Reduced expression of the psychiatric risk gene DLG2 (PSD93) impairs hippocampal synaptic integration and plasticity

Simonas Griesius1, Cian O’Donnell2, Sophie Waldron3,4, Kerrie L. Thomas3,5, Dominic M. Dwyer3,4, Lawrence S. Wilkinson3,4,6, Jeremy Hall3,5,6, Emma S. J. Robinson3,4, and Jack R. Mellor1

© The Author(s) 2022

INTRODUCTION

Genetic variations at the DLG2 gene locus are linked to multiple psychiatric disorders including schizophrenia [1, 2], bipolar [3, 4], autism spectrum disorder, and intellectual disability [5–7]. Reduced expression of the psychiatric risk gene DLG2 (PSD93) that interacts with NMDA receptors, potassium channels, and cytoskeletal regulators but the net impact of these interactions on synaptic plasticity, likely underpinning cognitive impairments associated with these conditions, remains unclear. Here, hippocampal CA1 neuronal excitability and synaptic function were investigated in a novel clinically relevant heterozygous Dlg2+/− rat model using ex vivo patch-clamp electrophysiology, pharmacology, and computational modelling. Dlg2+/− rats had reduced supra-linear dendritic integration of synaptic inputs resulting in impaired associative long-term potentiation. This impairment was not caused by a change in synaptic input since NMDA receptor-mediated synaptic currents were, conversely, increased and AMPA receptor-mediated currents were unaffected. Instead, the impairment in associative long-term potentiation resulted from an increase in potassium channel function leading to a decrease in input resistance, which reduced supra-linear dendritic integration. Enhancement of dendritic excitability by blockade of potassium channels or activation of muscarinic M1 receptors with selective allosteric agonist 77-LH-28-1 reduced the threshold for dendritic integration and 77-LH-28-1 rescued the associative long-term potentiation impairment in the Dlg2+/− rats. These findings demonstrate a biological phenotype that can be reversed by compound classes used clinically, such as muscarinic M1 receptor agonists, and is therefore a potential target for therapeutic intervention.

Neuropsychopharmacology; https://doi.org/10.1038/s41386-022-01277-6
of the gene but these do not accurately represent the heterozygous nature of DLG2 genetic variants in patient populations and potentially engage compensatory expression by other MAGUK proteins [32, 38] that is not present in heterozygous reduced gene dosage models (Supplementary Fig. S3). Therefore, here we investigate the combined impact of low gene dosage DLG2 on synaptic function, neuronal excitability and morphology using a novel CRISPR-Cas9 engineered heterozygous Dlg2+/− (het) rat model to understand the interactions that lead to impaired synaptic plasticity and cognitive function.
in hippocampus without effects on expression of other components of the postsynaptic density, including the closely related MAGUK DLG4 (PSD95) and the GluN1 NMDAR subunit (Supplementary Fig. S3). The specific reduction in DLG2 protein expression was replicated in tissue from prefrontal cortex, posterior cortex and cerebellum and mirrored by a ~50% reduction of dlg2 mRNA expression, without any change in dlg1, dlg3 or dlg4 mRNA [39].

**Associative LTP**

The learning of novel representations in CA1 is thought to arise from the dendritic integration of spatiotemporally coherent inputs from the entorhinal cortex (via the temporoammonic (TA) pathway) and CA3 (via the Schaffer collateral (SC) pathway) that can summate supralinearly to drive associative LTP (aLTP) [40–47]. LTP in CA1 of hippocampus is impaired in homozygous Dlg2−/− mice [33] but the interpretation of these results is complicated by the potential for compensation by other MAGUK proteins [32, 38]. The heterozygous Dlg2+−/− rat offers the opportunity to test whether LTP is impaired in the absence of any MAGUK compensation.

aLTP was assessed in the CA1 region of hippocampal slices by stimulating the SC and TA pathways simultaneously with a theta burst stimulation pattern whilst recording from CA1 pyramidal neurons (Supplementary Fig. 1A, B). An additional independent SC pathway was also stimulated as a negative control and a pathway check was done to confirm pathway independence (Supplementary Fig. S4). The induction protocol resulted in robust aLTP in the wts but reduced aLTP in the Dlg2+/− hets in both SC and TA test pathways (Fig. 1C–E). During induction the number of elicited action potential bursts and single spikes was reduced in the Dlg2+/− hets, despite baseline EPSC amplitudes being unchanged indicating that all neurons received similar inputs, but there was a trend suggesting reduced overall depolarisation in response to synaptic stimulation (Fig. 1F–J). Both spike number and depolarisation during induction correlated with LTP in the SC pathway but not the TA pathway (Supplementary Fig. S5). There was no effect of genotype on the after-hyperpolarisation (Fig. 1K). This suggests that in the Dlg2+/− hets, the integration of synaptic inputs from the SC and TA pathways is impaired, reducing dendritic depolarisation and action potential spiking which are the drivers of aLTP.

