui et al., 1995; Schell et al., 1997). It is apparent that in vivo the NMDA glycine site is not saturated in all brain regions (Fuchs et al., 2005), so it is possible that increasing glycine or D-serine levels could potentially modulate responses of the NMDAR.

The NMDAR hypofunction hypothesis of schizophrenia is principally based on the psycho-mimetic effects of the NMDAR antagonists phencyclidine (PCP) and ketamine. PCP induces a schizophrenia-like spectrum of symptoms in healthy subjects (positive, negative and cognitive aspects) and exacerbates symptoms in schizophrenia patients (Javitt and Zukin, 1991; Olney and Farber, 1995). Enhancing NMDAR function could therefore be of therapeutic benefit in schizophrenia. This has been tested clinically with glycine, D-serine and the glycine-site partial agonist D-cycloserine being administered as add-on therapy to conventional anti-psychotics. The results, although mixed, are encouraging with D-serine having benefit for the positive, negative and cognitive domains of schizophrenia (Heresco-Levy, 2005). There are issues with administration of D-serine or glycine, in particular with the large quantities of compound that need to be dosed to increase levels of these amino acids in the brain. Alternative approaches include inhibiting transporters of these amino acids to block cellular uptake, for example, by inhibiting the glycine Glyt1 transporter or the high affinity D-serine transporter Asc-1 (Sur and Kinney, 2004). Additionally, D-serine degradation can be prevented by inhibiting D-amino acid oxidase (DAO), which degrades a range of D-amino acids to their corresponding keto-acids (Konno and Yasumura, 1992).

A strain of mice has been identified lacking DAO activity (ddY/DAO$^-$) due to a single point mutation (G181R) in DAO (Sasaki et al., 1992). ddY/DAO$^-$ mice have elevated levels of D-amino acids, including increased D-serine in serum and brain compared to wild-type mice (Hashimoto et al., 1993; Konno and Yasumura, 1983; Morikawa et al., 2001). Recently, they have also been shown to have enhanced hippocampal LTP and improved performance in a

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test of spatial learning (Maekawa et al., 2005). This suggests inhibiting DAO could have therapeutic impact on the cognitive deficits found in schizophrenics. ddY/DAO$^-$ mice provide an important tool in validating DAO inhibition as a therapeutic target for schizophrenia. Therefore, we have further characterized ddY/DAO$^-$ mice and shown for the first time that they have increased occupancy of the NMDAR glycine site resulting in increased NMDAR function in vivo. Analysis of the expression of related biochemical partners to DAO shows that there is no apparent compensation in response to a decrease in DAO activity. Combined with further novel behavioral data this provides encouraging support for DAO inhibition as a therapeutic option in treating schizophrenia.

Results

Confirmation that the ddY/DAO$^-$ mouse strain lacks DAO activity

To confirm that the mouse strain ddY/DAO$^-$ lacks DAO activity, we examined DAO activity by two different methods. Histological studies with DAB staining in the kidney clearly show that there is no enzyme activity in ddY/DAO$^-$ mice (Fig. 1A; left-hand panel) compared to ddY/DAO$^+$ (right-hand panel). This was confirmed independently by a tissue homogenate assay of the kidney of both strains. The same assay also showed no DAO activity in the cerebellum of ddY/DAO$^-$ mice (Fig. 1B) compared with the wild-type strain (Fig. 1B). Interestingly, we did not see activity in the liver of either ddY/DAO$^+$ or ddY/DAO$^-$ mice (Fig. 1B), confirming earlier studies which have shown that there is no DAO in mouse liver (Konno et al., 1997). As a further control, we incubated kidney from ddY/DAO$^+$ mice with a DAO inhibitor, obtained from a high-throughput screen for DAO inhibitors (Brandish et al., in press), and demonstrated inhibition to a similar level as seen in the ddY/DAO$^-$ kidney.

Unaltered expression of DAO and NMDAR-related proteins in ddY/DAO$^-$ mice

We wanted to confirm that DAO protein was still expressed at the same level in ddY/DAO$^+$ and ddY/DAO$^-$ mice. Furthermore, we wanted to investigate the impact of the reduction of DAO activity, if any, on the expression of other proteins which play a role in D-serine metabolism and NMDAR neurotransmission. We initially looked at the expression of a range of relevant genes at the mRNA level in four different brain regions—cerebellum, cortex, hippocampus and hypothalamus. DAO mRNA levels remained unchanged across the two strains in all brain regions examined (data not shown). Serine racemase (SR) which creates D-serine from L-serine and Asc-1, the putative high affinity transporter of D-serine in the brain, also remained unchanged. The NR1 subunit of the NMDAR complex and the glycine transporter, GlyT1, were also similarly unaffected (data not shown). These findings were confirmed at the protein level using quantitative immunoblotting.

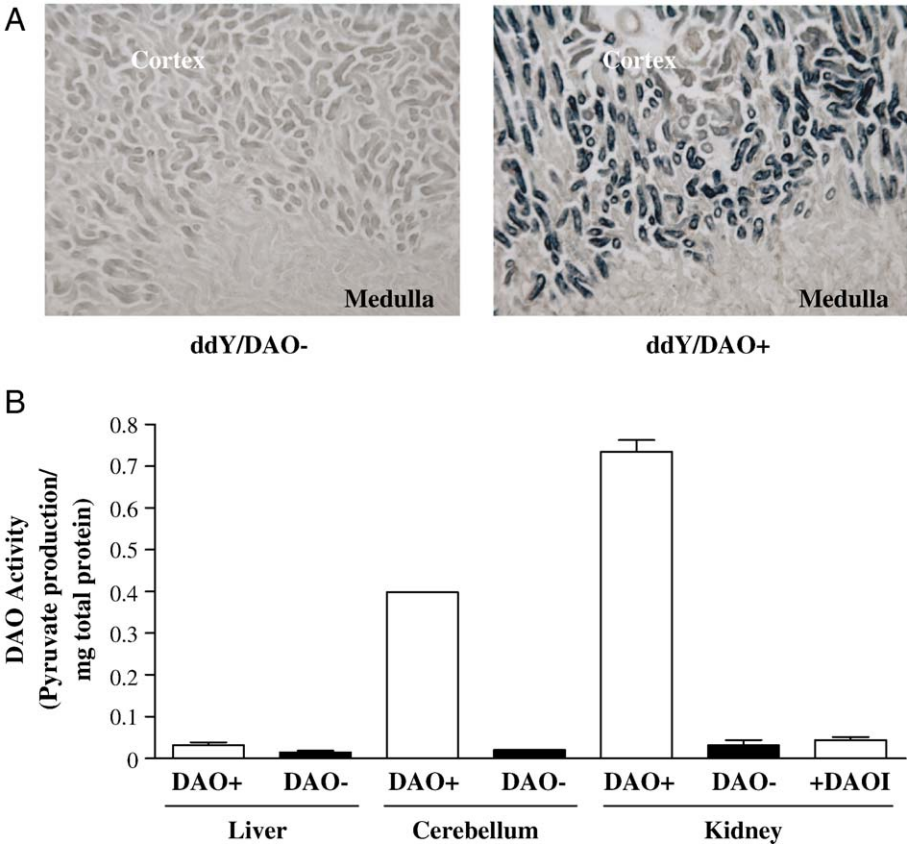

Fig. 1. DAO Activity in ddY/DAO$^+$ and ddY/DAO$^-$ mice. (A) Sections of kidneys from ddY/DAO$^+$ (right-hand panel) and ddY/DAO$^-$ (left-hand panel) mice were stained for DAO activity. DAB staining shows areas of DAO activity. (B) Liver, cerebellum and kidney extracts from ddY/DAO$^+$ and ddY/DAO$^-$ mice were assayed for DAO activity utilizing pyruvate production as a read-out. As a positive control kidney extract was incubated with a potent DAO inhibitor. $n = 3$ (except for cerebellum where tissue was pooled).

DAO protein expression was unaltered, as were the levels of SR, NR1 and Asc-1 when compared with the expression levels of α-tubulin and DISC1, which were both unchanged (Fig. 2A). This suggests there are no obvious compensatory changes for the lack of DAO activity in proteins which might be expected to be altered.

Neither was there any difference in D-serine uptake in cerebellar synaptosomes between the two strains (Fig. 2B), suggesting that there are no functional changes in D-serine uptake in response to a lack of DAO activity and consequent elevation of D-serine.

