Developmental vitamin D deficiency alters MK-801-induced behaviors in adult offspring

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Abstract

Rationale Developmental vitamin D (DVD) deficiency is a candidate risk factor for developing schizophrenia in humans. In rodents DVD-deficiency induces subtle changes in the way the brain develops. This early developmental insult leads to select behavioural changes in the adult, such as an enhanced response to amphetamine-induced locomotion in female DVD-deficient rats but not in male DVD-deficient rats and an enhanced locomotor response to the N-Methyl-D-Aspartate (NMDA) receptor antagonist, MK-801, in male DVD-deficient rats. However, the response to MK-801 induced locomotion in female DVD-deficient rats is unknown. Therefore, the aim of the current study was to further examine this behavioural finding in male *and* female rats and assess NMDA receptor density.

Methods DVD-deficient Sprague-Dawley rats were assessed for locomotion, ataxia, acoustic startle response (ASR) and prepulse inhibition of the ASR (PPI) to multiple doses of MK-801. The NMDA receptor density in relevant brain regions was assessed in a drug naïve cohort.

Results DVD-deficiency increased locomotion in response to MK-801 in both sexes. DVD-deficient rats also showed an enhanced ASR compared with control rats, but PPI was normal. Moreover, DVD-deficiency decreased NMDA receptor density in the caudate putamen of both sexes.

Conclusions These results suggest that a transient prenatal vitamin D deficiency has a long lasting effect on NMDA-mediated signalling in the rodent brain and may be a plausible candidate risk factor for schizophrenia and other neuropsychiatric disorders.

Keywords Schizophrenia, MK-801, vitamin D, locomotion, prepulse inhibition, animal model, NMDA

Introduction

The centrality of dopamine (DA) to the pathophysiology and treatment of schizophrenia is well described. This understanding is based on the correlation between clinical doses of antipsychotic drugs and their potency to block dopamine 2 receptors and ameliorate symptoms (Seeman and Lee 1975). It is also based on the psycho-stimulant effects of DA enhancing drugs such as amphetamine in both patients and nonpsychotic individuals (Angrist and Vankammen 1984). In addition, there is evidence that schizophrenia is associated with a persistent dysfunction of glutamate transmission involving N-methyl-D-aspartate (NMDA) receptors (Jentsch and Roth 1999; Laruelle et al. 2003; Olney and Farber 1995). For example, psychomimetic agents, such as dizocilpine (MK-801), ketamine and phencyclidine (PCP), acting on the glutamatergic system via inhibition of NMDA receptors, have been shown to cause psychotic-like symptoms in healthy controls (Krystal et al. 1994; Lahti et al. 2001). These NMDA receptor antagonists also induce a wider range of schizophrenia-like symptoms (e.g. cognitive deficits) in comparison to dopaminergic agonists such as amphetamine (Lahti et al. 2001), which tend to be associated strictly with the positive symptoms of schizophrenia (e.g. hallucinations, delusions). This broader symptom profile lends weight to the hypothesis that NMDA and glutamate systems are also involved in the pathogenesis of schizophrenia (Rung et al. 2005).

In rats, NMDA receptor antagonists induce a robust behavioural phenotype including hyperlocomotion in the open field and, at high doses, motor ataxia (motor/balance impairment) (Andine et al. 1999; Hiramatsu et al. 1989; Loscher and Honack 1992).

Furthermore, NMDA receptor antagonists are also known to impair prepulse inhibition (PPI) in rats (Bast et al. 2000). PPI is a form of sensorimotor gating where patients with schizophrenia and other neuropsychiatric disorders often show deficits (Swerdlow and Koob 1987). A deficit in PPI is considered an inability to 'gate out' irrelevant stimuli. With respect to animal models of schizophrenia, psycho-stimulant induced locomotor and PPI deficits have therefore been extensively investigated in animal models of the disorder (Al-Amin et al. 2000; Kesby et al. 2006; Meyer et al. 2008; Moore et al. 2006; Varty et al. 2000).

Based on various clues from epidemiology, prenatal vitamin D deficiency has been proposed as a candidate risk factor for schizophrenia (McGrath 1999). There is now also good clinical support for this proposal (McGrath et al. 2010). In order to explore the impact of transient low prenatal vitamin D on brain development we have developed a rodent model of Developmental Vitamin D (DVD) deficiency. DVD-deficiency alters brain development in the rat (Eyles et al. 2003) and mouse (Harms et al. 2008). Vitamin D deficiency during development alters both gene expression and the rates of mitosis and cell death in the developing brain (Eyles et al. 2003; Ko et al. 2004). The newborn offspring of DVD-deficient rats have larger lateral ventricles, decreased cortical thickness and altered DA metabolism compared to controls (Eyles et al. 2003; Kesby et al. 2009).

