Sensitivity to MK-801 in phospholipase C-β1 knockout mice reveals a specific NMDA receptor deficit

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Abstract

Phospholipase C-β1 (PLC-β1) is a critical component of multiple signalling pathways downstream of neurotransmitter receptors. Mice lacking this enzyme display a striking behavioural phenotype with relevance to human psychiatric disease. Glutamatergic dysfunction is strongly associated with several abnormal behavioural states and may underlie part of the phenotype of the phospholipase C-β1 knockout (KO) mouse. A heightened response to glutamatergic psychotomimetic drugs is a critical psychosis-related endophenotype, and in this study it was employed as a correlate of glutamatergic dysfunction. Control (n = 8) and PLC-β1 KO mice (n = 6) were treated with MK-801, a NMDA receptor (NMDAR) antagonist, following either standard housing or environmental enrichment, and the motor function and locomotor activity thus evoked was assessed. In addition, MK-801 binding to the NMDAR was evaluated through radioligand autoradiography in post-mortem tissue (on a drug-naive cohort). We have demonstrated a significantly increased sensitivity to the effects of the NMDA antagonist MK-801 in the PLC-β1 KO mouse. In addition, we found that this mouse line displays reduced hippocampal NMDAR expression, as measured by radioligand binding. We previously documented a reversal of specific phenotypes in this mouse line following housing in an enriched environment. Enrichment did not alter this heightened MK-801 response, nor NMDAR expression, indicating that this therapeutic intervention works on specific pathways only. These findings demonstrate the critical role of the glutamatergic system in the phenotype of the PLC-β1 KO mouse and highlight the role of these interconnected signalling pathways in schizophrenia-like behavioural disruption. These results also shed further light on the capacity of environmental factors to modulate subsets of these phenotypes.

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Introduction

Glutamatergic dysfunction is thought to be a key aspect underlying the aetiology of several psychiatric disorders, in particular schizophrenia (Coyle, 2006; Konradi & Heckers, 2003; Krystal et al. 2003; Moghaddam, 2003; Tamminga, 1998). The glutamate signalling system is intrinsic to both the motor regulation and higher brain functions affected in psychiatric disease (Goldman-Rakic & Selemon, 1997; Harrison, 1999; Lewis & Anderson, 1995; McCullumsmith & Meadow-Woodruff, 2002; Millan, 2005; Weinberger et al. 2001). Particularly striking is the ability of psychotomimetic drugs, such as phencyclidine (PCP)
and ketamine, to reproduce psychosis-like symptomatology, or exacerbate symptoms in patients, eliciting these symptoms through blockade of the NMDA receptor (NMDAR) an ionotropic glutamate receptor widely expressed in the brain (Adler et al. 1999; Javitt & Zukin, 1991; Krystal et al. 1994). Administration of NMDAR antagonists to mice results in a striking behaviour phenotype that is useful as both a model of certain psychiatric symptoms and as an index of glutamatergic function. Psychiatric endophenotypes can also be recapitulated in some genetic animal models. Mice lacking the constitutive subunit of the NMDAR, NR1, exhibit distinctive behavioural abnormalities (including hyperactivity) and display disrupted cortical development (Datwani et al. 2002a, b; Iwasato et al. 1997, 2000; Li et al. 1994).

A recent microarray study has identified PLC-β1 as being down-regulated in a cohort of individuals with schizophrenia (E. Thomas, personal communication) suggestive of a role in this disorder. PLC-β1, a second messenger directly activated by muscarinic, serotonergic and metabotropic glutamate receptors, cleaves PIP2 to form diacylglycerol (DAG) and inositol triphosphate (IP3). DAG stimulates protein kinase C (PKC), facilitating specific protein phosphorylation, whilst IP3 binds to its receptor, mobilizing intracellular stores of Ca2+ (Exton 1996; Nishizuka et al. 1998a, b). The increase in intracellular Ca2+ activates calcium-dependent protein kinases with the potential to phosphorylate numerous substrates including NMDARs, in turn initiating other cellular machinery including long-term changes such as long-term potentiation (LTP) and long-term depression (LTD). The role of PLC in NMDAR-dependent LTD has been demonstrated to be crucial in several neural regions including the hippocampus, visual and prefrontal cortex (Choi et al. 2005; Horne & Dell’Acqua, 2007; Otani et al. 2002; Reyes-Harde & Stanton, 1998). Hence, mice lacking the PLC-β1 isoform demonstrate virtually no NMDAR-dependent LTD (Choi et al. 2005). Notably, inhibitors of PLC activity inhibit glutamate neurotoxicity induced by overstimulation of NMDARs (Llansola et al. 2000).