To test the necessity of action potentials for aLTP, a paired theta burst LTP induction protocol was used, where action potentials were driven by somatic current injection to bypass dendritic integration, and spikes were paired with simultaneous SC pathway stimulation (Fig. 1L). Under these conditions, robust LTP was induced in the SC pathway in both genotypes, with the TA pathway acting as negative control (Fig. 1M–O). Similarly, when
AUC was measured. The right inset depicts a zoomed-in view of the compound EPSP decay, where the trace represents the response at maximal stimulation intensity in the presence of 50 µM D-APV. The left inset depicts a zoomed-in view of the single EPSP followed by a compound EPSP at increasing stimulation intensities (light to dark) over consecutive recording sweeps. The red main effect: genotype, with the change points marked by black circles. Change point (EPSP rising subthreshold slope mV/mS) (3-way ANOVA: genotype main effect: \( F_{1, 34} = 12.625, P < 0.001 \) (C) and AUC/slope ratio (3-way ANOVA: genotype main effect: \( F_{1, 34} = 8.003, P = 0.009 \) (D) across genotype. Hets: 13 cells, 9 animals and wts: 19 cells, 12 animals. Summary values depicted as mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001 (3-way ANOVA between subject effect).

Fig. 2 Increased threshold for supra-linear dendritic integration in the Dlg2−/− hets in the SC pathway. A Example traces depicting a single EPSP followed by a compound EPSP at increasing stimulation intensities (light to dark) over consecutive recording sweeps. The red trace represents the response at maximal stimulation intensity in the presence of 50 µM D-APV. The left inset depicts a zoomed-in view of the single EPSP, where rising subthreshold slope was measured. The right inset depicts a zoomed-in view of the compound EPSP decay, where the AUC was measured. B Example relationships between the single EPSP rising subthreshold slope and the compound EPSP decay AUC across genotype, with the change points marked by black circles. Change point (EPSP rising subthreshold slope mV/mS) (3-way ANOVA: genotype main effect: \( F_{1, 34} = 12.625, P = 0.001 \)) (C) and AUC/slope ratio (3-way ANOVA: genotype main effect: \( F_{1, 34} = 8.003, P = 0.009 \)) (D) across genotype. Hets: 13 cells, 9 animals and wts: 19 cells, 12 animals. Summary values depicted as mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001 (3-way ANOVA between subject effect).

aLTP was tested using baseline EPCs doubled in amplitude, maximal LTP was induced and there was no effect of genotype (Supplementary Figs. S6 and 7). As expected, aLTP was greater in ventral slices whereas theta burst LTP was greater in dorsal slices (Supplementary Figs. S8 and 9). This indicates that the hets are fundamentally able to undergo LTP but their ability to integrate inputs is impaired.

**Synaptic integration**

To directly test synaptic integration, the number of activated synapses required to generate supra-linear summation of EPSPs across multiple dendrites was assessed, which is a measure of the ability for synapses to integrate across the dendritic arbor [40, 42, 47] and allowed comparison of synaptic integration between genotypes. To activate increasing numbers of synapses, the SC pathway was stimulated with increasing intensity to activate synapses with a random spatial distribution across the proximal and basal dendritic arbor. The number of activated synapses was measured by the slope of a single EPSP, and the integration of synapses assessed by the amplitude and duration of a compound summated EPSP (area under the curve – AUC) elicited by repetitive high frequency synaptic stimulation (Fig. 2A).

As stimulation intensity was increased the number of active synapses increased in a linear relationship with the amplitude and durations of the summed compound EPSP until a “change point” was reached (see methods) after which the relationship became supra-linear because the duration of the compound EPSP increased (Fig. 2B), indicative of activation of regenerative or plateau potentials within the dendrites [40, 42, 47]. The inhibition of these regenerative potentials by D-APV demonstrated their dependence on NMDAR activation (Fig. 2A, B). The change point was increased in the Dlg2−/− hets (Fig. 2B, C), indicating that het neurons required more synaptic inputs to undergo the transition to supra-linear integration. Additionally, the maximum duration of the compound EPSP as a ratio to the corresponding slope of the single EPSP was reduced in the Dlg2−/− hets (Fig. 2D). This again indicates that Dlg2−/− hets require more synaptic input to integrate dendritic inputs and produce the supra-linear regenerative potentials important for aLTP.