Neurological examination of ddY/DAO$^+$ and ddY/DAO$^-$ mice

ddY/DAO$^+$ and ddY/DAO$^-$ mice underwent a neuroscreen to look for any obvious abnormalities caused by the lack of DAO activity that may have confounded further behavioral testing (Fig. 3). There was no significant differences between either ddY/DAO$^+$ or ddY/DAO$^-$ or male or female animals in the swim test, grip strength or time taken to cross a beam (Figs. 3A–C). There was a significant effect of genotype ($F_{(1,44)} = 15.73$, $P < 0.001$) on the number of footslips committed (Fig. 3D) with ddY/DAO$^-$ mice committing fewer overall footslips. No effects of gender were seen in any of the measures used.

Acoustic startle and prepulse inhibition are unaltered in ddY/DAO$^-$ mice

Acoustic startle (AS) is used as a prescreen for prepulse inhibition (PPI) to determine whether any animals are suffering from hearing impairments or are unable to respond to the tones presented to them. Any animals not reaching criteria were excluded from PPI.

There was a significant genotype × sound intensity interaction ($F_{(5,110)} = 2.53$, $P < 0.05$) in AS with the ddY/DAO$^-$ mice showing a higher startle amplitude (Fig. 4A) at several of the presented stimuli. However, there was no significant difference between the two strains in PPI (Fig. 4B).

ddY/DAO$^-$ mice show reduced locomotor activity

To determine the effects of DAO activity on locomotor activity, we measured initial activity in response to a novel environment over a period of 20 min. Mobile time is considered to be a measure of true locomotor activity, and it was found that there was a significant effect of genotype ($F_{(1,22)} = 6.92$, $P < 0.05$), time ($F_{(9,198)} = 22.91$, $P < 0.001$) and a genotype × time interaction ($F_{(9,198)} = 4.11$, $P < 0.001$) with the ddY/DAO$^-$ animals showing hypolocomotion in comparison with the ddY/DAO$^+$ animals (Fig. 5A). Measurement of activity includes behaviors such as grooming, rearing and stereotypy, and there was a significant effect of time ($F_{(9,198)} = 25.32$, $P < 0.001$) and a genotype × time interaction ($F_{(9,198)} = 3.69$, $P < 0.001$) with the effects of genotype alone just missing significance ($F_{(1,22)} = 4.01$, $P = 0.06$). Once again, the ddY/DAO$^-$ animals were hypoactive in comparison with ddY/DAO$^+$ mice (Fig. 5B).

The effects of a PCP challenge are attenuated in the ddY/DAO$^-$ mice

It was hypothesized that DAO mice may display altered NMDAR function. To further investigate this, the NMDAR antagonist PCP was used to challenge the mice in an assay of habituated locomotor activity. When PCP was administered, it was found that the ddY/DAO$^-$ mice did not respond to the PCP challenge, whereas the ddY/DAO$^+$, as expected, became hyperlocomotive. There was a significant genotype × treatment × time interaction ($F_{(12,528)} = 3.79$, $P < 0.001$) in mobile time (Fig. 6A). However, there was no significant effect of genotype on activity (Fig. 6B), as both the ddY/DAO$^+$ and ddY/DAO$^-$ mice became hyperactive. Upon closer inspection of the data (Fig. 6B), it is

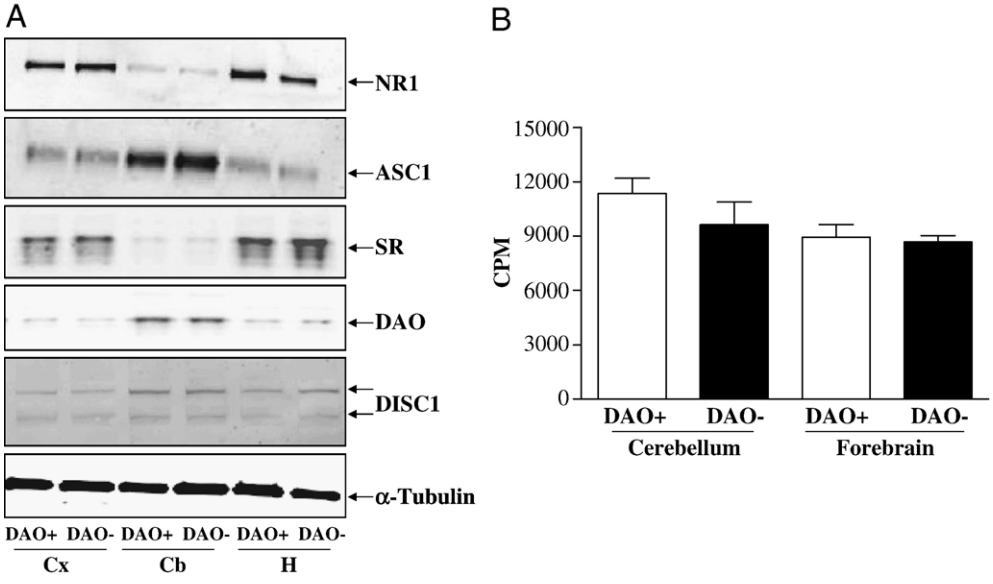

Fig. 2. Expression of DAO and related molecules in ddY/DAO$^+$ and ddY/DAO$^-$ mice. (A) Levels of protein expression of NMDAR1, Asc-1, SR, DAO, DISC1 and α-tubulin were quantitated by Western blotting and the Li-Cor Odyssey™ system (Licor, USA). A range of CNS tissues were analyzed (Cx = cortex, Cb = cerebellum and H = hippocampus). (B) D-Serine uptake in +/+ and −/− mice. [^{3}H]D-serine uptake was quantitated in synaptosomes of cerebellum and forebrain from ddY/DAO$^-$ and ddY/DAO$^+$ mice ($n = 4$). There was no significant difference between the 2 strains.

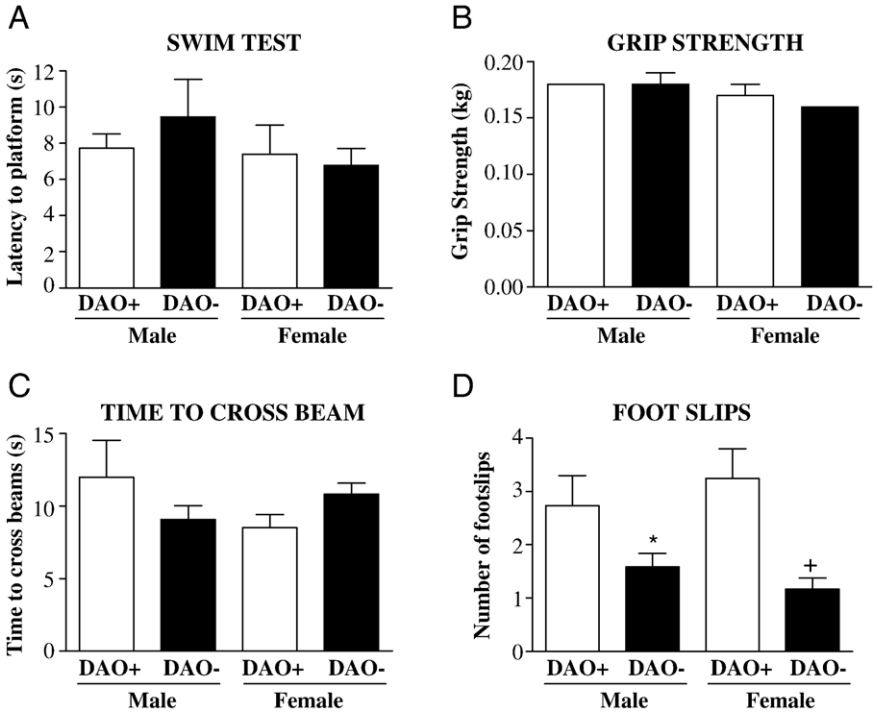

Fig. 3. Neuroscreen data for ddY/DAO$^+$ and ddY/DAO$^-$ mice. (A) Swim test was measured as time (s) to reach a visible platform in a 60-cm-long tank. (B) Grip strength was measured as weight pulled–body weight (kg). (C) Time to cross beam was measured as time (s) taken across an 8-mm × 60-cm-long round, wooden beam suspended 30 cm from the bench (D) Number of footslips was measured across the same beam as (C). *$P < 0.05$ compared with +/+ male animals, +$P < 0.05$ compared with +/+ female animals. $n = 11–12$.

clearly apparent that the effect of PCP on activity was partially attenuated in ddY/DAO$^-$ animals.