Adult male DVD-deficient animals show a complex behavioural phenotype. They have normal prepulse inhibition (Kesby et al. 2006). In the open field, DVD-deficient animals display a novelty-induced hyperlocomotion (Burne et al. 2004a; Burne et al. 2006; Kesby et al. 2006). Of particular relevance to the current study, adult male

DVD-deficient rats have been shown to be sensitive to the locomotor-enhancing effects of MK-801 (Kesby et al. 2006; O'Loan et al. 2007) and this could be ameliorated with the antipsychotic drug, haloperidol (Kesby et al. 2006). A later study revealed DVD-deficiency induces sex specific effects in adult rats. Female (but not male) DVD-deficient rats were shown to be more sensitive to amphetamine-induced locomotion than controls (Kesby et al. 2010). Thus it appears that both DA and NMDA neurotransmitter systems are perturbed in DVD-deficient rats and in some cases this is also dependent on sex.

Therefore, the current study aimed to further examine behaviour and brain neurochemistry in male *and* female Sprague-Dawley rats. We examined multiple doses of MK-801 on locomotion, ataxia, acoustic startle response (ASR) and PPI of the acoustic startle response. In addition, MK-801, dopamine receptor and dopamine transporter (DAT) binding in relevant brain regions including the prefrontal cortex (PFC), caudate putamen (CPu) and nucleus accumbens (Acb) was investigated.

Materials and methods

Animals

All procedures were performed with approval from the Queensland University Animal Ethics Committee, under the guidelines of the National Health and Medical Research Council of Australia. To induce vitamin D depletion, four-week old female Sprague-Dawley rats (Herston Animal Facility, Queensland, Australia) were kept on a vitamin D deficient diet (Dyets Inc., Bethlehem, Pennsylvania). Animals were housed on a

12-h light/dark cycle (lights on at 06:00 h) using incandescent lighting, to avoid ultraviolet radiation within the vitamin D action spectrum. These conditions were maintained for 6 weeks prior to mating and throughout gestation. This breeding protocol is sufficient to deplete serum vitamin D in Dams and offspring without altering neonatal blood calcium levels (Eyles et al. 2003; O'Loan et al. 2007). Control animals were kept under similar conditions except they received a standard vitamin D containing rat chow (AIN93G; Dyets Inc., Bethlehem, Pennsylvania). At birth all dams (and corresponding litters) were placed under control conditions for the remainder of the experiment. Only litters between 6 and 18 animals were included in this study (Zorrilla 1997). At weaning (postnatal day 21) all animals were housed in same sex groups of 2-4 and kept on standard animal house chow. Animals were tested at 5-6 months of age.

Behavioural Testing

All behavioural testing occurred between 08:00 –18:00 h under dim red light. Dizocilpine Maleate (MK-801; Sigma Aldritch, MO, USA) was administered via intraperitoneal injection (i.p.) at 1 ml/kg. MK-801 was made up in a 0.9% saline vehicle. MK-801 was administered 15 min before ASR/PPI testing. Females received lower doses of MK-801 relative to males (males- 0.2 or 0.5 mg/kg; females- 0.05 or 0.1 mg/kg) because of their reduced ability to metabolise this drug (Andine et al. 1999). Arenas and startle chambers were cleaned between tests with 30% ethanol and dried with paper towel.

Open field behaviour

Locomotion and exploration were assessed simultaneously in six black PVC arenas, 80 x 40 cm wide and 45 cm deep under red light. Distance travelled was assessed using automated video-tracking software (Ethovision, Noldus Information Technology, Wageningen, The Netherlands) via a centrally placed video camera. Rats were placed into the arena for 30 min a week before the first drug exposure to habituate animals to the testing arenas and control for the recognised effects of handling and novelty in DVD-deficient animals (Burne et al. 2006). Over the following three weeks, rats were placed into the arena and habituated for 30 min prior to administration of either saline or a dose of MK-801 (males- 0.1, 0.2 mg/kg; females- 0.05 or 0.1 mg/kg) after which rats were monitored for a further 90 min. Rats were tested using a repeated injection protocol with all rats receiving all doses of MK-801 and a saline injection with a minimum of seven days between injections. To control for procedural variables, all rats were treated repeatedly within the same arena and at the same time of day. Using this protocol, the response to MK-801 could be compared to baseline behaviour within animal. Groups comprised n= 8-13 animals per sex per test group. A separate cohort of males was tested with a higher dose of MK-801 because the previous locomotor sensitivity found in response to MK-801 (0.2 mg/kg) (Kesby et al. 2006; O'Loan et al. 2007) was not apparent. This cohort of male adult rats (n= 12-13) was tested over two weeks with saline and 0.5 mg/kg MK-801.