Modelling environmental modulation of symptomatology and determining the underlying aetiology is particularly critical in psychiatric disorders as they are generally thought to result from complex interactions between genetic and environmental factors (Gray & Hannan, 2007). In a previous report, we described several behavioural disruptions in phospholipase C-β1 (PLC-β1) knockout (KO) mice including locomotor hyperactivity and impaired prepulse inhibition, in addition to cognitive deficits. The key endophenotypes of locomotor hyperactivity and sensorimotor gating deficits were reversed by exposure to environmental enrichment (EE) – an intervention which provides increased sensory and cognitive stimulation (Nithianantharajah & Hannan, 2006) – along with the expression of M1/M4 muscarinic acetylcholine receptors (Gray et al. 2008; McOmish et al. 2007). Nonetheless, EE was not sufficient to improve the cognitive performance of these animals prompting the conclusion that the cognitive deficits may result from an alternate mechanism. These differential responses call into question the role of NMDARs, as they have been strongly implicated in cognitive processes and have also been shown to indirectly regulate PLC-β1. A caveat to this line of thought is that studies in other animal models have demonstrated modulation of glutamatergic signalling in response to EE (Andin et al. 2007; Bredy et al. 2003; Mora et al. 2007; Naka et al. 2005; Tang et al. 2001) suggesting that disruptions resulting from NMDA dysfunction could be ameliorated following enriched housing.

Given that the PLC-β1 KO mouse has been demonstrated to exhibit several abnormal behaviours, we wished to extend these studies, assessing whether these mice also display the critical endophenotype of sensitivity to the glutamatergic psychostimulant MK-801. This analysis also sheds light on the interactions between PLC-β1 and the NMDAR, and the possible involvement of the NMDAR in the observed behavioural deficits in PLC-β1 KO mice. In addition, the ability of EE to modify other aspects of the PLC-β1 KO phenotype raises the possibility that this environmental modification may also regulate the glutamatergic system and therefore the response to the glutamate-mediated psychotropic drug MK-801. Hence, both standard-housed and enriched PLC-β1 mice, along with wild-type (WT) littermates, were administered MK-801, and the locomotion, stereotypy and ataxia thus evoked were measured.

Methods

Animals

The PLC-β1 KO line was bred from mice heterozygous for the null mutation, on a SV129/C57Bl6 background strain (Kim et al. 1997). Genotypes were determined by PCR (Hannan et al. 2001; Kim et al. 1997). Mice were housed in single-sex groups of 5–6, with food and water available ad libitum. The animal holding area was maintained on a 12-h light/dark cycle (lights on 07:30). Testing was performed blind to genotype, and all procedures were approved by the institutional Animal Ethics Committee.
Environmental enrichment

At weaning, mice for the environmental-enrichment cohort were separated into standard housing (SH) or environmentally enriched (EE) groups. SH groups were housed in standard mouse cages (34 x 16 cm), with bedding and nesting materials. EE groups were housed in larger cages (40 x 28 cm), with a variety of objects including tunnels, ladders, ropes, platforms and other toys. The items in EE boxes had varying texture, shape and size, and were changed weekly to maintain novelty. EE groups were also exposed, for 1 h five times per week, to a larger chamber (94 L) containing a greater variety of novel objects. A minimum of 5-wk enrichment was conducted before behavioural testing commenced; all mice were aged ≥8 wk at the point of first behavioural testing.

