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MicroRNA-137 Controls AMPA-Receptor-Mediated Transmission and mGluR-Dependent LTD

Graphical Abstract

Highlights

- AMPA receptor subunit GluA1 is a direct target of miR-137
- miR-137 represses AMPA-receptor-mediated synaptic transmission
- miR-137 is required for mGluR5-mediated long-term depression

Authors

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In Brief

Olde Loohuis et al. reveal that microRNA miR-137, which is associated with intellectual disability and schizophrenia, controls AMPA-receptor-mediated transmission by targeting AMPA receptor subunit GluA1. miR-137 levels are regulated by mGluR5 activation, and, consequently, acute interference with miR-137 function impedes mGluR5-dependent synaptic plasticity.
MicroRNA-137 Controls AMPA-Receptor-Mediated Transmission and mGluR-Dependent LTD

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SUMMARY

Mutations affecting the levels of microRNA miR-137 are associated with intellectual disability and schizophrenia. However, the pathophysiological role of miR-137 remains poorly understood. Here, we describe a highly conserved miR-137-binding site within the mRNA encoding the GluA1 subunit of AMPA-type glutamate receptors (AMPARs) and confirm that GluA1 is a direct target of miR-137. Postsynaptic downregulation of miR-137 at the CA3-CA1 hippocampal synapse selectively enhances AMPAR-mediated synaptic transmission and converts silent synapses to active synapses. Conversely, miR-137 overexpression selectively reduces AMPAR-mediated synaptic transmission and silences active synapses. In addition, we find that miR-137 is transiently upregulated in response to metabotropic glutamate receptor 5 (mGluR5), but not mGluR1 activation. Consequently, acute interference with miR-137 function impedes mGluR-LTD expression. Our findings suggest that miR-137 is a key factor in the control of synaptic efficacy and mGluR-dependent synaptic plasticity, supporting the notion that glutamatergic dysfunction contributes to the pathogenesis of miR-137-linked cognitive impairments.

INTRODUCTION

Learning and memory processes rely on activity-dependent changes in synaptic strength. A fundamental mechanism for the modification of synaptic strength is the insertion or removal of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) at the postsynaptic membrane (Bassani et al., 2013; Citri and Malenka, 2008; Huganir and Nicoll, 2013). Long-term potentiation (LTP) and long-term depression (LTD) are two central mechanisms of synaptic plasticity depending on changes in AMPAR that mediate the majority of fast synaptic transmission (Malinow and Malenka, 2002). In the hippocampus, two independent forms of LTD coexist, the N-methyl-D-aspartate receptor (NMDAR)-LTD and the group I metabotropic glutamate receptor (mGluR)-LTD, each induced through distinct signaling cascades (Collingridge et al., 2010; Oliet et al., 1997). Translation from dendritically localized mRNAs is a primary process for the induction of mGluR-LTD (Huber et al., 2000; Klann and Dever, 2004; Snyder et al., 2001). The prevailing model is that group I mGluRs rapidly trigger the local synthesis of new dendritic proteins that are required for LTD by increasing the rate of AMPAR endocytosis at active synapses (Lüscher and Huber, 2010; Waung and Huber, 2009). Among the locally translated proteins are Arc/Arg3.1 (Jakkamsetti et al., 2013; Park et al., 2008; Waung et al., 2008), Map1b (Davidikova and Carroll, 2007), STEP1 (Zhang et al., 2008), and oligophrenin-1 (Nadif Kasri et al., 2011). Interestingly, different forms of intellectual disability (ID) have been linked to defective mGluR-LTD, confirming the tight regulation of AMPAR function at mature synapses through local protein synthesis as an essential form of plasticity and cognition (Aschrafi et al., 2005; Auerbach et al., 2011; Bateup et al., 2011; Lüscher and Huber, 2010).

Local translation-dependent synaptic LTD is tightly controlled by a number of different post-transcriptional mechanisms, such as the ubiquitin-proteasome system (Hou et al., 2006; Yashiro et al., 2009), translation initiation and elongation (Costa-Mattioli et al., 2005), and by microRNAs (miRNAs) (Manakov et al., 2009). MiRNAs are small, evolutionarily conserved signaling molecules that act as silencing regulators of mRNA targets. Disruption of miRNA expression or signaling impairs synaptic plasticity and has been associated with severe cognitive impairment in mice (Hansen et al., 2013; Konopka et al., 2010) and humans (Ripke et al., 2013; Willemsen et al., 2011). Consistent with these notions, inducing LTP or LTD rapidly alters the levels of many different miRNAs in hippocampal neurons (Park and Tang, 2009; Wirbrand et al., 2010). MiRNAs affect synaptic plasticity by directly targeting synaptic receptors or their downstream targets (Dutta et al., 2013; Fiore et al., 2014; Kocerha et al., 2009;
amp-Knockdown engenders reduced AMPAR-mediated transmission (Figure 2C), indicating that modulation of miR-137 levels is associated with opposing effects toward AMPAR-mediated synaptic transmission.