Levels of ataxia were also recorded to determine whether group differences in locomotion were compromised by ataxia. Ataxia was scored from video recordings blind to treatment using a ‘scan sampling’ technique. Each 90 min behavioural recording (6 arenas) was assessed over three time bins of 5 min; 0-5, 40-45 and 85-

90 min after drug/saline administration. These time points were selected from a pilot experiment to indicate baseline (0-5), peak (40-45) and final (85-90) levels of ataxia. Within the 5 min time bins rats were scored each 30 sec to produce 10 behavioural observations per time bin. The ataxia rating scale follows the recommendation of Cho et al (Cho et al. 1991), (0) inactive or coordinated movements; (1) awkward or jerky movements or loss of balance while rearing; (2) frequent falling or partial impairment of antigravity reflexes; (3) the inability to move beyond a small area and to support body weight; (4) inability to move except for twitching movements. Using this protocol the maximum score that can be attained is 120, which would indicate that the rat was unable to move for the entirety of the experiment.

Prepulse inhibition of the acoustic startle response

ASR and PPI of the ASR were assessed using six startle chambers (SR-LAB, San Diego Instruments, CA, USA) consisting of a plexiglass cylinder (9 cm diameter) mounted on a plexiglass frame with a piezoelectric accelerometer mounted below; in a ventilated, light-free enclosure (30 x 30 x 30 cm). Background noise was maintained at 70 dB and acoustic bursts of white noise were delivered via a speaker mounted 20 cm above the animal. PPI was tested over two days using an identical protocol. The first day was used to habituate the animals to the apparatus (Faraday and Grunberg 2000). The second day was used in combination with drug treatment, saline or MK-801 (males- 0.2 or 0.5 mg/kg; females- 0.05 or 0.1 mg/kg), at the same doses used in the locomotor study. This method for assessing ASR, PPI and habituation of ASR within the same testing session, has been adopted previously within this lab (Burne et al. 2004b; Kesby et al. 2006). Briefly, testing sessions consisted of a 5 min acclimation period at 70 dB to assess background level of

activity, followed by five startling pulses (110 dB), each 20 s apart. Subsequently, five blocks of 24 trials were presented, including six pulse-alone trials to assess ASR (70, 80, 90, 100, 110 and 120 dB) and 18 different trial types with a pre-pulse to assess PPI. Pre-pulses had intensities of 74, 78 or 86 dB with a range (8, 16, 32, 64, 128 or 256 ms) of pre-pulse to pulse intervals to assess maximal PPI. The pulse was presented for 40 ms and the pre-pulse presented as a 20 ms broad band burst. The sequence of intertrial intervals, which varied between 10 to 20s, was initially allocated randomly, and then edited to ensure identical intervals did not occur consecutively. Testing ended with the replication of initial startling pulses (110 dB), allowing for the assessment of within-test habituation of the ASR. The startle response was a measure of the average amplitude, using the median value of the five blocks of each trial type. PPI was assessed using the formula [(amplitude of trial without pre-pulse – amplitude of trial with pre-pulse)/amplitude of trial without pre-pulse] x100. A separate group of rats to those tested in the open field were used for these studies. Groups comprised n= 10-19 animals.

Receptor Radioligand Binding

Tissue collection

A separate behavioural and drug naïve group of 5 month-old rats were sacrificed by lethal injection of sodium pentobarbital between 09:00 and 12:00 h to minimise circadian variations. Brains were rapidly removed and frozen in liquid nitrogen. Coronal brain sections were cut (14 µm) at -15^{o}C by cryostat and thaw mounted on glass slides (polysine; Thermo Scientific, CA, USA). Slides were stored at -80^{o}C prior to binding experiments.

MK-801 binding

Tissue sections were thawed and dried at RT briefly and then incubated for 2.5 h at RT in 30mM HEPES buffer (pH 7.5) containing 1mM EDTA, 100 μM glycine, 100 μM glutamate and 20 nM [^{3}H]MK-801 (specific activity: 27.5 Ci/mmol). Non-specific binding was determined by the addition of 20 μM MK-801 to adjacent sections. Sections were then washed twice for 20 min in ice-cold 30mM HEPES buffer (pH 7.5) containing 1mM EDTA, dipped in ice-cold distilled water and air-dried.

Dopamine 1 (D1) receptor binding

Tissue sections were thawed and dried at RT briefly and then pre-incubated in 50mM Tris-HCl buffer (pH 7.4) containing 120mM NaCl, 5mM KCl, 2mM CaCl2 and 1mM MgCl2 for 20 min at RT. They were then briefly air dried and incubated for 1.5 h at RT with 4 nM [3H]SCH23390 (specific activity: 84 Ci/mmol) and 30 nM spiperone to prevent non-specific binding to the D2 receptor. Non-specific binding was determined by the addition of 10 μM (+)-butaclamol to adjacent sections. Sections were then washed twice for 10 min in ice-cold buffer, dipped in ice-cold distilled water and air-dried.