**NMDAR currents**

Synaptic integration is driven by NMDARs and protein-protein interaction studies have reported DLG2 to interact directly with NMDAR subunits [17–19] and with AMPAR indirectly [21]. Further, DLG2 has also been shown to affect glutamatergic function in homozygous Dlg2−/− models, albeit with variable results in AMPA/NMDA ratio [32–34, 48, 49]. To investigate whether glutamatergic function was affected in Dlg2−/− rats and whether this might explain the impairment in synaptic integration and aLTP, the AMPA/NMDA ratio was measured in CA1 pyramidal neurons (Fig. 3A). Dlg2−/− hets had a reduced AMPA/NMDA ratio in the SC pathway, with no effect in the TA pathway (Fig. 3B–D). AMPAR-mediated miniature excitatory postsynaptic currents (mEPSCs, Supplementary Fig. S10) resulting from the activity of single synapses were recorded to probe whether the reduction in AMPA/NMDA ratio resulted from a reduction in AMPA, an increase in NMDA, or a combination of the two. The slow kinetics and small amplitude of NMDAR-mediated mEPSCs make them difficult to detect accurately. AMPAR-mediated mEPSCs from synapses on proximal dendrites are more detectable than those from more distal synapses due to signal attenuation and therefore recorded mEPSCs will arise from the most proximal synapses [50]. There was no difference in the distributions of mEPSC amplitude, interevent interval, or decay tau across genotype (Fig. 3E–I). Paired-pulse facilitation, measured in the AMPA/NMDA ratio experiment, was also not different across genotype in either pathway (Supplementary Fig. S11). Together, these results show no
change in postsynaptic AMPAR function and presynaptic glutamate release probability in the SC pathway. It follows that the AMPA/NMDA ratio effect in the SC pathway was due to an increase in NMDAR function. This could result from either an increase in NMDAR number or a change in subunit composition between GluN2A and GluN2B. To test subunit composition, NMDAR currents were isolated (Fig. 3J) and the selective GluN2B negative allosteric modulator RO256981 was applied. RO256981 decreased EPSC amplitude (Fig. 3J–L) and increased the EPSC decay time in both the SC and the TA pathways (Fig. 3M, N). There was a trend toward a genotype...
Fig. 3 Glutamatergic function is altered in theDlg2+/−/−hets due to an increase in NMDAR current, with no change in AMPAR current or GluN2b subunit expression. A Schematic representation of the hippocampal slice recording setup, with the CA3 removed and stimulating electrodes in the stratum radiatum and in the stratum lacunosum moleculare. B AMPA/NMDA EPSC ratio example traces. The primarily AMPA-mediated EPSCs were recorded at a holding potential of −70 mV, whilst the AMPA- and NMDA-mediated EPSCs were recorded at a holding potential of 40 mV. The AMPA/NMDA ratio was derived by dividing the peak EPSC amplitude at −70 mV by the EPSC amplitude 45 ms after stimulation at 40 mV. AMPA/NMDA EPSC ratio across genotype in the SC (3-way ANOVA: genotype main effect: F_{1,34} = 0.583, P = 0.452) (C) and the TA (3-way ANOVA: genotype main effect: F_{1,34} = 0.583, P = 0.452) (D) pathways. Hets: 30 cells, 18 animals and wts: 38 cells, 19 animals for the SC data set and hets: 18 cells, 9 animals and wts: 16 cells, 8 animals for the TA data sample. Example mEPSC traces (E) and mean mEPSCs (F) across genotype. Cumulative frequency plots of amplitude (Kolmogorov–Smirnov Test: P = 0.028) (G), interevent interval (Kolmogorov–Smirnov Test: P = 0.999) (H), and decay tau (Kolmogorov–Smirnov Test: P = 0.067) (I). Hets: 25 cells, 9 animals and wts: 21 cells, 5 animals. J GluN2b example EPSC traces. The traces following RO256981 1 µM administration were also peak scaled to better illustrate changes in decay kinetics. EPSC amplitude in the SC (3-way repeated-measures ANOVA: drug main effect: F_{1,38} = 260.603, P < 0.001. Genotype x drug interaction: F_{1,38} = 2.952, P = 0.057) (K) and TA (3-way repeated-measures ANOVA: drug main effect: F_{1,27} = 42.076, P < 0.001. Genotype x drug interaction: F_{1,26} = 1.738, P = 0.185) (L) pathways. Decay tau 1/decay tau 2 ratio in SC (3-way repeated-measures ANOVA: drug main effect: F_{1,45} = 9.715, P = 0.003. Genotype x drug interaction: F_{1,45} = 0.272, P = 0.605) (M) and TA (3-way repeated-measures ANOVA: drug main effect: F_{1,27} = 3.410, P = 0.076. Genotype x drug interaction: F_{1,27} = 0.831, P = 0.370) (N) pathways. Hets: 24 cells, 6 animals and wts: 29 cells, 8 animals for the SC data set and hets: 19 cells, 6 animals and wts: 18 cells, 8 animals for the TA data set. Summary values depicted as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 (3-way ANOVA between subject effect).