Behavioural tests

For the MK-801 sensitivity testing, locomotor behaviour was assessed following an acute dose of MK-801 (Sigma-Aldrich Pty Ltd, Australia), as described below. Mice were placed into photo-beam activity arenas (E63-10, TruScan Coulbourn Instruments, USA), and allowed to habituate for 30 min before the injection of saline or MK-801 (baseline). Activity was monitored for a further 60 min, and the resulting distance travelled quantified using TruScan 2.0 software (Coulbourn Instruments), and expressed as total distance moved after drug injection. To establish the optimal level of MK-801, a dose–response curve was generated using MK-801 at 0.02, 0.1, 0.2 and 0.3 mg/kg (Fig. 1).

To assess the levels of stereotypy and ataxia induced by MK-801, each animal received a single administration of saline, 0.1, or 0.3 mg/kg MK-801. Six KO and 7/8 WT animals were used in each drug group. An infrared camera was mounted above each photo-beam activity arena, allowing measurement of locomotor activity, stereotypy and ataxia from a single session. The recordings were assessed manually according to predetermined criteria (Table 1) (Harkin et al. 2001; Hashimoto et al. 2005; Wu et al. 2005). The assessor was blind to genotype and drug group. Both stereotypy and ataxia were assessed at 15-, 30- and 40-min post-injection, and the scores presented as the sum of the three time-points.

For the second cohort, assessing the effects of enriched housing, a single dose of 0.3 mg/kg was used, with naive animals receiving a single injection of either saline or MK-801. Six KO, four WT and four heterozygous mice were used in each group. Stereotypy and ataxia were assessed as described in Table 1.

[3H]MK-801 autoradiography

In-situ radioligand binding with autoradiography was performed upon a separate, drug-naive, cohort of SH and EE animals. Animals were euthanized by cervical dislocation. Whole brains were dissected out, immersed in Tissue Tek OCT (Sakura Finetek, Japan), frozen, and stored at −80 °C. Serial coronal sections (14 μm), were cut at −20 °C on a Leica CM 3050 S cryostat (Leica, Germany), mounted onto gelatin/chrome alum-subbed glass slides, and stored at −80 °C. Sections between bregma −1.46 and −2.06

Table 1. Analysis of stereotypy and ataxia – methods

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
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<tbody>
<tr>
<td>Stereotypy</td>
<td>Criteria</td>
</tr>
<tr>
<td>0</td>
<td>Normal movement patterns</td>
</tr>
<tr>
<td>1</td>
<td>Some repetitive grooming or sniffing (longer than 3 s continuously)</td>
</tr>
<tr>
<td>2</td>
<td>Severe repetitive grooming or sniffing with head weaving or circling</td>
</tr>
<tr>
<td>3</td>
<td>Intense and continuous grooming, sniffing, weaving or circling</td>
</tr>
<tr>
<td>4</td>
<td>Essentially immobile</td>
</tr>
</tbody>
</table>

Ataxia

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>Normal movement patterns</td>
</tr>
<tr>
<td>1</td>
<td>Slightly unsteady gait; some spreading of hind limbs</td>
</tr>
<tr>
<td>2</td>
<td>Clearly unsteady gait; falling whilst walking or rearing</td>
</tr>
<tr>
<td>3</td>
<td>Inhibited movement, twitching or convulsive movements</td>
</tr>
<tr>
<td>4</td>
<td>Essentially immobile</td>
</tr>
</tbody>
</table>
were selected for in-situ radioligand binding and autoradiography.

Binding was performed as previously described (Pavey et al. 2002; Scarr et al. 2005). Briefly, [³H]MK-801 binding was carried out in 50 mM Tris–acetate buffer (pH 7.4), containing 100 µM glutamate, 50 µM glycine and 50 µM spermidine. Each animal was assessed using quadruplicate sections with n = 4–8 per group. The affinity of radioligand binding to mouse CNS was first determined using six concentrations of [³H] radioligand (1, 2, 4, 8, 16, 32 nM). Non-specific binding for [³H]MK-801 was taken as the binding of the radioligand in the presence of non-radiolabelled MK-801. The affinity and density for [³H]MK-801 were established from the concentration curve and calculated using the Hill equation. A concentration of three times the Kₐ value was used to ensure saturation binding. Thus, in the single point saturation analyses in WT and KO mice, the concentration of [³H]MK-801 was 10 nM.