The observed changes are likely due to a postsynaptic effect, since only CA1 neurons were genetically manipulated at the CA3-CA1 synapse and we did not observe any change in presynaptic release probability, as measured by a paired-pulse stimulation paradigm (Figure 2C). Collectively, these results suggest that miR-137 controls excitatory synaptic strength in a cell-autonomous manner.

The changes in AMPAR-mediated transmission could be based on a change in synaptic AMPARs levels at individual synapses, a change in the number of functional synapses, or both. We therefore measured miniature excitatory postsynaptic currents (mEPSCs) in the sponge-miR-137- and the miR-137-infected neurons as compared to non-infected cells. While sponge-miR-137-infected neurons exhibited no change in amplitude, they showed an increase in the frequency of mEPSCs. In contrast, neurons overexpressing miR-137 showed a decreased mEPSC frequency without exhibiting any changes in amplitude (Figures 2D and 2E). A change in frequency usually reflects a change in the number of active synapses or presynaptic release probability. However, since alterations in miR-137 levels did not elicit any changes in presynaptic release probability, these results suggest that modulation of miR-137 levels in hippocampal slices resulted in a change in the number of functional synapses.

**RESULTS**

**AMPA Subunit GluA1 Is a Direct Target of miR-137**

Bioinformatics studies revealed the presence of a highly conserved miR-137 binding site in the 3'-UTR of AMPAR subunit GluA1, but not for other AMPAR subunits (Figure 1A), suggesting that this miRNA targets GluA1. We transfected hippocampal neurons at 14 days in vitro (DIV) with either a luciferase reporter plasmid containing the GluA1 3'-UTR (wild-type [WT]) or an miR-137 binding-site-mutated GluA1 3'-UTR (mutant) (Figure 1B). Luciferase activity was assessed using an miR-137-sequestering sponge vector (Figure S1). Neurons infected with the sponge-miR-137 virus exhibited an increased luciferase signal when expressing the GluA1-3'UTR WT neurons were exposed to a previously validated locked nucleic acid LNA-miR-137 (Willemsen et al., 2011), whereas this increase was absent in the mutant condition, suggesting a targeting of this binding site by miR-137 (Figure 1B).

Western blot analysis revealed that GluA1 protein expression was significantly increased in the sponge-miR-137-expressing neurons, whereas GluA1 expression was significantly reduced in the miR-137-expressing neurons (Figures 1C and 1D). Of note, GluA1 mRNA expression was unaffected by miR-137, suggesting that the changes in GluA1 protein levels by miR-137 is regulated at the level of translation rather than transcription (Figure 1E).

We observed a significant increase in surface GluA1 (sGluA1) expression at synapses of sponge-miR-137-expressing hippocampal neurons, whereas sGluA1 levels were reduced during miR-137 overexpression (Figures 1F and 1G), supporting the notion that AMPAR subtype GluA1 is directly targeted by miR-137.

**miR-137 Regulates Excitatory Synaptic Transmission at the Hippocampal CA3-CA1 Synapse**

Next, we examined the role of endogenous miR-137 on synaptic function by assessing the effects of reduced miR-137 expression on synaptic transmission at the hippocampal CA3-CA1 synapse. Simultaneous whole-cell recordings of evoked excitatory postsynaptic currents (eEPSCs) from an 8-10-DIV CA1 pyramidal neuron expressing sponge-miR-137 and an adjacent non-infected neuron were performed. miR-137 downregulation resulted in a potentiation of AMPAR-mediated transmission, but not NMDAR-mediated transmission (Figure 2A). Conversely, miR-137 overexpression significantly depressed AMPAR-mediated transmission, but not NMDAR-mediated transmission (Figure 2B), indicating that modulation of miR-137 levels is associated with opposing effects toward AMPAR-mediated synaptic transmission.

The observed changes are likely due to a postsynaptic effect, since only CA1 neurons were genetically manipulated at the CA3-CA1 synapse and we did not observe any change in presynaptic release probability, as measured by a paired-pulse stimulation paradigm (Figure 2C). Collectively, these results suggest that miR-137 controls excitatory synaptic strength in a cell-autonomous manner.