Increased occupancy of the NMDAR glycine site in the ddY/DAO$^-$ mice as shown by the attenuated effects of a NMDAR glycine site antagonist

The lack of DAO activity in ddY/DAO$^-$ mice leads to an increase in tissue D-serine (Hashimoto et al., 1993). It is currently unclear whether this increase is purely intracellular or is also observed extracellularly. To determine whether this is the case we challenged ddY/DAO$^+$ and ddY/DAO$^-$ mice with a potent NMDA glycine-site antagonist, L-701,324 (Bristow et al., 1996), to determine whether elevated D-serine can block the ataxic effects of the NMDA glycine site antagonist suggesting that a fraction of the increased D-serine is extracellular. As expected when assessed in beam walking, L-701,324 caused an increase in both the number of footslips (Fig. 7A) and falls (Fig. 7B) in ddY/DAO$^+$ animals ($F_{(1,8)} = 17.05$, $P < 0.01$ and $F_{(1,8)} = 18.78$, $P < 0.01$ respectively). However, L-701,324 did not significantly impair the performance of ddY/DAO$^-$ mice, implying they were protected from its ataxic effects compared to ddY/DAO$^+$ animals, due to the increased occupancy of the glycine-site by D-serine.

Elevated NMDAR activity in ddY/DAO$^-$ mice as measured by Cerebellar cGMP levels

To determine whether any tonic and stimulated changes in NMDAR function are present, basal and harmaline stimulated cerebellar cGMP were measured. No differences in basal cGMP were seen between ddY/DAO$^+$ and ddY/DAO$^-$ mice. Harmaline produced significant increases in cGMP in both ddY/DAO$^+$ and ddY/DAO$^-$ mice (2-fold and 3-fold increase respectively), with a significantly greater response in mice lacking DAO (Fig. 8). This suggests no tonic differences in NMDAR-mediated neurotransmission but that increased D-serine in ddY/DAO$^-$ mice contributes to enhanced NMDAR function under conditions of stimulation.

Discussion

This current study establishes for the first time that mice with reduced D-amino acid oxidase activity (ddY/DAO$^-$) have enhanced NMDAR function in vivo. NMDAR hypofunction is currently a leading hypothesis in explaining the etiology of schizophrenia (Olney and Farber, 1995; Heresco-Levy, 2005). Potentiating NMDAR activity through inhibiting DAO would therefore be predicted to have therapeutic utility in the clinical setting.

We have been able to obtain evidence of enhanced NMDAR function in the ddY/DAO$^-$ mice by measuring enhanced cGMP elevation in the cerebellum of these mice in response to harmaline (Fig. 8). Cerebellar cGMP is an in vivo indicator of NMDA-receptor-mediated activity (Wood et al., 1994). This has been shown pharmacologically by increases of cerebellar cGMP in mice after D-serine administration (Emmett et al., 1991; Wood et al., 1994) with harmaline-induced increases in cGMP being attenuated by NMDAR glycine-site

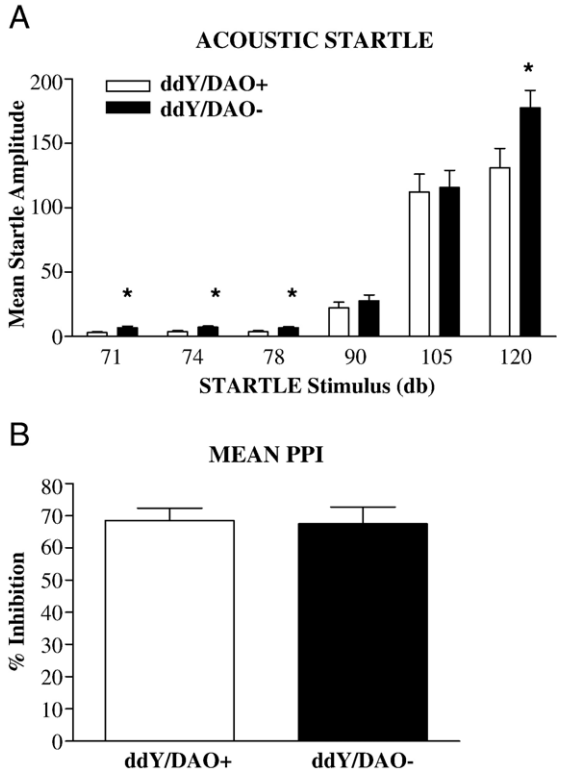

Fig. 4. Acoustic startle of ddY/DAO$^+$ and ddY/DAO$^-$ mice. (A) Response to acoustic startle for ddY/DAO$^+$ and ddY/DAO$^-$ mice. (B) Response to PPI for ddY/DAO$^+$ and ddY/DAO$^-$ mice expressed as percentage inhibition of the acoustic startle produced by pulse alone (120 dB). The mean level of PPI response obtained is presented. *$P < 0.05$ compared with ddY/DAO$^+$ animals ($n = 12$).

antagonists (Baron et al., 1997; Lehmann et al., 1988). In this study, no differences were seen in basal cGMP between ddY/DAO$^+$ and ddY/DAO$^-$ mice, indicating that the increased concentrations of D-serine present in the brain of ddY/DAO$^-$ mice do not cause increased NMDAR receptor activation during normal neurotransmission or possibly that desensitization of the nitric-oxide-mediated response to any chronic enhancement of NMDAR transmission occurs. When the NMDAR was stimulated with harmaline, 2- to 3-fold increases in cGMP were seen, in alignment with previous publications in adult mice (Bansinath et al., 1994; Moskal et al., 2005), but there was clear potentiation in the ddY/DAO$^-$ animals. The potentiated increase in cGMP levels and hence NMDAR activity in ddY/DAO$^-$ compared to ddY/DAO$^+$ mice in vivo corroborates previous reports that these mice demonstrate enhanced synaptic activity. ddY/DAO$^-$ mice have been shown to have increased NMDA receptor-mediated excitatory postsynaptic currents in spinal cord dorsal horn neurons and enhanced hippocampal LTP in vitro (Maekawa et al., 2005; Wake et al., 2001). At the behavioral level, the increase in hippocampal LTP is manifested by improved cognitive performance in the Morris water maze compared with wild-type mice (Maekawa et al., 2005). The critical role of the NMDAR in LTP and related processes is widely accepted with these latter processes considered as the best molecular correlates of learning and memory (Bliss and Collingridge, 1993; Collingridge and Bliss, 1995; Malenka and Bear, 2004; Bear and Malenka, 1994). Together, this suggests that decreased levels of DAO activity could improve cognitive performance through enhancing NMDAR activity.

We have also shown for the first time, albeit indirectly, that a reduction in DAO activity results in an extracellular increase in D-serine. Previously it had been shown that ddY/DAO$^-$ mice have elevated levels of D-serine in whole brain tissue only (Hashimoto et al., 1993). This new information is critical in the evaluation of the role of DAO in NMDAR regulation and schizophrenia as it is paramount that D-serine is able to access the extracellular space to increase NMDAR function as supported by our evidence. We have been able to demonstrate that a fraction of this increase in D-serine is extracellular. ddY/DAO$^-$ mice are protected from the ataxic effects of the potent NMDAR glycine site antagonist L-701,324 (Fig. 7) (Bristow et al., 1996) implying increased D-serine is competing for glycine-site occupancy. We elected to use pharmacological methods to measure increased extracellular D-serine as we were unable to measure increased extracellular D-serine satisfactorily by microdialysis in rat cerebellum in pilot experiments. In these rat assays, we were constrained by the analytical limits of detection of D-serine due to the small sample size and low extracellular D-serine levels in the cerebellum (caused by high DAO activity in this brain region). From our initial experience, we were confident that the consequences for the ddY/DAO$^-$ experiments would be that baseline D-serine could not be calculated for comparison purposes between ddY/DAO$^+$ and ddY/DAO$^-$ mice, making the data difficult to interpret.