Sections were air-dried for 60 min at room temperature, prior to incubation in buffer for 30 min. Sections were again air-dried preceding incubation in [³H]MK-801 (for total binding) or [³H]MK-801 + MK-801 for non-specific binding. All sections were incubated at room temperature for 60 min, washed 2 × 2 min in buffer (4°C), rinsed in ice-cold H₂O then dried under a stream of cool air. All slides were fixed in a desiccator containing paraformaldehyde vapour, and aspoed to Fuji BAS tritium-sensitive phosphor imaging plates (Fuji Photo Film Co., Japan). Following 6 d exposure time, plates were read using the Fujifilm BAS-5000 bioimaging analyser (Fuji Photo Film Co.), and densities were assessed using the AIS image analysis system, normalized using ³H microscales (Amersham Pharmacia Biotech AB, Sweden). Coronal sections were chosen to include a hippocampal cross-section, in addition to the most caudal area of the caudate putamen. Brain regions were selected for analysis based upon the binding pattern of the radioligand.

Statistics

All data were assessed for normality using the Kolmogorov–Smirnov test. A two-way ANOVA was employed for analysis of locomotor activity in response to MK-801, with Tukey’s post-hoc tests. Assessment of stereotypy and ataxia generated ordinal data so non-parametric Mann–Whitney tests were employed. For the enrichment cohort, both WT and heterozygous mice were included in the control group. Heterozygous mice do not display a phenotype at either the behavioural or molecular level (McOmish et al. 2007); however, heterozygote data was initially compared to that from WT mice for each test, with no variation observed. The WT and heterozygote data was subsequently combined. Assessment of MK-801-induced locomotion required a three-way ANOVA, followed by Tukey’s post-hoc tests. Again, Mann–Whitney tests were employed for the stereotypy and ataxia data. Autoradiographic data was analysed by two-way ANOVA followed by Tukey’s post-hoc tests for pairwise analyses.

Results

MK-801-induced locomotor activity

Measurement of MK-801-induced locomotor activity was conducted in a naive cohort with simultaneous recording for the assessment of stereotypy and ataxia. The KO mice showed significant hyperactivity compared to WT animals both at baseline (data not shown) and after a saline injection (Fig. 2; two-way ANOVA, genotype: F₃₄ = 25.74, p < 0.0001; drug: F₂₄ = 4.07, p = 0.026; interaction: F₂₄ = 11.31, p = 0.0002. WT saline vs. KO saline, Tukey’s multiple comparison test: p < 0.05). MK-801 administration induced elevated activity in the WT animals at 0.3 mg/kg (Tukey’s test, p < 0.05). However, MK-801 administration induced a significant reduction in activity in the KO animals, at both 0.1 and 0.3 mg/kg (Tukey’s tests, KO saline vs.
KO 0.1 or 0.3 mg/kg, p < 0.05). No effect of sex was observed (F = 0.254, p = 0.618).

**MK-801-induced stereotypy and ataxia**

KO animals showed significantly higher levels of stereotypy than WT animals in response to both 0.1 and 0.3 mg/kg MK-801 (Fig. 3a; Mann–Whitney test: 0.1 mg/kg, p = 0.009; 0.3 mg/kg, p = 0.009). Similarly, the levels of ataxia were elevated in KO mice at both 0.1 mg/kg (Fig. 3b; Wilcoxon signed rank test, p = 0.035), and 0.3 mg/kg (Mann–Whitney test: 0.3 mg/kg, p = 0.003); in fact no ataxia was observed in WT mice in response to the lower dose. No stereotypy or ataxia was observed in either WT or KO mice prior to injection or in response to saline (data not shown). No effect of sex was observed on either stereotypy (p = 0.853) or ataxia (p = 0.552).

**EE and MK-801 response in PLC-β1 KO mice**

Assessment of the response to 0.3 mg/kg MK-801 was conducted in a naive cohort after 5 wk of EE. As observed previously (McOmish et al. 2007), EE induced significant changes in distance travelled (Fig. 4; three-way ANOVA, housing: F_{1,49} = 4.90, p = 0.032). Namely, EE significantly decreased the locomotor hyperactivity observed in KO animals, while having no clear effect upon control mice (main effect, genotype × drug: F_{1,49} = 22.05, p < 0.0001). The main effects of genotype and drug were not significant (genotype: F_{1,49} = 0.40, p = 0.532; drug: F_{1,49} = 0.83, p = 0.367); however, the interaction between housing and drug response approached significance (F_{1,49} = 3.77, p = 0.058).