Letellier et al., 2014; Saba et al., 2012; Schratt et al., 2006), including LTD proteins such as Arc and Map1b (Chen and Shen, 2013; Wibrand et al., 2012). These deletions suggest that variation in miRNA expression affects synaptic protein levels and, consequently, synaptic plasticity by controlling the stability and translation of dendritically localized transcripts.

Using SNP microarray analysis, we have identified microdeletions encompassing MIR137 in a number of unrelated families with genetic forms of ID (Willemsen et al., 2011). These deletions cause reduced miR-137 expression and increased transcript levels for miR-137 target genes such as KLF4, MITF, and EZH2 in ID patient-derived cell lines. Moreover, a SNP linked to MIR137 has shown genome-wide significant association with cognition in ID patient-derived cell lines. Moreover, a SNP linked to MIR137 has shown genome-wide significant association with cognitive impairments.
miR-137 Promotes Silencing of Active Synapses

miR-137 was shown to play a critical role in neuronal morphogenesis during the development of newborn neurons in the DG in vivo (Smrt et al., 2010).

We therefore examined the role of miR-137 in dendritic spine formation or maintenance in 21-DIV hippocampal neuronal cultures. Image analysis revealed that, while no changes were observed in spine type and size by altering miR-137 levels, spine density of sponge-miR-137-infected cells was increased, whereas no effects on spine density were seen upon miR-137 overexpression (Figures 3A and 3B). Together with the finding that AMPAR mEPSC frequency is significantly altered by miR-137, we conclude that miR-137 specifically affects the number of active synapses rather than changes spine morphology at the CA3-CA1 synapse.

Next, we examined the mechanism through which miR-137 mediates synaptic depression. A plausible mechanism could involve silencing of active synapses; i.e., synapses with NMDARs but no functional AMPARs (Hanse et al., 2013). The proportion of these silent synapses rapidly decreases during the first 2 weeks of post-natal development in the hippocampus, coinciding with the maturation process of the synapses (Liao et al., 1999). We therefore measured the amount of surface NMDAR subunit NR1 co-localizing with surface AMPAR subunit GluA1 in hippocampal neurons infected with control (GFP alone), sponge-miR-137, or miR-137 at 8 DIV; g-tubulin was used as the loading control.

D) Mean GluA1 protein levels in the different conditions as indicated, normalized to g-tubulin levels in the same sample, are shown (n = 6; *p < 0.05, one-way ANOVA). (E) Mean GluA1 mRNA levels in the different conditions as indicated are shown (n = 6; one-way ANOVA).

To directly probe for a role of miR-137 in synapse silencing, we measured the failure rate using a minimum stimulation protocol (Isaac et al., 1995; Liao et al., 1995). Whole-cell patch-clamp recordings were obtained from CA1 pyramidal neurons, clamped...
at their resting membrane potential (−60 mV), and excitatory synaptic transmission was elicited with a weak stimulus that produced failures in ±50% of the trials (Figure 3E). Fifty trials per session were recorded at −60 and +40 mV for each cell, and the failure rate at these two holding potentials was computed. In non-infected 8-DIV CA1 neurons, the failure rate was larger at −60 than at +40 mV (Figure 3F), indicating a substantial fraction of the synapses are still silent at this stage of development (Figure 3I). Neurons infected with the sponge-miR-137 showed a similar failure rate measured at −60 mV compared with that at +40 mV (Figure 3G), indicative of fewer silent synapses due to a reduction in miR-137 levels (Figure 3I). Conversely, in CA1 hippocampal neurons overexpressing miR-137, we observed a much higher rate of failures at −60 mV compared with that at +40 mV (Figure 3H), indicative of a high fraction of silent synapses in this condition. Together these data show that the amount of silent synapses during development is significantly altered upon miR-137 expression manipulation, and they suggest that miR-137 acts as a synaptic break during development by preventing the unsilencing of silent synapses.

miR-137 Is Required for mGluR-LTD Expression

Recent findings have shown that both LTP or LTD induction rapidly alters the level of more than 50 different miRNAs in hippocampal neurons (Park and Tang, 2009). Given our results showing that miR-137 regulates AMPAR-mediated synaptic strength, we hypothesized that miR-137 might also be required for synaptic plasticity. We examined the role of miR-137 in mGluR-LTD, a form of synaptic plasticity depending on local protein translation at hippocampal neurons. We first examined the temporal expression pattern of miR-137 after mGluR-LTD induction. Using qPCR we found that LTD induced by the mGluR group I agonist (S)-3,5-dihydroxyphenylglycine (DHPG) rapidly increased mature miR-137 levels within 15 min after DHPG application. Interestingly, the levels of miR-137 were at baseline after 45 min (Figure 4A), suggesting a temporal regulation. The finding that precursor (pre)-miR-137 levels are concomitantly decreased 15 min after DHPG application (Figure 4B) suggested that this rapid regulation is possibly due to an increased processing of pre-miR-137 into mature miR-137 rather than enhanced miRNA transcription. Furthermore, using specific miGluR1 or miGluR5 antagonists (LY367385 and MPEP), we found that a DHPG-induced increase in mature miR-137 levels was dependent on mGluRs, but not on mGluR1, activation (Figure 4C).