Interestingly, ddY/DAO$^-$ mice showed fewer footslips when assessed on beam walking in the neurological screen. The predominance of DAO in the hindbrain and major role of the cerebellum in coordination of motor activity (Sanchez-Perez et al., 2005) indicates the possibility that enhanced NMDAR activity in the cerebellum of the ddY/DAO$^-$ mice contributes to improved motor coordination. This corroborates previous studies which show that dosing of D-serine derivatives reduces the number of falls in ataxic mutant mice which the authors correlate with increased levels of cerebellar D-serine (Saigoh et al., 2006). Hypoactivity of ddY/DAO$^-$ animals in response to a novel environment was also observed despite the fact that beam walking data do not indicate sedation or motor impairment. An interpretation of this finding is that increased glutamatergic transmission augments tonic GABAergic inhibition of nucleus accumbens dopamine release. The fact that ddY/DAO$^-$ mice are partially protected from the effects of PCP on locomotor activity (Fig. 6) indicates differences between the two strains of mice in this pathway. These behavioral observations may imply enhanced synaptic transmission in ddY/DAO$^-$ mice and warrant further investigation to interpret them in context of the neurochemical differences between ddY/DAO$^-$ and ddY/DAO$^+$ mice.

There are a number of preclinical assays of anti-psychotic activity/positive symptomology including prepulse inhibition (PPI) and reversal of PCP-induced hyperlocomotion. PPI, a measure of sensorimotor gating which, is disrupted in patients suffering from schizophrenia and other schizotypal personality disorders, was not altered in the ddY/DAO$^-$ mice compared to ddY/DAO$^+$ mice (Braff et al., 1995, 1999;Cadenhead et al., 1993; Swerdlow et al., 1994; Stevens et al., 2003). This may suggest that DAO inhibition would not show efficacy in treating the positive symptoms of schizophrenia but it is possible that the

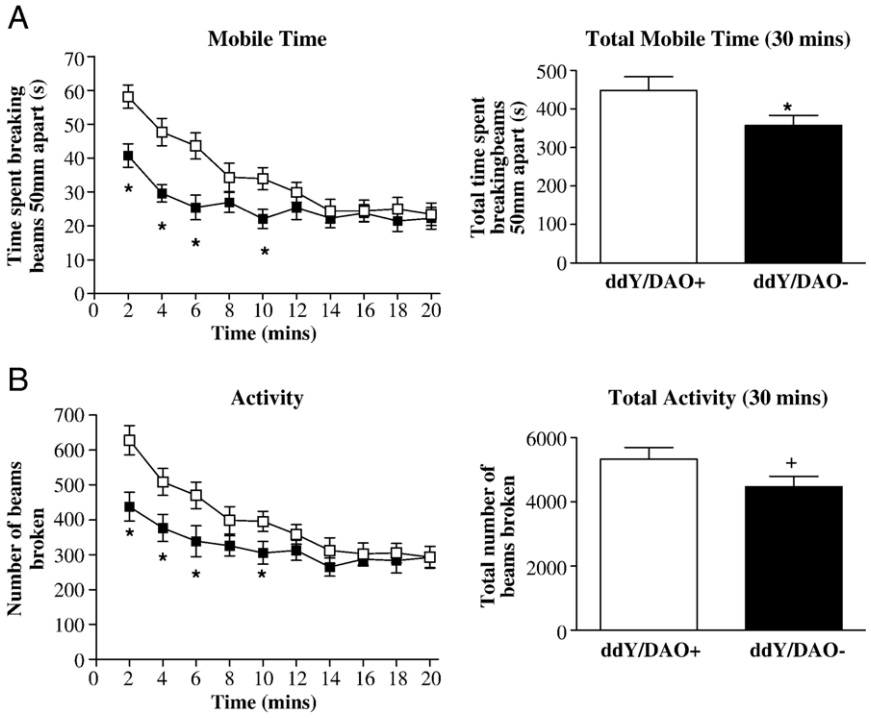

Fig. 5. Spontaneous Locomotor activity of ddY/DAO$^+$ and ddY/DAO$^-$ mice. Locomotor activity was collected in 2-min time bins over a 30-min time period. Mobile time (A) is calculated time spent breaking beams 50 mm apart and activity (B) is calculated as number of beams broken. ddY/DAO$^-$ mice show hypolocomotion and hypoactivity when compared with ddY/DAO$^+$ mice during the first 20 min of the test. *$P < 0.05$ and +$P = 0.06$ compared with ddY/DAO$^+$ ($n = 12$).

protocol used here may have been flawed—a more relevant experiment would have been to look at the effects on PPI deficits induced by an NMDAR antagonist in ddY/DAO$^-$ mice. In rodents, PCP causes a deficit in PPI (Andersen and Pouzet, 2004), and it may be that reduced DAO activity would protect against this deficit. This is supported by the locomotor response

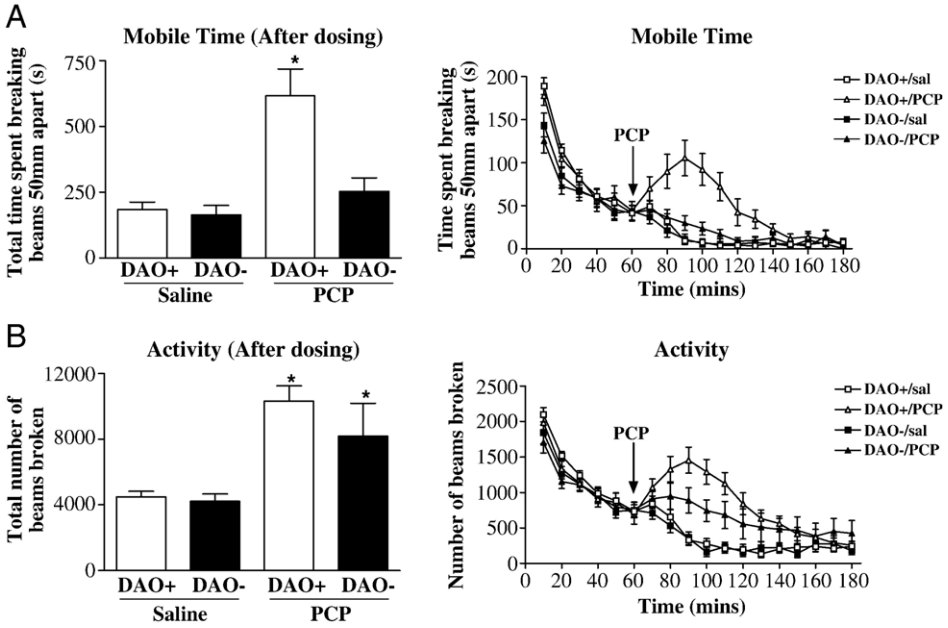

Fig. 6. Effects of PCP on spontaneous locomotor activity in ddY/DAO$^+$ and ddY/DAO$^-$ mice. Mice were dosed with either vehicle (saline) or PCP (3 mg/kg) and their activity was analyzed for 2 h post-dosing. Data were recorded as mobile time (A; time spent breaking beams 50 mm apart) and activity (B; number of beams broken). ddY/DAO$^-$ do not show hyperlocomotion as recorded in mobile time but do show hyperactivity as shown by activity. *$P < 0.05$ compared with vehicle-treated animals ($n = 12$).

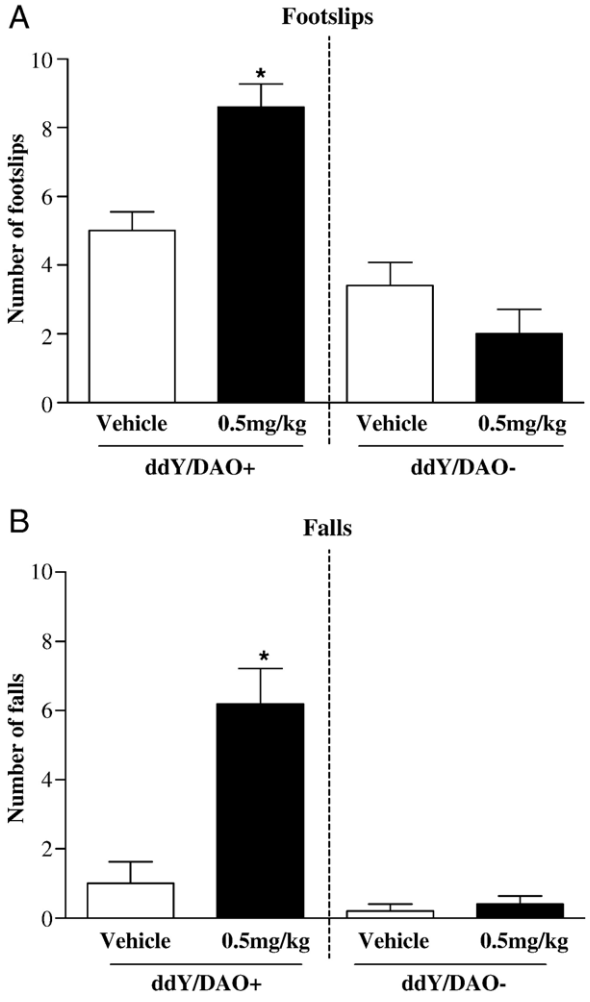

Fig. 7. Effect of the NMDAR glycine site antagonist L-701,324 on beam walking in ddY/DAO$^+$ and ddY/DAO$^-$ mice. Mice were dosed with the glycine site antagonist L-701,324 (0.5 mg/kg, i.p.) and footslips were then analyzed as they crossed a wooden beam (8 mm diameter, 60 cm long). *$P < 0.05$ compared with vehicle-treated animals. $n = 5$.