SH saline-treated KO mice displayed significant hyperactivity compared to SH WT mice (Tukey’s
multiple comparison test, \( p < 0.05 \)). Housing the KO animals in an enriched environment resulted in a suppression of activity relative to SH KO mice (McOmish et al. 2007), at both baseline (data not shown), and after a saline injection (Tukey’s test, \( p < 0.05 \)). Enrichment therefore reversed the locomotor hyperactivity of KO mice, but did not alter control group activity.

The SH mice responded to MK-801 as described in the previous cohort, with control mice displaying a mild elevation of activity, whilst the KO group displayed a significant suppression (\( p < 0.05 \)). Enrichment did not alter the WT response to MK-801, with significant hyperactivity observed (\( p < 0.05 \)). Administration of MK-801 to the enriched KO mice did not alter the distance moved, indicating that the drug does not suppress locomotor activity in KO mice and that EE does not improve KO responsiveness to MK-801. No effect of sex on locomotor activity was observed (\( F_2.467, p = 0.124 \)).

In both SH and EE groups, the KO mice demonstrated higher levels of stereotypy in response to MK-801 than WT mice (Fig. 5a; Mann–Whitney tests, SH control vs. KO: \( p = 0.0062 \); EE control vs. KO: \( p = 0.0007 \)). No effect of housing was observed. The same pattern was observed for ataxia (Fig. 5b), with KO mice displaying heightened ataxia in response to MK-801 compared to controls (Mann–Whitney tests, SH control vs. KO: \( p = 0.0062 \); EE control vs. KO: \( p = 0.0007 \)). Again, no effect of housing condition was observed. No stereotypy or ataxia was observed in any group prior to injection or in response to saline (data not shown). No effect of sex was observed on either stereotypy (\( p = 0.627 \)) or ataxia (\( p = 0.602 \)).

**EE and NMDAR expression in PLC-\( \beta \)1 KO mice**

Binding was assessed in 12 hippocampal regions; the pyramidal, radiatum and oriens layers of cornu ammonis (CA) 1, 2 and 3 were measured, in addition to the lacunomus, and the granular and molecular layers of the dentate gyrus (Fig. 6). With the exception of the pyramidal layer of CA3, all regions demonstrated a significant effect of genotype with no statistically significant effect of environment, as described in Table 2, revealing significant differences between WT and PLC-\( \beta \)1 KO animals. Overall a decrease in NMDAR binding by tritiated MK-801 is observed throughout the hippocampus; however, this effect is most strongly observed in the oriens layer of CA and the granular layer of dentate gyrus, where significant differences between PLC-\( \beta \)1 KO animals and their WT littermates are observed under both SH and EE conditions. No significant impact of environment was observed. MK-801 binding in the cortex and striatum showed no significant changes either by genotype or by environment (Table 2).

**Discussion**

The present study has documented a striking hypersensitivity to the NMDA antagonist MK-801 in PLC-\( \beta \)1 KO mice. Providing critical insight into the interactions between PLC-\( \beta \)1 and the NMDAR, this suggests that at least part of the behavioural disruption in PLC-\( \beta \)1 KO mice is due to abnormal glutamatergic signalling.

Our initial finding in the present study demonstrated a significant reduction in activity in response to
even very low doses of MK-801 in KO mice, whilst WT mice responded with the expected hyperactive response. This reduced activity corresponded with substantially higher levels of stereotypy and ataxia in KO animals, which displayed a severe lack of coordination and eventual paresis and immobility. This behavioural response to MK-801 is similar to that documented in WT animals at much higher doses (Frantz & Van Hartesveldt, 1999; Irifune et al. 1995), and represents a substantial leftward shift of the dose–response curve. Importantly, the observed hypersensitivity to MK-801 correlates with a significant reduction in hippocampal NMDAR binding in KO animals. This suggests that a reduced baseline level of hippocampal NMDAR activity might prime KO mice to increased responsiveness to the effects of the antagonist.

