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Laminar analysis of the role played by GluR1 in experience-dependent and synaptic depression of sensory responses in barrel cortex

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Abstract

Several synaptic depression mechanisms have been described for the hippocampus, cerebellum and neocortex *in vitro*, but little is known about which if any are engaged during experience-dependent depression (EDD). Here we show that EDD in the barrel cortex depends on the AMPA subunit GluR1 in layers II/III and IV but not layer V and that LTD is also GluR1 dependent in the IV -II/III but not the II/III -V pathway.

Sensory deprivation can cause a long-lasting depression of sensory responses to stimulation of the deprived input in both the visual and barrel cortices. The mechanisms underlying experience dependent depression (EDD) are not well understood but are thought to be synaptic and may rely on similar mechanisms to LTD. However, there are a large number of candidate LTD mechanisms including NMDA, mGluR and cannabinoid dependent forms and expression can occur via changes in the phosphorylation states of GluR1, GluR2, NMDA receptors, presynaptic release and internalisation of AMPA receptors. As a simple test of whether a GluR1 dependent process is involved in EDD we looked at the effect of whisker deprivation in GluR1 knockout mice^{1, 2}.

To study depression in cortical layer II/III (LII/III) in the absence of depression in layer IV (LIV) we spared a single whisker and removed the others unilaterally. This form of depression is specific to the deprived whisker, dependent on cortical activity, does not occur in the thalamus or cortical LIV when induced at 4 weeks of age, but is present in layers II/III³. In wild-types, principal whisker responses were depressed to 48.3% of control undeprived values (mean±sem = 58.4±5.1 spikes/50 stimuli for undeprived wild-types; 28.2±7.2 for deprived wild-types, $Q=2.1$, $\alpha<0.05$). Cells in barrels located far from the spared barrel were depressed as strongly as those located closer (Supplementary Figure 1B). However, in GluR1 knockouts, principal whisker responses were indistinguishable from undeprived values at 98.9% of control levels (mean±sem = 64.9±7.2 spikes/50 stimuli for undeprived GluR1 knockouts; 64.2±6.5 for deprived GluR-1 knockouts). In contrast, LIV responses were the same in deprived and undeprived animals for both wild-types (101%) and GluR1 knockouts (104%) (Figure 1).

To test whether plasticity was GluR1 dependent in LIV we used chessboard pattern of deprivation (Figure 2). As a preliminary analysis, we ran a 3-way ANOVA on the data (total df=76), which showed an effect of genotype ($F(1,1) = 4.7$, $p<0.05$) and deprivation ($F(1,1) = 4.1$, $p<0.05$) but no effect of layer ($F(1,1)=0.14$, $p=0.71$). While depression occurred in LIV

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of wild-types (72%), there was no depression in GluR1 knockouts (95%). Short-latency responses appeared to be slightly depressed in deprived wild-types at 82% of undeprived levels, but were not significantly different from control values ($F(1,54) = 1.5$, $p = 0.22$), while long-latency responses (≥ 10 ms) were depressed to 65% of undeprived levels, which was statistically significant ($F(1,52) = 5.6$, $p < 0.05$).

Responses were also depressed in LII/III of wild-types but not in GluR1-knockouts. Deprived principal whisker responses were depressed to 68.1% of undeprived levels (mean $\pm$ sem = 62.1 ± 5.3 spikes/50 stimuli for undeprived; 42.3 ± 3.8 for deprived wild-types), which was statistically significant ($Q = 1.99$, $\alpha < 0.05$), while in GluR1 knockouts, deprived principal whisker responses were indistinguishable from undeprived levels (96.2%) (mean $\pm$ sem = 64.6 ± 6.5 spikes/50 stimuli for undeprived; 62.1 ± 4.3 for deprived GluR-1 knockouts, Figure 2).

While EDD was prevented in GluR1 knockouts, potentiation was not (Figure 2B). Responses from the two spared whiskers evoking the most spikes in each cell were significantly greater in deprived barrel-columns than in undeprived animals, independent of genotype. A two way ANOVA for spared surround whisker responses revealed an effect of deprivation for S1 ($F(1,1) = 7.5$, $p < 0.02$), and S2 ($F(1,1) = 5.69$, $p < 0.05$) but not of genotype ($p > 0.4$ in both cases). Averaged across genotypes the S1 whisker potentiated almost 2 fold from 28.2 ± 3.2 spikes/50 stimuli to 53.7 ± 8.0 . The potentiated value for S1 was not different between GluR1 knockouts (mean $\pm$ sem = 49.4 ± 9.7 spikes/50 stimuli) and wild-types (57.6 ± 12.8 ; $t(17) = 0.5$, $p = 0.62$). Although GluR1 is normally important for experience-dependent potentiation⁴, nitric oxide dependent potentiation may compensate in GluR1 knockout animals⁵.

Finally, we also tested depression in layer V. A two way ANOVA (total df = 21) revealed an effect of deprivation ($F(1,1) = 23.52$, $p < 0.0001$) but not of genotype ($F(1,1) = 0.02$, $p = 0.88$) indicating that depression occurred in both wild type and GluR1 knockouts (Figure 2A). In wild-types, the principal whisker response was reduced to 32% of undeprived control levels by deprivation (undeprived mean $\pm$ sem = 43.4 ± 4.7 , deprived = 14.9 ± 5.8) similar to the levels of depression seen in GluR1 knockouts of 47.5% (undeprived mean = 40.1 ± 5.2 , deprived = 19.0 ± 4.3).