of the ddY/DAO$^-$ mice when challenged with PCP. The ddY/DAO$^-$ mice were completely protected against the PCP effect on mobile time and partially protected against the stereotypic effects of PCP. ddY/DAO$^-$ mice have also been recently shown to be protected from the stereotypy and ataxia induced by another NMDAR antagonist MK-801, but there was no effect on locomotor activity (Hashimoto et al., 2005). Together the PPI and locomotor data do not allow a clear-cut conclusion to be made on the role of diminished DAO activity for the treatment of the positive symptoms of schizophrenia.

We have also investigated the effects of inhibiting DAO activity on its closest related biochemical partners and provide the first data set which indicates that there are no obvious compensatory mechanisms triggered by DAO inhibition. This is of immense importance for a drug discovery effort where apart from DAO inhibition, there are a number of alternative mechanisms to increase NMDAR function. These include blocking Asc-1 and GlyT1 (transporters for D-serine and glycine respectively). GlyT1 expression overlaps with that of the NMDAR and so is of major interest (Smith et al., 1992). GlyT1 and Asc-1 functions have been studied by generating knockout mice. GlyT1−/− and Asc-1−/− mice die soon after birth, GlyT1−/− with severe respiratory and motosensory deficits (Gomeza et al., 2003) and Asc-1−/− from tremors and seizures (Xie et al., 2005). The GlyT1 heterozygote (GlyT1+/−) is viable and has been shown to have enhanced NMDAR function in CA1 pyramidal cells and improved performance in a hippocampal-dependent spatial memory task (Tsai et al., 2004). Similarly, hyperexcitability was observed in hippocampal slices from Asc-1−/− mice and shown to be due to enhanced NMDAR function (Xie et al., 2005). No behavioral data on the Asc-1+/− mice are currently available. On comparison of ddY/DAO$^-$ mice to these knockouts it is clear that all three mechanisms result in elevations in NMDAR function. However, chronic reduction of only DAO throughout life is viable, though it is possible that the lethality observed in Asc-1−/− and GlyT1−/− mice is related to a role in the early stages of development. Clearly, it will require the generation of efficacious DAO, Asc-1 and GlyT-1 inhibitors to ultimately test this in the clinic. We were thus interested in whether the lack of DAO activity would result in compensatory changes in these related systems and proteins. We did not see any changes in the expression of the GlyT1 or the NMDA NR1 subunit in membrane preparations in the ddY/DAO$^-$ line (Fig. 2), although we did not look to see if there were differences in surface expression and hence function of these molecules. D-Serine is synthesized by serine racemase (SR) from L-serine (Wolosker et al., 1999a,b). The levels of SR expression remained unchanged in ddY/DAO$^-$ mice however we did not measure its activity so there may be functional changes in this enzyme (Fig. 2A). It was also hypothesized that D-serine uptake capacity might be changed in the ddY/DAO$^-$ mice to clear elevated D-serine. However, we did not observe any changes in the expression of Asc-1 or any functional changes as measured by [^{3}H]-D-serine uptake suggesting that this is not the case (Fig. 2B). Taken together, these data suggest that loss of DAO activity does not result in any obvious changes in NMDAR-related molecular

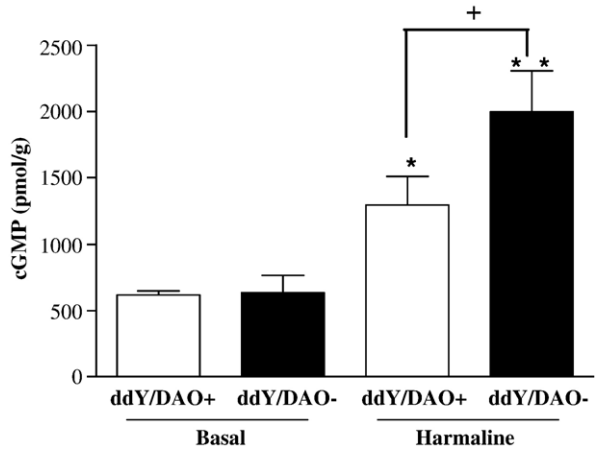

Fig. 8. In vivo NMDAR activity under basal and activated conditions in ddY/DAO$^+$ and ddY/DAO$^-$ mice. Basal and harmaline stimulated cerebellar cGMP was measured in cerebellum from dosed mice. No differences were seen in basal cGMP between ddY/DAO$^+$ and ddY/DAO$^-$ mice. NMDA stimulation by harmaline (80 mg/kg, s.c.) produced an elevation in cerebellar cGMP which was augmented in ddY/DAO$^-$ mice. *$P < 0.05$, compared with vehicle treated ddY/DAO$^+$ mice, **$P < 0.01$ compared with vehicle treated ddY/DAO$^-$ mice, +$P < 0.05$ between genotypes ($n = 4–5$).

systems and that DAO inhibition remains a potentially safe mechanism to enhance NMDAR function.

To further support a role for DAO in schizophrenia, there have been a number of genetic association studies recently reported (Chumakov et al., 2002; Craddock et al., 2005; Kirov et al., 2005). In the initial report, four single-nucleotide polymorphisms (SNPs) in DAO were shown to be associated with schizophrenia in a French–Canadian sample. Furthermore, this same study identified risk associated with a novel gene G72, now renamed D-amino acid oxidase activator (DAOA). The putative protein product of G72 was shown to potentiate DAO activity in an in vitro enzyme assay and polymorphisms in DAO and G72 to act in combination to induce risk (Chumakov et al., 2002). These finding have been corroborated by further positive association studies for DAO in cohorts from Germany and China (Liu et al., 2004; Schumacher et al., 2004). There have been an even greater number of positive findings for DAOA (see Kirov et al., 2005, for review). G72/DAOA is a primate specific gene so we were unable to study the effects of DAO inactivation on its function in the ddY/DAO$^-$ mice but it would be of interest to identify any other similar DAO activating proteins. DAO and DAOA join an ever-increasing list of genes which are being tagged as schizophrenia risk genes. The rationale for a role for DAO in schizophrenia is strengthened over many of the alternative candidates as it has a clear functional pathway to the disease through the NMDAR (Kirov et al., 2005; Harrison and Weinberger, 2005).

In summary, we have been able to show a reduction in DAO activity, through enzyme inactivation by a natural mutation in the ddY/DAO$^-$ line, leads to enhanced NMDAR activity in vivo. Schizophrenia provides the most likely disease areas where this type of activity may have therapeutic utility with NMDAR antagonists known to mimic all symptomatic domains.

Experimental methods

Animals

ddY/DAO$^-$ and ddY/DAO$^+$ mice were obtained from the laboratory which initially isolated this strain (Konno and Yasumura, 1983). ddY/DAO$^-$ mice have been shown to have a missense mutation (Gly-181–Arg) in the DAO gene and so lack DAO activity (Sasaki et al., 1992).

All animals were group or singly housed (depending on fighting behavior) in solid-bottomed cages with sawdust bedding and environmental enrichment. Food and water were available ad libitum. Temperature and humidity were maintained at 21 ± 2°C and 55 ± 10% respectively. Lights were on a 12:12-h light cycle with lights coming on at 07.00 am. All procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act (1986) and its associated guidelines.

Drug preparation

Phencyclidine HCl (PCP; Sigma) was dissolved in 0.9% saline and administered intraperitoneally (i.p.) in a volume of 10 ml/kg.