Dopamine 2 (D2) receptor binding

Tissue sections were thawed and briefly dried at RT and then pre-incubated in 50mM Tris-HCl buffer (pH 7.4) containing 120mM NaCl, 5mM KCl, 2mM CaCl2, 1mM MgCl2 and 0.001% ascorbic acid for 30min at RT. They were then briefly air dried and incubated for 60min at RT with 5 nM [3H]raclopride (specific activity: 60.1 Ci/mmol). Non-specific binding was determined by the addition of 10 μM (+)-

butaclamol to adjacent sections. Sections were then washed twice for 5 min in ice-cold buffer, dipped in ice-cold distilled water and air-dried. Due to low expression in the PFC, this region was not assessed for D2 receptors.

DAT binding

Tissue sections were thawed and briefly dried at RT and then pre-incubated in 50mM Tris-HCl buffer (pH 7.4) containing 120mM NaCl and 0.1% bovine serum albumin for 20min at 4^{o}C. They were then briefly air dried and incubated for 2 h at 4^{o}C with 15 nM [^{3}H]WIN35428 (specific activity: P70 85.9 Ci/mmol, P140 85.6 Ci/mmol). Non-specific binding was determined by the addition of 10 μM GBR12909 to adjacent sections. Sections were then washed twice for 1 min in 4^{o}C buffer, dipped in ice-cold water and air-dried. Due to low expression in the PFC, this region was not assessed for the DAT.

Beta-imaging and analysing samples

Autoradiographic images for receptors and transporter were taken using a Beta-Imager^{TM} camera (Biospace, Paris, France). Sections were scanned for 3.5 h at a high-resolution setting. Quantitative analysis of these images was performed using the program β-Image Plus (version 4, Biospace). The radioligand-binding signal was expressed in counts per minute per square millimetre (cpm/mm2) and with the use of radio-chemical standards this was converted to fmol/mg tissue equivalents. Anatomical location was determined using well-known landmarks (Paxinos and Watson 2005). Binding was assessed in mesolimbic structures including the CPu and Acb shell (AcbSh) and core (AcbC) subregions (Bregma +1.6 mm). The PFC included both the prelimbic and infralimbic cortex (Bregma +3.3 mm). Specific

binding was calculated by subtracting non-specific binding from total binding. Non-specific binding was less than 15% of total binding. The data presented are the averages of 2-3 brain sections from each rat (n= 6-7).

Statistical analysis

All locomotion data were assessed over the total 90 min of testing. Locomotor responses to MK-801 were analysed as a percentage of each individual animal's saline treatment to control for variability in baseline locomotion and procedural variables (such as time of day). Behavioural data were assessed with a two-way analysis of variance (ANOVA) with main effects of maternal diet and MK-801 dose where appropriate. Binding data were assessed with a two-way ANOVA with main effects of sex and maternal diet. To investigate main effects or interactions t tests were used.

Results

Open Field behaviour

DVD-deficiency had no effect on body weight of male or female rats. There was also no significant effect of DVD-deficiency on baseline locomotion during the initial habituation test of 30 min or after saline in either sex.

There was a significant dose-dependent effect of MK-801 on the locomotor response of both female ($F_{(1,19)}$ = 34.9, p < 0.001; Fig. 1a) and male rats ($F_{(1,15)}$ = 6.8, p = 0.02;

Fig. 1b). In female rats there was also a significant main effect of maternal diet ($F_{(1,19)}$ = 7.2, p = 0.015). Moreover, there was a significant interaction of dose x maternal diet ($F_{(1,19)}$ = 8.4, p = 0.009). Female DVD-deficient rats showed a greater locomotor response to MK-801 after 0.1 mg/kg (t_{19} = 3.0. p = 0.007; Fig. 1a). In male rats there was no significant main effect of maternal diet. However, in the separate cohort of male rats, treated with a the higher dose of MK-801 (0.5 mg/kg), male DVD-deficient rats showed a significantly greater locomotor response than control male rats ($F_{(1,23)}$ = 6.9, p = 0.015; Fig. 1c).

<<Insert Fig. 1 near here>>

Ataxia scores were low for both male and female rats with the doses of MK-801 administered failing to induce more than a slight impairment of balance. In male rats ataxia scores showed a significant main effect of dose ($F_{(1,36)}$ = 6.9, p = 0.012) but no effect of maternal diet (ataxia scores after 0.2mg/kg MK-801; control = 1.0 $\pm$ 0.6, DVD-deficient = 1.0 $\pm$ 0.4). There was also no significant effect of maternal diet in male rats after 0.5 mg/kg MK-801 (ataxia scores after 0.5mg.kg MK-801; control = 14.4 $\pm$ 5.5, DVD-deficient = 14.8 $\pm$ 5.5). In female rats there was also a significant main effect of dose ($F_{(1,34)}$ = 6.6, p = 0.015) but again, no significant effect of maternal diet (ataxia scores after 0.1mg.kg MK-801; control = 0.6 $\pm$ 0.3, DVD-deficient = 0.8 $\pm$ 0.4).