PLC-β1 is a critical component of the signalling pathways downstream of NMDA (Choi et al. 2005; Horne & Dell’Acqua, 2007; Otani et al. 2002; Reyes-Harde & Stanton, 1998). [3H]MK-801 binds the open, active form of the NMDAR, thus its binding levels are an indirect correlate of the functional activity of this receptor. In the absence of PLC-β1, the NMDAR is either reduced in expression or in a less active state. This outcome may be mediated by interactions between the NMDAR and other receptors with which PLC-β1 is directly coupled, including the metabotropic glutamate receptor 5 (mGluR5) (Chuang et al. 2001). Disruption of mGluR5 signalling results in altered NMDAR activity (Gray et al. 2009; Lecourtier et al. 2007; Nevian & Sakmann, 2006); hence the absence of PLC-β1 may result in altered mGluR5 signalling...

Fig. 6. [3H]MK-801 autoradiography in the brains of PLC-β1 and control mice under standard housing (SH) and environmentally enriched (EE) conditions. (a) [3H]MK-801 binding revealed a striking pattern across the brains of the mice allowing analysis of cortex and striatum, as well as the hippocampus including the distinction of the pyramidal, oriens and radiatum layers. (b, c) No significant differences in estimated tissue equivalents (ETE) were observed for genotype or environment in the cortex or the striatum. (d, e) An overall effect of genotype was observed in the pyramidal layer of CA1 and CA2 (*p < 0.05). Pairwise comparison revealed a significant difference between SH wild-type (WT) and SH knockout (KO) in CA2 (c) (*p < 0.05); however, no pairwise comparisons returned significant for the pyramidal layer of CA1. (f) No differences were documented in the pyramidal layer of CA3. (g–i) Significant overall effects of genotype were observed in the oriens layer of CA1 (*p < 0.001), CA2 (p < 0.001) and CA3 (p < 0.001). Pairwise comparisons confirmed significant differences between SH WT and SH KO...
As the imaging process differed slightly. In particular, the buffer used by Bohm and colleagues (2002) did not include co-stimulatory elements for NMDARs such as glycine, affording a less direct correlate of receptor functionality that may be more influenced by agonal state. Nonetheless, the observation of glutamatergic dysfunction parallels our findings.

Our observation that EE had no effect upon NMDAR binding in the cortex or hippocampus, whilst in agreement with our behavioural data, was unexpected in the context of the hypothesized role of glutamatergic signalling in eliciting the positive effects of enrichment.