L-701,324 (Terlings Park, MSD, UK) (Bristow et al., 1996) was dosed at 0.5 mg/kg in 70% PEG/30% H_20 pH 11 in a volume of 5 ml/kg, i.p. Harmaline HCl (Sigma, Dorset, UK) was dissolved in 0.9% saline and then administered subcutaneously (s.c.) in a volume of 10 ml/kg, at 80 mg/kg.

DAO histology activity assays

20 μm mouse kidney sections were preincubated for 30 min at 37°C in buffer A (0.1 M Tris maleate pH 7.8, containing 3 mM $CeCl_3$ and 100 mM sodium azide). Sections were then processed in buffer A containing 10 mM D-proline and incubated for a further 60 min at 37°C. They were then incubated for 30 min at 40°C in buffer B (50 mM sodium acetate pH 5.3 containing 0.05% DAB and 100 mM sodium azide). Finally, sections were washed in 50 mM sodium acetate, coverslipped with DPX and examined by light microscopy. This method was adapted from previous publications (Angermuller and Fahimi, 1988a,b; Sasaki et al., 1992).

DAO homogenate activity assays

Tissue from specified organs from ddY/DAO$^-$ and ddY/DAO$^+$ mice were prepared as previously published (D'Aniello et al., 1993). Essentially, tissues were homogenized in 50 μM Tris–HCl (pH 8.2; 0.25 g/ml) and centrifuged at 30,000×g for 30 min. 200 μl 0.1 M D-alanine (in 100 μM Tris–HCl, pH 8.2) was added to 200 μl of supernatant and incubated for 30 min at 37°C. 200 μl of the supernatant was then mixed with 200 μl of 20% trichloroacetic acid (TCA), followed by centrifugation at 14 000 rpm for 5 min. 400 μl supernatant was incubated with 400 μl 2,4-dinitrophenylhydrazine (1 mM in 1 M HCl) for 10 min at 37°C. Finally, 800 μl of 1.5 M NaOH was added to the supernatant and incubated for 10 min at 37°C. The absorbance of this final mixture was read at 445 nM on a TECAN Safire plate reader (Reading, UK).

Taqman analysis

Brain regions were rapidly dissected from ddY/DAO$^+$ and ddY/DAO$^-$ mice and snap frozen in isopentane. Tissues were stored at −80°C until analysis. The samples were thawed in lysis buffer (Applied Biosystems, CA, USA) and homogenized using a shaking mixer mill (Retsch, Leeds, UK). RNA was then extracted robotically in 96-well format (6700 Applied Biosystems, Reading, UK). RT-QPCR was performed using an ABI 7900 sequence detector (Perkin Elmer, MA, USA) on cDNA samples. The PCR reaction was carried out at 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95°C for 15 s, then 60°C for 1 min using the manufacturers Universal PCR Master Mix (Perkin Elmer, MA, USA).

Taqman primer and probe sets were designed from sequences in the GenBank database using Primer Express software (Perkin Elmer, MA, USA). Alpha tubulin was used to normalize for the amount of sample in a given reaction. Results are expressed as fold change as calculated relative to ddY/DAO$^+$ after normalization.

Taqman primers

Gene	Species	Forward primer	Reverse primer	Probe	Accession number
DAO	Mus	gaaaaacgcagttctgggattc	ccgtagccataatcagggaaca	aagctgacccccagtgagatggac	nm_010018
SerineRacemase	Mus	aacagaaggcatcttggtccat	agcacttccagggcaattgt	agcctgcagtgatagctggacaagg	nm_013761
Glyt1	Mus	ccgcctctcttcttccagatc	caccgtgaagatgagaatgaaaaa	ttggcgctttgtttctccggctat	nm_008135
Asc-1	Rat	catgattcatgtcagacgctgtac	tgtggcccccgcaaca	ccaatccctgccctccttgt	nm_053726

Antibodies

The following primary antibodies were used for immunoblotting: rabbit polyclonal to DAO (raised to the mouse DAO peptide GLWQPYLSDPSNPQEAEWSQQ; 1:1000), rabbit polyclonal to Asc-1 (raised to the mouse Asc-1 peptide PSPLPITDKPLKTQC; 1:1000), mouse monoclonal to Serine Racemase (BD Transduction, 1:500), rabbit polyclonal to NMDA R1 (Chemicon, CA, USA; 1:500), rabbit polyclonal to DISC1 (D27; 1:500 (Brandon et al., 2004)) and mouse monoclonal to α-tubulin (Sigma, 1:5000).

Western blot analysis

For soluble proteins, tissues from ddY/DAO$^+$ and ddY/DAO$^-$ mice were lysed in RIPA buffer (50 mM Tris–EDTA buffer, 150 mM NaCl, 0.1% SDS, 0.5% deoxycholic acid, 1% Igepal CA-630, 2 μl/ml protease inhibitor cocktail, Sigma). Lysates were solubilized for 1 h at 4°C and centrifuged at 14,000 rpm for 15 min. Protein concentrations were quantitated by BCA analysis as per manufacturers protocol (Pierce, Boston, USA). 100 μg of each sample was then separated by SDS-PAGE and analyzed by Western blotting. Protein levels in each sample were quantitated using the Li-Cor Odyssey™ system (Nebraska, USA).

For transmembrane proteins ddY/DAO$^+$ and ddY/DAO$^-$ mouse tissues were homogenized in ice cold 10 mM HEPES pH 7.4 containing 0.32 M sucrose using a glass teflon homogenizer and centrifuged at 3000 rpm for 10 min at 4°C. The resulting supernatant was centrifuged at 20,000 rpm for 30 min at 4°C and the pellet resuspended in phosphate buffered saline by trituration. Protein concentration was determined using a BCA protein quantification method, as per manufacturer's protocol (Pierce, Boston, USA). Samples (25 μg protein) were analyzed by Western blotting as above.

[^{3}H]D-serine uptake in synaptosomes of forebrain and cerebellum

Forebrain and cerebellum from ddY/DAO$^+$ and ddY/DAO$^-$ mice were dissected after decapitation and homogenized in 10 volumes of 5 mM HEPES, pH 7.4 containing 0.32 M sucrose. Homogenates were centrifuged at 800×*g* for 10 min, and the resulting supernatant was centrifuged at 40,000×*g* for 30 min. Synaptosome pellets were resuspended in ice-cold assay buffer (120 mM NaCl, 25 mM $NaHCO_3$, 1.5 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4, 10 mM D-glucose, 10 mM HEPES, pH 7.4) to a concentration of 25 mg original wet weight per milliliter. Membranes (2.5 mg) were added to 100 nM [^{3}H]D-serine (Perkin Elmer, MA, USA, specific activity 29 Ci mmol) in 96-well plates on ice. Uptake was initiated by transferring assay plates to a water bath at 25°C, and incubations were carried out for 8 min. Uptake was terminated via rapid filtration onto Unifilter GF/B glass fibre filters and washing three times with 1 ml ice-cold assay buffer. Radioactivity was detected using Microscint O scintillation fluid on a TopCount scintillation counter. Non-specific binding was determined using 100 mM D-serine.

Behavioral testing

Naïve male and female ddY/DAO$^+$ and ddY/DAO$^-$ mice aged 3–4 months were used for the neuroscreen battery, spontaneous locomotor activity and PCP-induced locomotor activity. Male animals were also used in acoustic startle (AS) and prepulse inhibition (PPI).

Neuroscreen

Animals underwent a swim test in which a 60-cm-long plastic trough was filled with water (24–28°C) and the animal placed at one end, pointing towards the wall. Time taken to reach a visible platform at the other end of the trough was recorded. Each animal underwent 4 trials with an inter-trial interval of 30 s.

To assess muscle tone of the animals a test of grip strength was used in which spring weigh scales (Fisher Scientific, Tustin, CA) with an attached trapeze were used. Mice were allowed to grasp the trapeze with their forepaws, while the observer pulled down gently on the mouse's tail. Animals were allowed 3 trials and (weight pulled–body weight) was used for analysis.

To assess motor co-ordination animals were placed on a round, wooden beam (8 mm diameter, 60 cm long, elevated 20 cm above the bench) and allowed to walk to a goal box (Stanley et al., 2005). Mice that fell were placed back on to the beam at the position they had fallen from and were allowed a maximum time of 60 s on the beam. The time taken to cross the beam as well as number of footslips committed was recorded.