Acoustic startle response

MK-801 increased the acoustic startle response compared to saline treatment in both males ($F_{(2,74)}$ = 19.9, p < 0.001; Fig. 2a-c) and females ($F_{(2,73)}$ = 16.6, p < 0.001; Fig. 2d-f). There was also a significant interaction of ASR x maternal diet in both males ($F_{(5,370)}$ = 3.7, p = 0.003) and females ($F_{(5,365)}$ = 2.7, p = 0.019). DVD-deficient rats showed an increased ASR to 120 dB compared with control rats at the lower dose of MK-801.

<<Insert Fig. 2 near here>>

Prepulse inhibition of the acoustic startle response

Maximal PPI responses were observed after saline treatment at an inter-stimulus interval of 64 ms and all analyses were performed using this interval (Fig. 3). There was a significant main effect of prepulse sound intensity on PPI in both males ($F_{(2,148)}$ = 43.3, p < 0.001) and females ($F_{(2,146)}$ = 23.8, p < 0.001). MK-801 significantly impaired PPI responses compared to saline treatment in males ($F_{(1,74)}$ = 14.1, p < 0.001; Fig. 3a-c) but not in females (Fig. 3d-f). There were no significant main effects of maternal diet or MK-801 dose on the PPI response. Male DVD-deficient rats appeared to have lower percent PPI scores than control rats after treatment with 0.5 mg/kg MK-801 (Fig. 3c), but this difference failed to reach significance ($F_{(1,23)}$ = 2.8, p = 0.11).

<<Insert Fig. 3 near here>>

MK-801 binding

Binding intensity for MK-801 was highest in the PFC being over 50% greater than any of the mesolimbic regions. In the CPu there was a significant main effect of maternal diet ($F_{(1,27)}$ = 5.1, p = 0.033) with DVD-deficient rats exhibiting lower binding levels (Fig. 4). There were no significant effects of maternal diet in any of the other regions assessed and no interactions of maternal diet x sex.

<<Insert Fig. 4 near here>>

Dopamine receptor and DAT binding

There were no significant effects of maternal diet in D1, D2 or DAT levels in ay of the regions assessed (Table. 1). DAT levels were significantly higher in the CPu ($F_{(1,26)}$ = 7.9, p = 0.010), Acb shell ($F_{(1,26)}$ = 8.3, p = 0.009) and Acb core ($F_{(1,26)}$ = 8.2, p = 0.009) of female rats compared with male rats.

<<Insert Table. 1 near here>>

Discussion

The main findings of this study were that male and female DVD-deficient rats had a greater sensitivity to MK-801-induced locomotion, an increase in the acoustic startle response and decreased MK-801 binding in the CPu. There was no effect of maternal diet on the sensitivity to MK-801 on measures of ataxia or percent PPI.

These results do not address the neural circuits involved in the behavioural response of control and DVD-deficient rats to MK-801. Although MK-801-induced locomotion is a widely used tool in animal models of schizophrenia (Al-Amin et al. 2000; Kesby et al. 2006; Meyer et al. 2008; Moore et al. 2006; Varty et al. 2000) the mechanism/s behind how MK-801 may increase locomotion remain obscure. Hyperlocomotion in rats could reflect either hyper-responsive DA or hypo-responsive NMDA signalling, a process thought to be crucial to psychotic symptoms in schizophrenia (Seeman 1987). There is, however, conflicting data on the centrality of DA to MK-801-induced hyperactivity. Increases in DA signalling have been implicated (Mathe et al. 1998; Moghaddam 1997) but the supporting data is far from convincing (Adams and Moghaddam 1998; Carlsson and Carlsson 1989; Druhan et al. 1996). Moreover, the lack of significant alterations in DA signalling found in this study would not appear to explain the locomotor sensitivity to MK-801 observed here and in two prior studies (Kesby 2006, O'loan 2007). We have previously shown an enhanced locomotor response to amphetamine and alterations in DA transporter density and affinity in DVD-deficient rats but this was present only in females (Kesby et al. 2010). The locomotor sensitivity described here in DVD-deficient rats occurred independent of sex. Although DA dysregulation may still be involved this suggests some common mechanism, perhaps glutamatergic as suggested by the small but significant reduction in MK-801 binding in the CPu, is present in both male and female DVD-deficient rats.

DVD-deficiency did not induce any PPI deficit consistent with previous studies (Kesby et al. 2006). Moreover, MK-801 impaired PPI equally in male DVD-deficient and control rats at the same dose where enhanced locomotor responses were

observed in DVD-deficient males. However, male and female DVD-deficient rats both showed an enhanced ASR to the high intensity acoustic pulses after MK-801. The neurobiology behind startle response and PPI are dissociable although there is overlap in the neural circuitry involved (Koch 1999). For example, MK-801 infused into the nucleus accumbens or dorsal medial thalamus increases the ASR without disrupting PPI (Bakshi and Geyer 1998). A similar dissociation between PPI and locomotion has also been observed. Blockade of gamma aminobutyric acid transmission in the ventral tegmental area with picrotoxin has been demonstrated to induce hyperlocomotion without impairing PPI (Schwienbacher et al. 2002). Our data suggest that DVD-deficiency results in an enhanced response to MK-801-induced motor activation but is not associated with pathways involved in the inhibitory control of the ASR.