**x** drug interaction in the EPSC amplitude measurement in the SC pathway but no genotype **x** drug interaction in the EPSC decay kinetics. Together, these results show similar NMDAR subunit composition across genotype and therefore the enhancement in synaptic NMDAR function likely arises from increased receptor number. SC = somatic current injection. However, despite reduced input resistance in theDlg2+/−/−hets, there was no effect of genotype on spike output to current injection (Fig. 4E, F). This could be explained by a depolarised resting membrane potential (Fig. 4G) and a trend towards hyperpolarised action potential spike threshold (Fig. 4H) in theDlg2+/−/−hets indicating that smaller membrane potentials and depolarisation thresholds were required to initiate spikes. There was no effect of genotype on spike half-width, maximum spike slope, spike amplitude, or capacitance, and a slight decrease in latency to spike in theDlg2+/−/−hets (Supplementary Fig. S16).

Reduced input resistance in theDlg2+/−hets could be explained via two mechanisms: i) increased membrane area through greater dendritic branching and extent [42, 67] or ii) increased membrane conductance, most likely caused by increased potassium channel expression. To test the first mechanism, a subset of neurons from the intrinsic excitability experiments were filled with neurobiotin to allow post hoc morphological analysis. Analysis of these neurons revealed that het neurons were smaller than wt neurons (Fig. 4I) and had reduced dendritic arborisation overall, with the most striking reductions in the DG2+/−/−hets could be filled with neurobiotin to allow post hoc morphological analysis. Analysis of these neurons revealed that het neurons were smaller than wt neurons (Fig. 4I) and had reduced dendritic arborisation overall, with the most striking reductions in

To assess input resistance, measurements were analysed from voltage clamp experiments (using identical conditions to the LTP experiments in Fig. 1) and in current clamp experiments. In both these separate and independent data setsDlg2+/−/−hets had reduced input resistance (Fig. 4A–D). This increase in electrical leak in theDlg2+/−/−hets is predicted to reduce cross-talk between synapses and their integration leading to a reduced spike output but it is also expected to reduce the spike output in response to somatic current injection. However, despite reduced input resistance in theDlg2+/−/−hets, there was no effect of genotype on spike output to current injection (rheobase) (Fig. 4E, F). This could be explained by a depolarised resting membrane potential (Fig. 4G) and a trend towards hyperpolarised action potential spike threshold (Fig. 4H) in theDlg2+/−/−hets indicating that smaller membrane potentials and depolarisation thresholds were required to initiate spikes. There was no effect of genotype on spike half-width, maximum spike slope, spike amplitude, or capacitance, and a slight decrease in latency to spike in theDlg2+/−/−hets (Supplementary Fig. S16).

Reduced input resistance in theDlg2+/−hets could be explained via two mechanisms: i) increased membrane area through greater dendritic branching and extent [42, 67] or ii) increased membrane conductance, most likely caused by increased potassium channel expression. To test the first mechanism, a subset of neurons from the intrinsic excitability experiments were filled with neurobiotin to allow post hoc morphological analysis. Analysis of these neurons revealed that het neurons were smaller than wt neurons (Fig. 4I) and had reduced dendritic arborisation overall, with the most striking differences in the basal and proximal apical regions (Fig. 4M). Contrary to the predicted neuronal size – input resistance relationship, there was no correlation between total dendritic branch length and input resistance (Fig. 4N). Therefore, reduced neuronal arborisation in theDlg2+/−/−hets cannot explain the observed reduced input resistance and instead increased potassium channel expression is the most likely explanation.

Computational modelling of synaptic integration in representative reconstructed pyramidal neurons also predicted increased potassium channel expression as the mechanism underlying reduced input resistance (Supplementary Fig. S19) and enabled exploration of the likely potassium channel subtypes mediating reduced synaptic integration. DLG2 interacts with potassium inward rectifier Kir2.3 [22] and Kir2.2 [23] as well as A-type Kv1.4 [24] channels which therefore represent potential candidates to underpin decreased input resistance and synaptic integration. The model suggested that A-type potassium channels are the most likely candidates upregulated in theDlg2+/−/−hets to underly the dendritic integration deficits (Supplementary Fig. S19). However, any mechanism that reduces input resistance is predicted to facilitate dendritic integration in theDlg2+/−/−hets.