Acoustic startle (AS)

To ascertain that all animals showed a normal startle response, AS was performed. Animals were habituated in their home cages for 1 h to 65-dB white noise. They were then placed into standard startle chambers (SR-Lab system, San Diego Instruments). Each session was initiated with a 5-minute acclimatization period of white noise at 65 dB followed by five successive 100-dB habituation tones (40-ms long inter-trial interval = 30 s). These trials were not included in the analysis. Six different trial types were then presented: 90, 95, 100, 105, 110 and 120 dB (40-ms long inter-trial interval = 30 s) with the background noise at 65 dB. Each of these was presented 10 times in a pseudorandom order and the average response to each trial calculated.

Prepulse inhibition (PPI)

Animals were habituated in their home cages for 1 h to 65-dB white noise. They were then placed into standard startle chambers. Each session was initiated with a 5-minute acclimatization period of white noise at 65 dB followed by 37 trials. Trials 1–6 and 32–37 were the 120-dB pulse alone (40-ms inter-trial

interval = 11–19 s) and were excluded from analysis and used to determine the degree of habituation occurring during the assay. Trials 7 to 31 consisted of three different trial types containing a prepulse followed by the startle pulse (71 + 120, 74 + 120 and 78 + 120 dB) which were presented 5 times and a 120-dB pulse alone trial that was presented 10 times. PPI was calculated as ((startle to pulse only − startle to prepulse and pulse)/startle to pulse) × 100).

Spontaneous locomotor activity

Animals were taken from their home cages and placed into Benwick locomotor activity monitors (Linton Instrumentation, Diss, Norfolk, UK). These consisted of clear Perspex boxes with gridded lids containing a thin layer of sawdust. A grid of 36 infrared beams on two levels was used to measure movement. Mobile time (s) (time spent breaking beams 50 mm apart) and activity (number of beams broken) were recorded.

Challenge of ddY/DAO$^-$ and ddY/DAO$^-$ mice with PCP

Animals were placed into the Benwick locomotor boxes and allowed to habituate for 60 min and then administered PCP (3 mg/kg, i.p.). Locomotor activity was recorded for a further 2 h with no other intervention.

Challenge of ddY/DAO$^-$ and ddY/DAO$^-$ mice with L-701,324

Animals were trained to cross a round, wooden beam (8 mm diameter, 60 cm long and elevated 20 cm above the bench) as described in the neuroscreen. The minimum effective dose of the glycine site antagonist L-701,324 causing robust ataxia (0.5 mg/kg) was determined through a dose–response curve in stock animals due to a lack of transgenic mice (data not shown). Animals were administered vehicle or L-701,324 (0.5 mg/kg, i.p.) with a pretreatment time of 30 min and their performance assessed on the beam as described in the neuroscreen.

Basal and harmaline stimulated cyclic GMP

Cerebellar cyclic GMP (cGMP) was measured under basal conditions and 15 min after administration of harmaline 80 mg/kg (Wood et al., 1982). Naïve and drug-treated animals were euthanased by decapitation and the cerebellum removed and immediately frozen in isopentane cooled on dry ice. Frozen cerebella were ground to a fine powder using a stainless steel pestle and mortar cooled on dry ice and the powder homogenized in 10 volumes of ice-cold 0.1 M HCl. Following centrifugation at 10,000 rpm for 15 min at 4°C, supernatants were frozen at −80°C until analysis for cGMP by enzyme immunoassay (low pH, R&D Systems Europe).

Statistical analysis

Data are presented as the mean ± SEM. All test data were analyzed using either Statistica (StatSoft Inc., Tulsa, USA) or GraphPad Prism (San Diego California USA). Data were analyzed using one-way or repeated measure ANOVAs and all post hoc analysis was done using Student–Newman–Keuls tests.

References

Andersen, J.D., Pouzet, B., 2004. Spatial memory deficits induced by perinatal treatment of rats with PCP and reversal effect of D-serine. Neuropsychopharmacology 29, 1080–1090.

Angermuller, S., Fahimi, H.D., 1988a. Light microscopic visualization of the reaction product of cerium used for localization of peroxisomal oxidases. J. Histochem. Cytochem. 36, 23–28.

Angermuller, S., Fahimi, H.D., 1988b. Heterogenous staining of D-amino acid oxidase in peroxisomes of rat liver and kidney. A light and electron microscopic study. Histochemistry 88, 277–285.

Bansinath, M., Arbabha, B., Turndorf, H., Garg, U.C., 1994. Methodological variables during analysis of in vivo cerebellar cyclic GMP, an indirect marker of nitric oxide release. J. Pharmacol. Toxicol. Methods 31, 107–112.

Baron, B.M., Harrison, B.L., Kehne, J.H., Schmidt, C.J., van Giersbergen, P.L., White, H.S., Siegel, B.W., Senyah, Y., McCloskey, T.C., Fadayel, G.M., Taylor, V.L., Murawsky, M.K., Nyce, P., Salituro, F.G., 1997. Pharmacological characterization of MDL 105,519, an NMDA receptor glycine site antagonist. Eur. J. Pharmacol. 323, 181–192.

Bear, M.F., Malenka, R.C., 1994. Synaptic plasticity: LTP and LTD. Curr. Opin. Neurobiol. 4, 389–399.

Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39.

Braff, D.L., Swerdlow, N.R., Geyer, M.A., 1995. Gating and habituation deficits in the schizophrenia disorders. Clin. Neurosci. 3, 131–139.

Braff, D.L., Swerdlow, N.R., Geyer, M.A., 1999. Symptom correlates of prepulse inhibition deficits in male schizophrenic patients. Am. J. Psychiatry 156, 596–602.

Brandish, P.E., Chiu, C.S., Schneeweis, J., Brandon, N.J., Leech, C.L., Kornienko, O., Scolnick, E.M., Strulovici, B., Zheng. W., in press. A Cell-based Ultra-High Throughput Screening Assay for Identifying Inhibitors of D-amino Acid Oxidase. Journal of Biomolecular Screening.

Brandon, N.J., Handford, E.J., Schurov, I., Rain, J.C., Pelling, M., Duran-Jimeniz, B., Camargo, L.M., Oliver, K.R., Beher, D., Shearman, M.S., Whiting, P.J., 2004. Disrupted in Schizophrenia 1 and Nudel form a neurodevelopmentally regulated protein complex: implications for schizophrenia and other major neurological disorders. Mol. Cell. Neurosci. 25, 42–55.

Bristow, L.J., Hutson, P.H., Kulagowski, J.J., Leeson, P.D., Matheson, S., Murray, F., Rathbone, D., Saywell, K.L., Thorn, L., Watt, A.P., Tricklebank, M.D., 1996. Anticonvulsant and behavioral profile of L-701,324, a potent, orally active antagonist at the glycine modulatory site on the *N*-methyl-D-aspartate receptor complex. J. Pharmacol. Exp. Ther. 279, 492–501.

Cadenhead, K.S., Geyer, M.A., Braff, D.L., 1993. Impaired startle prepulse inhibition and habituation in patients with schizotypal personality disorder. Am. J. Psychiatry 150, 1862–1867.

Chumakov, I., et al., 2002. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 99, 13675–13680.

Collingridge, G.L., Bliss, T.V., 1995. Memories of NMDA receptors and LTP. Trends Neurosci. 18, 54–56.

Craddock, N., O'Donovan, M.C., Owen, M.J., 2005. The genetics of schizophrenia and bipolar disorder: dissecting psychosis. J. Med. Genet. 42, 193–204.