Locomotor sensitivity to MK-801 has been demonstrated in other animal models independent of changes in MK-801 binding (Al-Amin et al. 2001). How these MK-801 induced behavioural alterations in DVD-deficient animals relate to the small reduction in MK-801 binding within the CPu of DVD-deficient rats remains unknown (Al-Amin et al. 2001). However, it is of interest to note that deficits in MK-801 binding have been found in the CPu of rats after chronic exposure to both typical and atypical antipsychotics (Tarazi et al. 2003; Tarazi et al. 1996). The decreased MK-801 binding found in DVD-deficient rats may therefore represent a downstream compensatory response to enhanced endogenous glutamatergic signalling. Clearly studies are required to clarify whether there are alterations in glutamate signalling in the DVD-deficient brain at both the regional and functional levels.

Consistent with previous reports, the results of this study indicate that the developmental absence of vitamin D induces persistent alterations in brain function in the adult offspring. Investigations into NMDA receptor function and dopamine/glutamate release in response to MK-801 are warranted in the DVD-deficient rat model. The current study has further demonstrated that DVD-deficiency affects the subsequent development of neurotransmitter systems involved in psychomimetic-induced locomotion and acoustic startle responses, specifically dopamine/glutamate transmission. These results provide further support that DVD-deficiency modulates brain development and may be a plausible candidate risk factor for schizophrenia and other neuropsychiatric disorders.

Disclosure/Conflicts of Interest

The authors have no conflicts to disclose.

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Titles and legends to figures

Fig. 1 Locomotor response to MK-801 in female (**a**) and male (**b,c**) rats. Locomotor response is expressed as a percentage change from each rat's own saline response (dotted line). Developmental vitamin D (*DVD*)-deficient rats showed significantly elevated responses to the higher doses of MK-801 compared to control animals. Data are presented as mean ± SEM

* $p < 0.05$

Fig. 2 Acoustic startle response (*ASR*) to varying acoustic pulses in male (**a-c**) and female (**d-f**) rats after varying doses of MK-801 (males- **a** = 0, **b** = 0.2 or **c** = 0.5 mg/kg; females- **d** = 0, **e** = 0.05 or **f** = 0.1 mg/kg). There were significant interactions of ASR x maternal diet in males ($p = 0.003$) and females ($p = 0.019$). Data are presented as mean ± SEM of the ASR amplitude in millivolts (mV)

Fig. 3 Prepulse inhibition (*PPI*) of the acoustic startle response in male (**a-c**) and female (**d-f**) rats after varying doses of MK-801 (males- **a** = 0, **b** = 0.2 or **c** = 0.5 mg/kg; females- **d** = 0, **e** = 0.05 or **f** = 0.1 mg/kg). There were no significant main effect of maternal diet found in either males or females. Data are presented as mean ± SEM of the percentage PPI at a 64 msec prepulse to pulse interval

Fig. 4 MK-801 binding in control and developmental vitamin D (DVD)-deficient rats in the prefrontal cortex (PFC), caudate putamen (CPu) and the nucleus accumbens shell subregion (AcbSh) and core subregion (AcbC). There was significant main effect of maternal diet in the CPu with levels significantly decreased in DVD-deficient

rats compared with controls (p = 0.033). No main effect of maternal diet was found in any other region. Data are presented as mean ± SEM of MK-801 binding (fmol/mg of tissue).