**Rescue of synaptic integration and plasticity**

The aLTP, theta burst LTP, and dendritic integration results from Figs. 1 and 2 suggest that, given enough synaptic input,Dlg2+/−/−hets could be filled with neurobiotin to allow post hoc morphological analysis. Analysis of these neurons revealed that het neurons were smaller than wt neurons (Fig. 4I) and had reduced dendritic arborisation overall, with the most striking...
Fig. 4  Reduced input resistance and dendritic arborisation in the Dlg2+/− hets. A Current traces in response to a 2 mV voltage step for wt and het. B Input resistance (V clamp) across genotype (3-way ANOVA: genotype main effect: $F_{1,146} = 5.698$, $P = 0.018$). Hets: 73 cells, 28 animals and wts: 73 cells, 31 animals. C Voltage traces in response to a $-150$ pA current step across genotype. D Input resistance (I clamp) across genotype (3-way ANOVA: genotype main effect: $F_{1,78} = 4.209$, $P = 0.044$). The sets of cells in panels C and D are separate and represent independent analyses. E Voltage deflections in response to a current step of equal size across genotype. Rheobase (3-way ANOVA: genotype main effect: $F_{1,99} = 0.011$, $P = 0.916$) (F), resting membrane potential (RMP) (3-way ANOVA: genotype main effect: $F_{1,136} = 2.075$, $P = 0.009$) (G), and spike threshold (3-way ANOVA: genotype main effect: $F_{1,89} = 3.105$, $P = 0.082$) (H) across genotype. Hets: 83 cells, 21 animals and wts: 53 cells, 17 animals. I Example morphological reconstructions across genotype, cell bodies denoted with circles. Dendritic branch number (3-way ANOVA: genotype main effect: $F_{1,40} = 23.279$, $P < 0.001$) (J), total dendritic branch length (3-way ANOVA: genotype main effect: $F_{1,40} = 7.002$, $P = 0.013$) (K), and mean dendritic branch length (3-way ANOVA: genotype main effect: $F_{1,40} = 0.133$, $P = 0.718$) (L) across genotype. M Scholl analysis across genotype (3-way ANOVA: genotype main effect: $F_{1,31} = 5.532$, $P = 0.025$) Hets: 23 cells, 12 animals and wts: 17 cells, 9 animals. N Correlation between total dendritic branch length and input resistance dataset (Pearson correlation: $R^2 = 0.015$, $P = 0.600$). Summary values depicted as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (3-way ANOVA between subject effect).
Fig. 5  Potassium channel block and muscarinic M1 agonism lower dendritic integration thresholds and M1 agonism rescues associative LTP in the Dlg2+/– hets. A Example traces depicting a single EPSP followed by a compound EPSP at increasing stimulation intensities (light to dark) over consecutive recording sweeps before and after 4-aminopyridine 0.3 mM across genotype. Input resistance (3-way repeated-measures ANOVA: drug effect: $F_{1, 23} = 8.608, P = 0.007$, genotype x drug interaction: $F_{1, 23} = 0.418, P = 0.524$) (B), change point (3-way repeated-measures ANOVA: drug effect: $F_{1, 14} = 8.422, P = 0.012$, genotype x drug interaction: $F_{1, 23} = 0.042, P = 0.841$) (C), AUC/slope (3-way repeated-measures ANOVA: drug effect: $F_{1, 23} = 3.988, P = 0.058$, genotype x drug interaction: $F_{1, 23} = 1.570, P = 0.223$) (D), and resting membrane potential (RMP) (3-way repeated-measures ANOVA: drug effect: $F_{1, 24} = 57.899, P < 0.001$, genotype x drug interaction: $F_{1, 24} = 0.230, P = 0.636$) (E) as percent of control before and after the after 4-aminopyridine 0.3 mM across genotype. Hets: 19 cells, 10 animals and wts: 13 cells, 6 animals.  

F Example traces depicting a single EPSP followed by a compound EPSP at increasing stimulation intensities (light to dark) over consecutive recording sweeps before and after 77-LH-28-1 7 µM across genotype. Input resistance (3-way repeated-measures ANOVA: drug effect: $F_{1, 21} = 4.12, P = 0.055$, genotype x drug interaction: $F_{1, 21} = 1.270, P = 0.237$) (G), change point (3-way repeated-measures ANOVA: drug effect: $F_{1, 16} = 0.176, P = 0.680$. Genotype x pathway interaction: $F_{2, 32} = 0.09, P = 0.914$). N Baseline EPSC amplitude across genotype (3-way repeated-measures ANOVA: pathway effect: $F_{2, 32} = 12.169, P < 0.001$. Genotype main effect: $F_{1, 16} = 0.176, P = 0.680$. Genotype x pathway interaction: $F_{2, 32} = 0.09, P = 0.914$). O Example EPSP traces of LTP induction, with example EPSPs following post hoc spike truncation. Burst number (3-way ANOVA: genotype main effect: $F_{1, 16} = 0.100, P = 0.755$) (Q), and total spike number (3-way ANOVA: genotype main effect: $F_{1, 16} = 0.029, P = 0.867$) (R) across genotype during LTP induction. Hets: 13 cells, 7 animals and wts: 11 cells, 6 animals. Summary values depicted as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (3-way ANOVA between subject effect).
did not differ among pathways and across genotype (Fig. 5N), spike number disappearing. Taken together, Fig. 5 shows 77-LH-28-1 rescued synaptic summation and the indicating that the amount of synaptic input received was synaptic integration.