### Table 2. Analysis of MK-801 autoradiographic binding

<table>
<thead>
<tr>
<th>Region</th>
<th>Two-way ANOVA</th>
<th>Tukey’s <em>post-hoc</em> multiple comparison tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotype Environment</td>
<td>SH WT vs. EE WT vs. EE WT vs. EE KO vs. SH KO</td>
</tr>
<tr>
<td>CA1 Pyr</td>
<td>$p &lt; 0.05$ $F_{1,13} = 6.21$ $F_{1,13} = 1.35$</td>
<td>$p = 0.05$ $p = 0.156$ $p = 0.488$ $p = 0.386$</td>
</tr>
<tr>
<td>CA1 Rad</td>
<td>$p = 0.01$ $F_{1,13} = 14.8$ $F_{1,13} = 0.84$</td>
<td>$q = 2.96$ $q = 2.09$ $q = 1.00$ $q = 1.31$</td>
</tr>
<tr>
<td>CA1 Orn</td>
<td>$p = 0.001$ $F_{1,13} = 33.5$ $F_{1,13} = 0.02$</td>
<td>$q = 0.001^{***}$ $p = 0.012$ $p = 0.622$ $p = 0.130$</td>
</tr>
<tr>
<td>CA2 Pyr</td>
<td>$p = 0.05$ $F_{1,13} = 6.01$ $F_{1,13} = 1.41$</td>
<td>$q = 7.96$ $q = 3.89$ $q = 2.03$ $q = 1.39$</td>
</tr>
<tr>
<td>CA2 Rad</td>
<td>$p = 0.01$ $F_{1,13} = 13.3$ $F_{1,13} = 0.04$</td>
<td>$q = 0.01^{**}$ $p = 0.066$ $p = 0.678$ $p = 0.568$</td>
</tr>
<tr>
<td>CA2 Orn</td>
<td>$p = 0.001$ $F_{1,13} = 23.7$ $F_{1,13} = 0.22$</td>
<td>$q = 6.54$ $q = 3.41$ $q = 1.94$ $q = 0.71$</td>
</tr>
<tr>
<td>CA3 Pyr</td>
<td>$p = 0.067$ $F_{1,13} = 3.77$ $F_{1,13} = 3.20$</td>
<td>n.a. n.a. n.a. n.a.</td>
</tr>
<tr>
<td>CA3 Rad</td>
<td>$p = 0.001$ $F_{1,13} = 0.432$</td>
<td>$p = 0.01^{**}$ $p = 0.052$ $p = 0.661$ $p = 0.181$</td>
</tr>
<tr>
<td>CA3 Orn</td>
<td>$p = 0.001$ $F_{1,13} = 32.9$ $F_{1,13} = 0.04$</td>
<td>$q = 6.23$ $q = 2.94$ $q = 0.63$ $q = 1.96$</td>
</tr>
<tr>
<td>DG Mol</td>
<td>$p = 0.05$ $F_{1,13} = 7.82$ $F_{1,13} = 0.01$</td>
<td>$q = 0.001^{*<strong>}$ $p = 0.01^{</strong>}$ $p = 0.112$ $p = 0.970$</td>
</tr>
<tr>
<td>DG Gran</td>
<td>$p = 0.01$ $F_{1,13} = 12.3$ $F_{1,13} = 0.48$</td>
<td>$q = 7.27$ $q = 4.39$ $q = 2.36$ $q = 0.05$</td>
</tr>
<tr>
<td>Lacunosum</td>
<td>$p = 0.05$ $F_{1,13} = 4.48$ $F_{1,13} = 0.51$</td>
<td>$q = 0.05^{*}$ $q = 0.787$ $q = 0.441$ $q = 0.137$</td>
</tr>
<tr>
<td>Cortex</td>
<td>$p = 0.562$ $F_{1,13} = 0.347$ $F_{1,13} = 2.08$</td>
<td>n.a. n.a. n.a. n.a.</td>
</tr>
<tr>
<td>Striatum</td>
<td>$p = 0.307$ $F_{1,13} = 1.09$ $F_{1,13} = 1.99$</td>
<td>n.a. n.a. n.a. n.a.</td>
</tr>
</tbody>
</table>

SH WT, Standard housed wild-type; SH KO, standard housed knockout; EE WT, environmentally enriched wild-type; EE KO, environmentally enriched knockout.

Regions described include: molecular layer of the dentate gyrus (DG Mol); granular layer of the dentate gyrus (DG Gran); pyramidal layers of CA1, CA2, CA3 (CA1 Pyr, CA2 Pyr, CA3 Pyr, respectively); radiatum layers of CA1, CA2, CA3 (CA1 Rad, CA2 Rad, CA3 Rad, respectively); oriens layers of CA1, CA2, CA3 (CA1 Orn, CA2 Orn, CA3 Orn); lacunosum, cortex and striatum.

Where the two-way ANOVA performed revealed a significant effect, the relevant pairwise comparisons were performed.

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Capability, which in turn may flow through to changes in NMDAR function.

An earlier study of a second, independently generated, line of PLC-β1 KO mice demonstrated a reduction in NMDARs in the CA1 region (Bohm et al. 2002), which is in agreement with our results in this hippocampal region. These authors also found a deficit in spatial memory, but did not find alterations in NMDARs in other hippocampal subregions. This difference may be attributed to experimental technique, as the imaging process differed slightly. In particular,
EE has been shown to up-regulate certain subunits of the NMDAR, as well as other components of the glutamatergic system (Andin et al. 2007; Naka et al. 2005). However, what is currently uncertain, is to what extent this translates to NMDAR function. While some authors are in agreement with our findings, others have demonstrated that enrichment does not directly alter NMDAR function; Lehola et al. (2004) found that an enriched environment did not alter NMDAR function in the barrel cortex of rats.