* $p < 0.05$

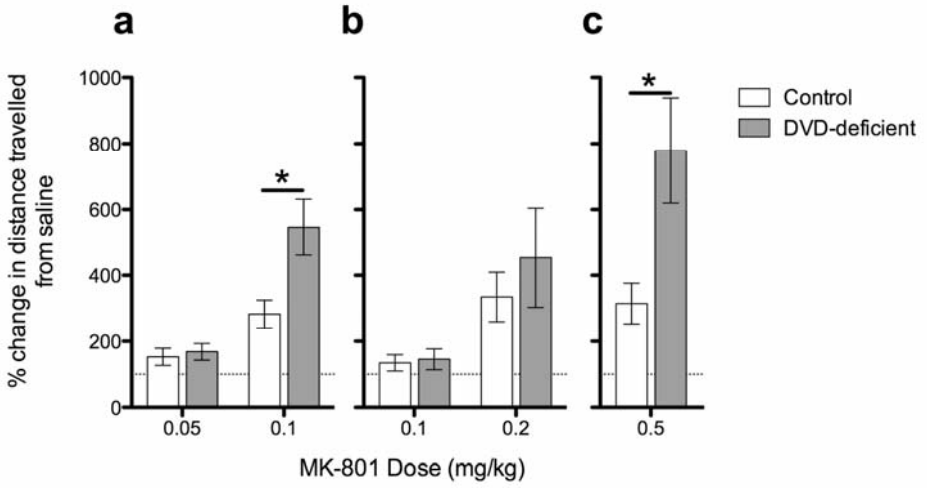

Fig. 1
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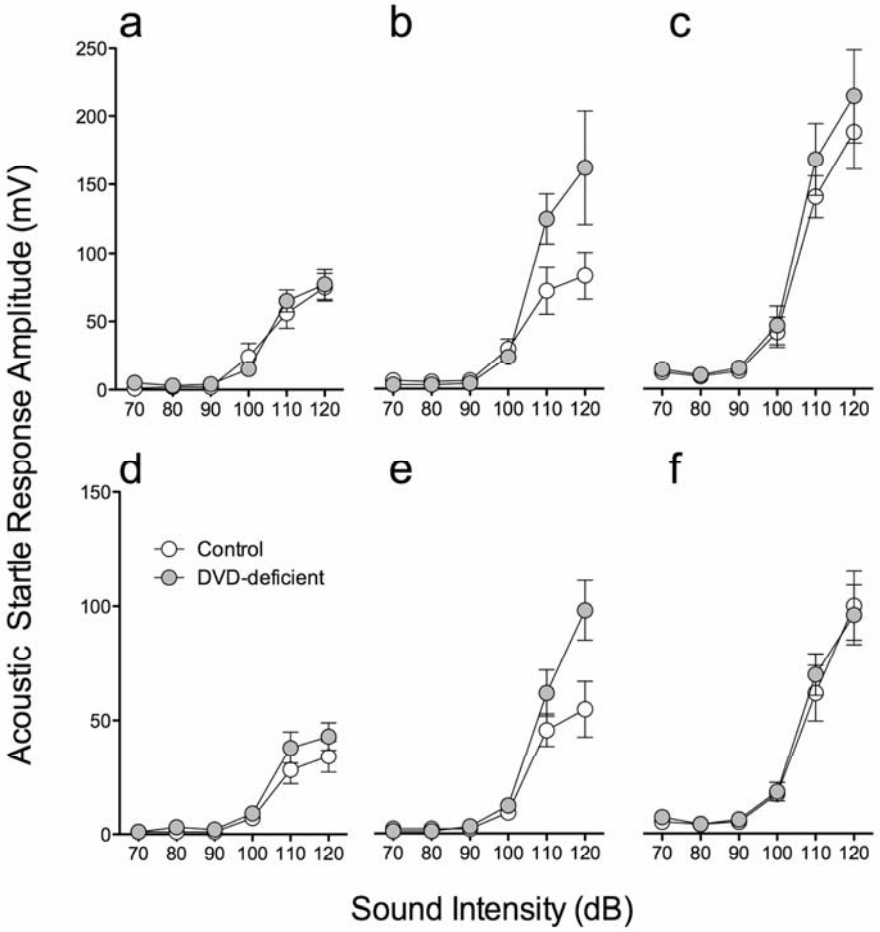

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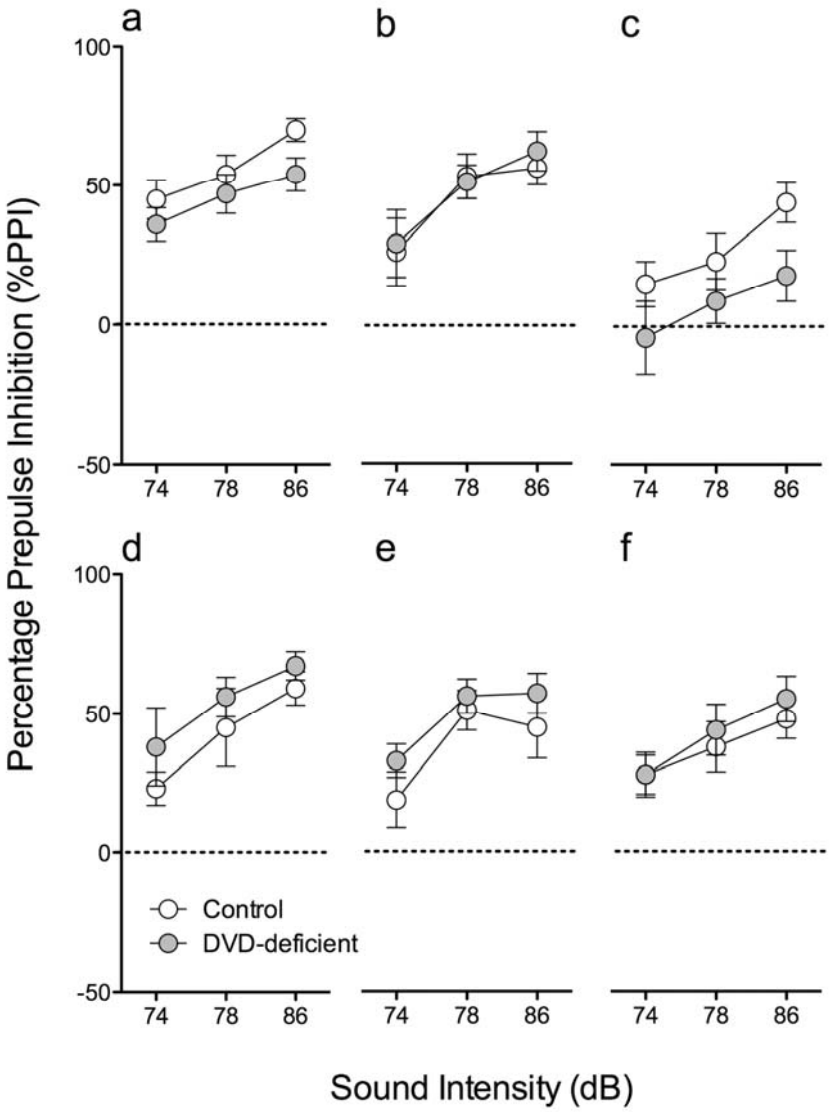