Dlg2 hets is more sensitive to increased dendritic excitability and synaptic integration. However, previous studies on Dlg2+/− full knockout models have either reported no changes in the AMPA/NMDA ratio or a reduction in the AMPA/NMDA ratio due to reduction in AMPAR function [32–34, 48, 49]. Here, AMPAR function was unchanged and instead we found an unexpected increase in NMDAR currents, likely caused by increased synaptic expression selectively at Schaffer collateral synapses. There is no evidence that Dlg2 is differentially expressed at Schaffer collateral vs temporoammonic synapses in CA1 so the mechanism for this selective enhancement of NMDAR expression is unknown. On its own, enhanced NMDAR currents predict enhanced aLTP, but we found the converse with aLTP impairment. This is similar to previous reports in Dlg2+/− mice. In homozygous Dlg2−/− mice CA1 LTP was normal in response to strong 100 Hz induction protocol but reduced in response to TBS given to just the SC pathway [33]. In our study using heterozygous Dlg2+/- rats, TBS-induced LTP pairing postsynaptic stimulation with SC input was normal and an LTP deficit only became apparent in the Dlg2+/- model when neurons were required to integrate converging inputs suggesting a nuanced and potentially behaviourally relevant phenotype in the clinically relevant Dlg2+/− model. Furthermore, synaptic integration and the initiation of non-linear dendritic events are key determinants of feature detection and selectivity in neuronal networks [41, 72, 73] and a deficit in detecting events and giving appropriate salience are important features of many psychiatric disorders [74].

The dichotomy between enhanced NMDAR currents and reduced NMDAR function in Dlg2+/− rats during aLTP highlights the dominant role played by changes to intrinsic neuronal excitability; in this instance reduced input resistance caused by increases in potassium channel function. Interestingly, in a Dlg2−/− full knockout model no changes in input resistance were reported [75] highlighting again the importance of using clinically relevant models. In our Dlg2+/− model this increase in potassium channel function does not appear to be caused by a direct interaction with Dlg2 but instead as a homeostatic regulatory mechanism perhaps to compensate for increased synaptic currents. A similar compensatory mechanism is found in other models of psychiatric disorders such as Fmr1−/− mice where changes in intrinsic neuronal excitability dominate the resulting perturbations in network processing including dendritic integration and synaptic plasticity [63, 76–78]. This raises the intriguing possibility that genetic disruptions to synaptic function may generally cause homeostatic compensations in intrinsic neuronal excitability that dominate neuronal function and present a common biological phenotype across multiple psychiatric disorders [79].

We have demonstrated in this study that the compensatory mechanisms affecting neuronal excitability can be ameliorated pharmacologically with the administration of selective agonists.
such as 77-LH-28-1 rescuing impairments in synaptic integration and plasticity, a proof of principle that may be applicable to other psychiatric disorder risk variants. For example, an increase in input resistance due to the administration of 77-LH-28-1 could facilitate spike backpropagation, potentially rescuing the plasticity impairment and network dysfunctions reported in the *Cacna1c*+/−/ and 22q11 deletion syndrome models of genetic vulnerability to schizophrenia [80, 78]. Highly selective muscarnic M1 receptor agonists have efficacy clinically with negligible side effects [81–84] making them attractive pharmaceutical tools. It remains to be seen whether behavioural impairments in DLG2 models can be rescued using similar pharmacological strategies.

**REFERENCES**

1. Kirov G, Pocklington AJ, Holmans P, Ivanov D, Ikeda M, Ruderfer D, et al. De novo CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia. Mol Psychiatry. 2012;17:142–53.

2. Marshall CR, Howrigan DP, Merico D, Thiruvahindrapuram B, Wu W, Greer DS, et al. Contribution of copy number variants to schizophrenia from a genome-wide study of 14,121 subjects. Nat Genet. 2017;49:27–35.

3. Noor A, Lionel AC, Cohen-Woods S, Moghimi N, Rucker J, Fennell A, et al. Copy number variant study of bipolar disorder in Canadian and UK populations implicates synaptic genes. Am J Med Genet Part B Neuropsychiatr Genet. 2014;165:303–13.

4. Georgieva L, Rees E, Moran JL, Chambert KD, Milanova V, Craddock N, et al. De novo CNVs in bipolar affective disorder and schizophrenia. Hum Mol Genet. 2014;23:607–83.

5. Cusco I, Medrano A, Gener B, Vilardell M, Gallastegui F, Villa O, et al. Autism-specific copy number variants further implicate the phosphatidylinositol signalling pathway and the glutamatergic synapse in the etiology of the disorder. Hum Mol Genet. 2009;18:1795–804.