In both this and a previous study (McOmish et al. 2007), a reduction in baseline PLC-β1 KO hyperlocomotion was observed in response to EE; confirming the efficacy and reproducibility of the intervention. However, whilst the reduction in baseline activity masked the inhibitory effects of MK-801 administration in the enriched KO mice, the level of stereotypy and ataxia evoked by MK-801 remained unchanged. It therefore appears that whilst EE can modulate some aspects of the PLC-β1 KO phenotype, the sensitivity to glutamatergic antagonists was not ameliorated. This dissociation confirms that the aberrant phenotype caused by the PLC-β1 null mutation is mediated by multiple pathways that are differentially responsive to the effects of enrichment. More specifically, the inability of EE to alter NMDAR binding strengthens the conclusion that the environmental rescue in PLC-β1 KO mice is specific to muscarinic M1/M4 receptors (McOmish et al. 2007), which show a high level of dependence upon PLC-β1 for neurotransmission in the adult brain (Hannan et al. 2001; Kim et al. 1997). These findings may go some way towards explaining how and why the symptomatology of disorders like schizophrenia, which are dependent upon environmental contribution, may manifest differently in disparate individuals. It emphasizes how environmental modulation of phenotype may variably alter the manifestation of symptomatology, alleviating some symptoms while not affecting others.

We have previously observed that whilst enrichment restored other aspects of the behavioural phenotype, it did not ameliorate the cognitive deficits, suggesting that NMDAR dysfunction could be the critical underlying factor (McOmish et al. 2007). CA1- and dentate gyrus-specific NMDAR KO animals show deficits in spatial memory (McHugh et al. 2007; Rampon et al. 2000), and the activation of PLC by NMDAR is required for the structural and functional changes underlying LTP (Horne & Dell’Acqua, 2007). Reinforcing the putative link between NMDAR deficit and learning and memory dysfunction are the findings that enrichment neither reversed the cognitive deficits nor restored NMDAR expression levels.

It should be noted that a critical limitation of this work is the inclusion of both male and female animals. This was necessitated by the limited availability of KO mice, but limits the capacity to observe subtle variations that may occur between sexes or across the oestrous cycle. All animals were group-housed, which raises the possibility of synchronization of oestrus cycles through Whitten or McClintock effects (McClintock, 1971; Whitten, 1957). In this case the effect of cycling should be minimal. Alternatively, were they not aligned and the effect of oestrous stage on MK-801 response significant, one may have expected variation in the response to drug administration across the multiple days of testing conducted for the dose–response curve. Figure 1 shows repeated treatment of MK-801 conducted at 1-wk intervals (groups were randomized). The response to MK-801 was consistent within the WT and KO groups, regardless of the day tested, suggesting that any variation with stage of the oestrous cycle was minimal. The finding of heightened response to MK-801 in KO mice is in accordance with both our autoradiographic findings and previously published findings in a distinct PLC-β1 KO mouse line (Bohm et al. 2002), where the sex of the animals used was not recorded. No effect of sex was observed within our studies, but the small number of animals within each subgroup, means that the potential effect of sex must remain as the subject of further investigation.

This specific model, the PLC-β1 null mouse, is of particular interest in the context of a recent microarray study that has identified PLC-β1 as being down-regulated in a schizophrenia cohort (E. Thomas, personal communication). This study provides us with some insight as to how disruption of this gene may result in abnormal behaviours that result from disruption of multiple neurotransmitter systems. These findings suggest that the sensitivity to the psychotomimetic drug MK-801, along with the previously described memory deficits observed in PLC-β1 KO mice are mediated by dysfunction in hippocampal glutamatergic signalling via NMDARs. Furthermore, EE is not sufficient to redress the deficit, emphasizing the notion that disruption in a single gene may result in divergent representation of multiple endophenotypes, with variable dependence upon environmental factors.

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Statement of Interest

None.

References


MK-801 sensitivity reflects reduced NMDARs in PLC KOs