Internal controls in whisker deprived mice indicated that differences in depression were not attributable to systematic differences in anaesthesia between wild-types and GluR1-knockouts (Supplementary Figure 1 and Results). We also found no developmental abnormalities in either the receptive field structure or the overall architecture of the barrel cortex in GluR1 knockout mice (Supplementary Figure 2 and Results).

EDD and LTD may be related mechanistically because sensory deprivation occludes LTD in both visual and barrel cortex^{6, 7}. Therefore to test whether LTD mechanisms might also differ between layers, we compared LTD in the projection from LIV-II/III and LII/III-V, within the same barrel-column. In LII/III, an LTD spike-pairing protocol produced depression to $47 \pm 6\%$ below baseline in wild-types and $13 \pm 8\%$ in GluR1 knockouts (Supplementary Figure 3A,B). Depression in wild-types was significantly greater than in GluR1 knockouts ($t(50) = 3.3$, $p < 0.002$) and the level of depression in the GluR1 knockouts was not statistically significant ($t(54) = 1.7$, $p = 0.1$). In layer V, EPSPs depressed $37 \pm 6\%$ below baseline in wild-types and this was very similar to that seen in GluR1 knockout animals $40 \pm 6\%$ (Supplementary Figure 3C,D). LTD in wild-types was not significantly different from LTD in GluR1 knockouts ($t(29) = 1.18$, $p = 0.25$), but was highly significant from baseline ($t(29) = 5.22$, $p < 0.0001$) in both cases. These data suggest a difference in the

dependence of LTD on GluR1 in the two pathways might contribute to the difference in the level of experience-dependent depression seen in LII/III compared to layer V *in vivo*.

Synaptic transmission was normal in the GluR1 knockout animals both *in vivo* (Supplementary Figure 2 and Results) or *in vitro*⁵, which suggests that other glutamate receptor subunits might compensate for GluR1. To check for a difference in GluR expression levels we measured receptor expression using quantitative RT-PCR (Supplementary Figure 4). We found no difference in endogenous levels of expression in GluR2, GluR3 or GluR4 between undeprived knockouts and wild-type animals ($F(1,1) = 0.12$, $p = 0.73$). In general we found that whisker deprivation down-regulated expression of GluRs in both genotypes rather than compensating for the loss of GluR1. GluR3 levels were down regulated less in knockouts than in wild-types, which might contribute to a lack of depression. We cannot rule out differences in protein levels or in the distribution of GluRs as further contributory compensatory factors.

Are there plausible mechanisms by which GluR1 could affect synaptic depression? GluR1 phosphorylation could control depression by controlling channel conductance^{8, 9} and/or the dynamics of GluR endocytosis¹⁰. In the visual cortex, LTD causes dephosphorylation of the Serine 845 PKA site on the GluR1 subunit⁶, PKA is involved in ocular dominance plasticity¹¹ and studies in GluR1 point mutants show that both the serine 831 and the serine 845 site are required for LTD in LII/III cells¹². However, this is the first study to provide direct evidence for a causal role of GluR1 in EDD mechanisms *in vivo*. A cannabinoid dependent depression mechanism may not have been evident in these studies because it requires presynaptic NMDA receptors^{13, 14}, which are probably limited to early development and are not present in cortex beyond approximately P20¹⁵. In this study, mice were at least P26 at the start of deprivation. Therefore GluR1 dependent EDD would appear to be involved more in adult than developmental plasticity. In support of this idea, we did not find any developmental abnormalities in either the receptive field structure or the overall architecture of the barrel cortex in GluR1 knockout mice (Supplementary Figure 2 and Results).

In conclusion, this study provides the first evidence of a role for GluR1 in EDD in the neocortex *in vivo* and in addition reveals a major difference in depression mechanisms operating in different cortical layers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. The effect of the GluR1 knockout on principal whisker depression in animals subject to single whisker experience (D1 only) for 7 days

A. Response magnitude is unaffected by deprivation in layer IV in wild-type (grey bars) and GluR1 knockout animals (white bars). However, in layer II/III, responses are decreased in wild-types but unaffected in GluR1 knockouts. Dashed lines indicate control undeprived levels for wild-types in each layer (data averaged across animals; bars represent means $\pm$ sem). **B.** Cumulative distribution functions for responses of individual layer II/III cells pooled across animals. Again only the wild-type principal whisker responses show depression (curve shifted to the left). **C.** Distribution of penetrations for cells located in control and deprived animals of both genotypes, colour coded for response magnitude of layer II/III cells to principal whisker stimulation. Colour code: yellow = 1 spike per stimulus or more, green = 0.5 to 0.99 spikes per stimulus, blue = 0.1 to 0.49 spikes per stimulus. Dark grey indicates the barrel with the spared whisker (D1).

Figure 2. The effect of the GluR1 knockout on principal whisker depression in chessboard pattern deprived animals (7 days deprivation)

A. In wild-type mice (grey bars), whisker response magnitude is decreased in layer II/III, IV and V of deprived animals. In GluR1 knockout mice (white bars), responses are unaffected by deprivation in layers II/III and IV but depressed in layer V. Dashed lines indicate control undeprieved levels for each layer in wild-types (data averaged across animals; bars represent means $\pm$ sem). **B.** Responses of cells located in deprived barrel-columns to stimulation of spared surround whiskers are shown for undeprieved (grey lines, white squares) and chessboard deprived cases (black lines, black squares). Responses for each cell are ranked from the strongest (S1) to weakest (S5) surround whisker for each cell and then averaged across cells. Note that potentiation occurs in Wild-type (top) and GluR1 (bottom) animals by similar amounts. **C.** Distribution of penetrations for cells located in control and deprived animals of both genotypes. Colour codes the same as for Figure 1, except that in this Figure, dark grey indicates deprived barrels.