6. Egger G, Roetzer KM, Noor A, Lionel AC, Mahmood H, Schwarzbraun T, et al. Identification of risk genes for autism spectrum disorder through copy number variation analysis in Austrian families. Neurogenetics 2014;15:117–27.

7. Ruzzo EK, Pérez-Cano L, Jung Y-J, Wang L-K, Kase-Haghighi D, Harti C, et al. Inherited and de novo genetic risk for autism impacts shared networks. Cell 2019;178:850–66.e26.

8. Alemany S, Ribasés M, Vilor-Tejedor N, Buxtemante M, Sánchez-Mora C, Bosch R, et al. New suggestive genetic loci and biological pathways for attention function in adult attention-deficit/hyperactivity disorder. Am J Med Genet Part B Neuropsychiatr Genet. 2015;168:459–70.

9. Reggiani C, Coppens S, Sekhara T, Dimov I, Pichon B, Lu

10. Frank RAW, Komijama NH, Ryan TJ, Zhu F, O’Dell TJ, Grant SGN. NMDA receptors are selectively partitioned into complexes and supercomplexes during synapse maturation. Nat Commun. 2016;7:11264.

11. Dakoji S, Tomita S, Karimzadegan S, Nicoll RA, Bredt DS. Interaction of transmembrane AMPA receptor regulatory proteins with multiple membrane associated guanylate kinases. Neuropharmacology. 2003;45:849–56.

12. Irie M. Binding of neuroligins to PSD-95. Science 1997;277:1511–15.

13. Purcell SM, Moran JL, Fromer M, Ruderfer D, Solovieff N, Roussos P, et al. A cross-phenome study of 41,321 subjects. Nat Genet. 2017;49:92–7.

14. Fromer M, Pocklington AJ, Kavanagh DH, Williams HJ, Dwyer S, Gormley P, et al. Contribution of copy number variants to schizophrenia from a genome-wide study of 41,321 subjects. Nat Genet. 2017;49:27–35.

15. Gonzalez-Mantilla AJ, Moreno-De-Luca A, Ledbetter DH, Martin CL. A cross-phenome study of 41,321 subjects. Nat Genet. 2017;49:92–7.

16. Sanders SJ, Muntha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, et al. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature. 2012;485:237–41.