D'Aniello, A., D'Onofrio, G., Pischetola, M., D'Aniello, G., Vetere, A., Petrucelli, L., Fisher, G.H., 1993. Biological role of D-amino acid oxidase

and D-aspartate oxidase. Effects of D-amino acids. J. Biol. Chem. 268, 26941–26949.
Emmett, M.R., Mick, S.J., Cler, J.A., Rao, T.S., Iyengar, S., Wood, P.L., 1991. Actions of D-cycloserine at the *N*-methyl-D-aspartate-associated glycine receptor site in vivo. Neuropharmacology 30, 1167–1171.
Fuchs, S.A., Berger, R., Klomp, L.W., de Koning, T.J., 2005. D-amino acids in the central nervous system in health and disease. Mol. Genet. Metab. 85, 168–180.
Gomeza, J., Hulsmann, S., Ohno, K., Eulenburg, V., Szoke, K., Richter, D., Betz, H., 2003. Inactivation of the glycine transporter 1 gene discloses vital role of glial glycine uptake in glycinergic inhibition. Neuron 40, 785–796.
Harrison, P.J., Weinberger, D.R., 2005. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol. Psychiatry 10, 40–68.
Hashimoto, A., Oka, T., 1997. Free D-aspartate and D-serine in the mammalian brain and periphery. Prog. Neurobiol. 52, 325–353.
Hashimoto, A., Nishikawa, T., Konno, R., Niwa, A., Yasumura, Y., Oka, T., Takahashi, K., 1993. Free D-serine, D-aspartate and D-alanine in central nervous system and serum in mutant mice lacking D-amino acid oxidase. Neurosci. Lett. 152, 33–36.
Hashimoto, A., Yoshikawa, M., Niwa, A., Konno, R., 2005. Mice lacking D-amino acid oxidase activity display marked attenuation of stereotypy and ataxia induced by MK-801. Brain Res. 1033, 210–215.
Heresco-Levy, U., 2005. Glutamatergic neurotransmission modulators as emerging new drugs for schizophrenia. Expert Opin. Emerg. Drugs 10, 827–844.
Javitt, D.C., Zukin, S.R., 1991. Recent advances in the phencyclidine model of schizophrenia. Am. J. Psychiatry 148, 1301–1308.
Kirov, G., O'Donovan, M.C., Owen, M.J., 2005. Finding schizophrenia genes. J. Clin. Invest. 115, 1440–1448.
Konno, R., Yasumura, Y., 1983. Mouse mutant deficient in D-amino acid oxidase activity. Genetics 103, 277–285.
Konno, R., Yasumura, Y., 1992. D-Amino-acid oxidase and its physiological function. Int. J. Biochem. 24, 519–524.
Konno, R., Sasaki, M., Asakura, S., Fukui, K., Enami, J., Niwa, A., 1997. D-Amino-acid oxidase is not present in the mouse liver. Biochim. Biophys. Acta 1335, 173–181.
Lehmann, J., Chapman, A.G., Meldrum, B.S., Hutchison, A., Tsai, C., Wood, P.L., 1988. CGS 19755 is a potent and competitive antagonist at NMDA-type receptors. Eur. J. Pharmacol. 154, 89–93.
Liu, X., He, G., Wang, X., Chen, Q., Qian, X., Lin, W., Li, D., Gu, N., Feng, G., He, L., 2004. Association of DAAO with schizophrenia in the Chinese population. Neurosci. Lett. 369, 228–233.
Maekawa, M., Watanabe, M., Yamaguchi, S., Konno, R., Hori, Y., 2005. Spatial learning and long-term potentiation of mutant mice lacking D-amino-acid oxidase. Neurosci. Res. 53, 34–38.
Malenka, R.C., Bear, M.F., 2004. LTP and LTD: an embarrassment of riches. Neuron 44, 5–21.
Matsui, T., Sekiguchi, M., Hashimoto, A., Tomita, U., Nishikawa, T., Wada, K., 1995. Functional comparison of D-serine and glycine in rodents: the effect on cloned NMDA receptors and the extracellular concentration. J. Neurochem. 65, 454–458.
Morikawa, A., Hamase, K., Inoue, T., Konno, R., Niwa, A., Zaitsu, K., 2001. Determination of free D-aspartic acid, D-serine and D-alanine in the brain of mutant mice lacking D-amino acid oxidase activity. J. Chromatogr., B, Biomed. Sci. Appl. 757, 119–125.
Moskal, J.R., Kuo, A.G., Weiss, C., Wood, P.L., O'connor, H.A., Kelso, S., Harris, R.B., Disterhoft, J.F., 2005. GLYX-13: a monoclonal antibody-derived peptide that acts as an *N*-methyl-D-aspartate receptor modulator. Neuropharmacology 49, 1077–1087.
Olney, J.W., Farber, N.B., 1995. NMDA antagonists as neurotherapeutic drugs, psychotogens, neurotoxins, and research tools for studying schizophrenia. Neuropsychopharmacology 13, 335–345.
Saigoh, K., Matsui, K., Takahashi, K., Nishikawa, T., Wada, K., 2006. The stereo-specific effect of D-serine ethylester and the D-cycloserine in ataxic mutant mice. Brain Res. 808, 42–47.
Sanchez-Perez, A., Llansola, M., Cauli, O., Felipo, V., 2005. Modulation of NMDA receptors in the cerebellum: II. Signaling pathways and physiological modulators regulating NMDA receptor function. Cerebellum 4, 162–170.
Sasaki, M., Konno, R., Nishio, M., Niwa, A., Yasumura, Y., Enami, J., 1992. A single-base-pair substitution abolishes D-amino-acid oxidase activity in the mouse. Biochim. Biophys. Acta 1139, 315–318.
Schell, M.J., Brady, R.O. Jr., Molliver, M.E., Snyder, S.H., 1997. D-Serine as a neuromodulator: regional and developmental localizations in rat brain glia resemble NMDA receptors. J. Neurosci. 17, 1604–1615.
Schumacher, J., Jamra, R.A., Freudenberg, J., Becker, T., Ohlraun, S., Otte, A.C., Tullius, M., Kovalenko, S., Bogaert, A.V., Maier, W., Rietschel, M., Propping, P., Nothen, M.M., Cichon, S., 2004. Examination of G72 and D-amino-acid oxidase as genetic risk factors for schizophrenia and bipolar affective disorder. Mol. Psychiatry 9, 203–207.
Smith, K.E., Borden, L.A., Hartig, P.R., Branchek, T., Weinshank, R.L., 1992. Cloning and expression of a glycine transporter reveal colocalization with NMDA receptors. Neuron 8, 927–935.
Stanley, J.L., Lincoln, R.J., Brown, T.A., McDonald, L.M., Dawson, G.R., Reynolds, D.S., 2005. The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. J. Psychopharmacol. 19, 221–227.
Stevens, E.R., Esguerra, M., Kim, P.M., Newman, E.A., Snyder, S.H., Zahs, K.R., Miller, R.F., 2003. D-Serine and serine racemase are present in the vertebrate retina and contribute to the physiological activation of NMDA receptors. Proc. Natl. Acad. Sci. U. S. A. 100, 6789–6794.
Sur, C., Kinney, G.G., 2004. The therapeutic potential of glycine transporter-1 inhibitors. Expert Opin. Investig. Drugs 13, 515–521.
Swerdlow, N.R., Braff, D.L., Taaid, N., Geyer, M.A., 1994. Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. Arch. Gen. Psychiatry 51, 139–154.
Tsai, G., Ralph-Williams, R.J., Martina, M., Bergeron, R., Berger-Sweeney, J., Dunham, K.S., Jiang, Z., Caine, S.B., Coyle, J.T., 2004. Gene knockout of glycine transporter 1: characterization of the behavioral phenotype. Proc. Natl. Acad. Sci. U. S. A. 101, 8485–8490.
Wake, K., Yamazaki, H., Hanzawa, S., Konno, R., Sakio, H., Niwa, A., Hori, Y., 2001. Exaggerated responses to chronic nociceptive stimuli and enhancement of *N*-methyl-D-aspartate receptor-mediated synaptic transmission in mutant mice lacking D-amino-acid oxidase. Neurosci. Lett. 297, 25–28.
Wolosker, H., Blackshaw, S., Snyder, S.H., 1999a. Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-*N*-methyl-D-aspartate neurotransmission. Proc. Natl. Acad. Sci. U. S. A. 96, 13409–13414.
Wolosker, H., Sheth, K.N., Takahashi, M., Mothet, J.P., Brady, R.O. Jr., Ferris, C.D., Snyder, S.H., 1999b. Purification of serine racemase: biosynthesis of the neuromodulator D-serine. Proc. Natl. Acad. Sci. U. S. A. 96, 721–725.
Wood, P.L., Richard, J.W., Pilapil, C., Nair, N.P., 1982. Antagonists of excitatory amino acids and cyclic guanosine monophosphate in cerebellum. Neuropharmacology 21, 1235–1238.
Wood, P.L., Emmett, M.R., Wood, J.A., 1994. Involvement of granule, basket and stellate neurons but not Purkinje or Golgi cells in cerebellar cGMP increases in vivo. Life Sci. 54, 615–620.
Xie, X., Dumas, T., Tang, L., Brennan, T., Reeder, T., Thomas, W., Klein, R.D., Flores, J., O'Hara, B.F., Heller, H.C., Franken, P., 2005. Lack of the alanine-serine-cysteine transporter 1 causes tremors, seizures, and early postnatal death in mice. Brain Res. 1052, 212–221.