Fig 3.
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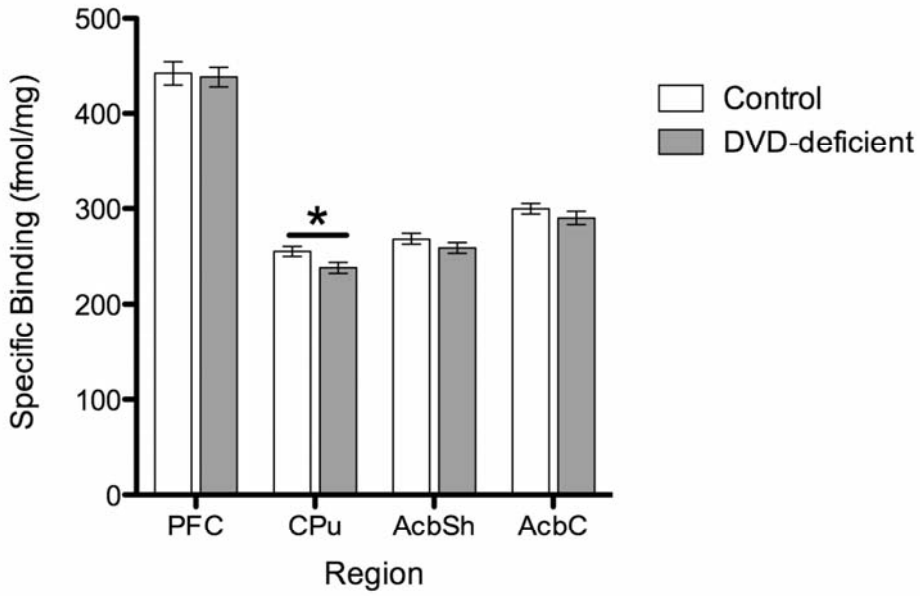

Fig. 4
133x91mm (300 x 300 DPI)

Table 1. Summary of dopamine receptor and transporter binding data

	Male		Female	
Binding/region	**Control**	**DVD-Deficient**	**Control**	**DVD-Deficient**
D1 receptor				
CPu	470.5 ± 21.2	418.8 ± 16.1	455.6 ± 26.1	440.7 ± 22.8
Acb shell	415.2 ± 23.4	379.3 ± 23.2	414.4 ± 22.8	388.1 ± 29.0
Acb core	411.1 ± 20.8	372.1 ± 20.6	407.7 ± 25.0	390.5 ± 28.5
medial PFC	63.2 ± 2.3	59.4 ± 1.4	62.2 ± 2.4	66.3 ± 4.2
D2 receptor				
CPu	43.6 ± 3.8	43.4 ± 5.1	49.6 ± 3.9	51.7 ± 4.0
Acb shell	18.0 ± 1.7	20.0 ± 1.8	24.2 ± 3.8	22.6 ± 3.4
Acb core	21.4 ± 2.1	21.9 ± 2.6	24.1 ± 4.0	25.2 ± 3.6
DAT				
CPu	99.9 ± 6.4	98.9 ± 3.3	121.4 ± 8.1	118.3 ± 8.0
Acb shell	47.8 ± 3.1	46.5 ± 2.1	57.8 ± 3.8	55.4 ± 3.1
Acb core	66.8 ± 4.0	66.1 ± 4.0	80.8 ± 5.2	79.5 ± 5.2

Abbreviations: *DVD*, Developmental vitamin D; *D1*, Dopamine 1; *D2*, Dopamine 2; *DAT*, Dopamine transporter; *CPu*, Caudate Putamen; *Acb*, Nucleus Accumbens; *PFC*, prefrontal cortex.

Data is presented as mean ± S.E.M. of specific binding in fmol/mg.