17. Irie M. Binding of neuroligins to PSD-95. Science 1997;277:1511–15.

18. Niethammer M, Kim E, Sheng M. Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. J Neurosci. 1996;16:2157–63.

19. Chen B-S, Gray John A, Sanz-Clemente A, Wei Z, Thomas Eleanor V, Nicoll Roger A, et al. SAP102 mediates synaptic clearance of NMDA Receptors. Cell Rep. 2012;2;1120–28.
45. Larkum ME, Zhu JJ, Sakmann B. A new cellular mechanism for coupling inputs arriving at different cortical layers. Nature. 1999;398:338–41.
46. Magee JC. A symptomatically controlled, associative signal for Hebbian plasticity in hippocampal neurons. Science. 1997;275:209–13.
47. Stuart GJ, Spruston N. Dendritic integration: 60 years of progress. Nat Neurosci. 2015;18:1713–21.
48. Eavas PO, Huang X, Hosang L, Stodieck S, Cui L, Liu YZ, et al. An opposing function of paralogs in balancing developmental synapse maturation. PLoS Biol. 2018;16:e2006838.
49. Tao Y-X, Rumbaugh G, Wang G-D, Petralia RS, Zhao C, Kauer FW, et al. Impaired NMDA receptor-mediated postsynaptic function and blunted NMDA receptor-dependent persistent pain in mice lacking synaptic density-93 protein. J Neurosci. 2003;23:6703–12.
50. Stuart G, Spruston N. Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. J Neurosci. 1998;18:3501–10.
51. Magee JC. Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. J Neurosci. 1998;18:7613–24.
52. Lotnicz A, Notomi T, Tamas G, Shigemoto R, Nusser Z. Polarized and compartment-dependent distribution of HCN1 in pyramidal cell dendrites. Nat Neurosci. 2002;5:1185–93.
53. Harnett MT, Magee JC, Williams SR. Distribution and function of HCN channels in the apical dendritic tuft of neocortical pyramidal neurons. J Neurosci. 2015;35:1024–37.
54. Buchanan KA, Petrovic MM, Chamberlain SEL, Marrion NV, Mellor JR. Facilitation of long-term potentiation by muscarinic M1 receptors is mediated by inhibition of SK channels. Neuron. 2010;68:948–63.
55. Tigaret CM, Olivo V, Sadowski JHP, Ashby MC, Mellor JR. Coordinated activation of distinct Ca2+ sources and metabotropic glutamate receptors encodes Heb- bian synaptic plasticity. Nat Commun. 2016;7:10289.
56. Ngo-Anh TJ, Bloodgood BL, Lin M, Sabatini BL, Maylie J, Adelman JP. SK channels and NMDA receptors form a Ca2+-mediated feedback loop in dendritic spines. Nat Neurosci. 2005;8:642–49.
57. Harnett Mark T, Xu N-L, Magee Jeffrey C, Williams Stephen R. Potassium channels control the interaction between active dendritic integration compartments in Layer 5 cortical pyramidal neurons. Neuron. 2013;79:516–29.
58. Biró ÁA, Brémaud A, Falck J, Ruiz AJ. A-type K+ channels impede supralinear summation of clustered glutamatergic inputs in layer 3 neocortical pyramidal neurons. Neuronopharmacology. 2018;140:86–99.
59. Losonczy A, Magee JC. Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. Neuron. 2006;50:291–307.
60. Liu Y, Cui L, Schwarz MK, Dong Y, Schüller OM. Adrenergic gate release for spike timing-dependent synaptic potentiation. Neuron. 2017;93:394–408.
61. Makara Judit K, Magee Jeffrey C. Variable dendritic integration in hippocampal CA3 pyramidal neurons. Neuron. 2013;80:1438–50.
62. Dougherty KA, Nicholson DA, Diaz L, Buss EW, Neuman KM, Chetkovich DM, et al. Differential expression of HCN subunits alters voltage-dependent gating of h-channels in CA1 pyramidal neurons from dorsal and ventral hippocampus. J Neurophysiol. 2013;109:1940–57.
63. Booker SA, Domanski APF, Dando OR, Jackson AD, Isaac JTR, Hardingham GE, et al. Altered dendritic spine function and integration in a mouse model of fragile X syndrome. Nat Commun. 2019;10:4813.
64. Fan Y, Fricker D, Brager DH, Chen X, Lu H-C, Chitwood RA, et al. Activity-dependent decrease of excitability in rat hippocampal neurons through increases in Ih. Nat Neurosci. 2005;8:1542–51.
65. Brager DH, Lewis AS, Chetkovich DM, Johnston D. Short- and long-term plasticity in CA1 neurons from mice lacking h-channel auxiliary subunit TRIPbB. J Neurophysiol. 2013;110:5808–73.
66. Armstrong JF, Faccenda E, Harding SD, Pawson AJ, Southan C, Sharman JL, et al. The IUPHAR/BPS guide to PHARMACOLOGY in 2020: extending immunopharmacology content and introducing the IUPHAR/MMV Guide to MALARIA PHARMACOLOGY. Nucleic Acids Res. 2020;48:D1006–D21.
67. Nguyen A, Kath J, Hanson D, Biggers M, canniff P, Donovan C, et al. Novel nonpeptide agents potently block the C-type inactivated conformation of Kv1.3 and suppress T cell activation. Mol Pharmacol. 1996;50:1672–79.
68. Dennis SH, Pasqui F, Colvin EM, Sanger H, Mogg AJ, Felder CC, et al. Activation of muscarinic M1 acetylcholine receptors induces long-term potentiation in the hippocampus. Cereb Cortex. 2016;26:414–26.

ACKNOWLEDGEMENTS
We thank Jenny Carter for coordinating the initial generation and breeding of the Dlg2+/− line rat, Rachel Humphries for computational modelling discussions and Aleks Domanski and all members of the Robinson and Mellor groups for general discussions. We also thank Hannah Jones and Estela Michail for their input in the study of morphology.

AUTHOR CONTRIBUTIONS
Conceptualisation, SG, JH, LSW, ESJR, JRM; Methodology, SG, CÓD, JRM; Investigation and Analysis, SG, SW, Writing, SG, SW, CÓD, KLT, DMD, LSW, JH, ESJR, JRM; Supervision, KLT, DWD, LSW, JH, ESJR, JRM.

FUNDING
The authors gratefully acknowledge funding from Medical Research Council (UK) (CÓD, G4W4 BIOMED PhD studentship to SG), Biotechnology and Biological Science Research Council (UK) (JRM), Wellcome Trust (UK) (JRM, PhD studentship to SW). The Dlg2+/− rats were generated as part of a Wellcome Trust Strategic Award ‘DEFINE’ (JH and LSW) and the Wellcome Trust Strategic Award and the Neurosciences and Mental Health Research Institute, Cardiff University, UK provided core support. ER has received research funding from Boehringer Ingelheim, Eli Lilly, Pfizer, Small Pharma Ltd. and MSD, and DD has received research funding from Eli Lilly, but these companies were not associated with the data presented in this manuscript.
COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41386-022-01277-6.

Correspondence and requests for materials should be addressed to Jack R. Mellor.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022