Next, we examined whether mGluR5-induced miR-137 increase is required for mGluRs-LTD. We reasoned that acute delivery of LNA-anti-miR-137 would only interfere with the DHPG-induced miR-137 expression, without affecting basal levels of miR-137. Indeed, introducing LNA-anti-miR-137 or control LNA-anti-miRNA-NT, into CA1 neurons of organotypic hippocampal slices via whole-cell recording pipettes, did not affect basal synaptic transmission when neurons were treated with the respective LNA-anti-miRNAs for 60 min (Figure 4D). MGlur-LTD can be induced chemically, using DHPG or by synaptic activation of mGluRs, using paired pulses of low-frequency (1 Hz) synaptic stimulation (PP-LFS). Consistent with previous studies, DHPG application (100 μM, 5 min) or PP-LFS caused an acute depression in evoked EPSCs onto both neurons treated with LNA-anti-miRNAs (Figures 4E and 4F). However, only a persistent LTD was observed in LNA-anti-miRNA-NT-treated neurons, but not in LNA-anti-miR-137 neurons (Figures 4E–4G).
Finally, miR-137 was selectively required for mGluR-LTD, as no effect of manipulating miR-137 levels on NMDAR-induced LTD was observed (Figure S2). Together these data show that miR-137 is selectively required for mGluR5-mediated LTD.

**DISCUSSION**

**miR-137 Regulates AMPAR Subunits and Synaptic Transmission in Hippocampal Neurons**

Small non-coding miRNAs recently have emerged as key regulators of neuronal development and synaptic plasticity (Olde Loohuis et al., 2012). In this study, we identified AMPAR subunit GluA1 as a target of miR-137. By temporally and spatially manipulating miR-137 levels at the hippocampal CA3-CA1 pathway, we demonstrate that postsynaptic miR-137 controls maturation and plasticity of excitatory synaptic function by regulating AMPAR expression. miR-137 repressed the incorporation of AMPARs into the synaptic membrane, thereby preventing synaptic maturation and plasticity. In particular, overexpressed miR-137 in organotypic brain slices silenced active synapses, likely by the removal of sGluA1. Conversely, decreased miR-137 levels led to the accelerated maturation of excitatory synapses.
Our observations could have direct implications for how experience-driven activity drives early neuronal development, particularly in the context of neurodevelopmental disorders such as ID and autism spectrum disorder (ASD) (Hanse et al., 2013). Decreased levels of miR-137 during development may lead to disturbed activity-dependent selection of AMPAR-silent synapses by excessive and premature AMPAR unsilencing and subsequent synapse stabilization. Recently, in animal models of ASD, such indiscriminative unsilencing of synapses has been shown to lead to distorted neuronal network formation. For example, haplo-insufficiency of Syngap1 in mice leads to premature maturation of hippocampal and cortical synapses, resulting in a shortening of the critical period for plasticity (Clement et al., 2012, 2013).

The changes in synaptic AMPAR-mediated currents observed in our study are likely a result of altered total GluA1 expression by miR-137. However, we cannot exclude that molecular changes induced by altering miR-137 also could impact directly AMPAR trafficking or actin cytoskeleton dynamics. Of note, previous gene ontology (GO) analysis of the transcriptome data (Collins et al., 2014) suggests that the actin-remodeling pathway, including the Rho GTPase pathway (Govek et al., 2005), is a prominent signaling pathway that is targeted by miR-137. This is of interest since neuronal plasticity requires both actin cytoskeleton remodeling and local protein translation in response to extracellular signals (Zukin et al., 2009). Indeed, insertion and removal of AMPARs in the context of LTP and LTD has been shown to be dependent on actin polymerization (Bosch and Hayashi, 2012; Cingolani and Goda, 2008). miR-137 may thus trigger a selective removal of GluA1 from a subset of synapses that subsequently become silent by targeting directly the actin cytoskeleton pathway as well as impact directly the formation of dendritic spines. In this context, it is of note that the density of dendritic spines also were increased in sponge-miR-137-infected neurons. However, we did not observe any effects on spine density upon miR-137 overexpression, which is in accordance with a previous in vivo study in which miR-137 levels were altered in hippocampal neurons (Smrt et al., 2010). Alternatively, the effects on dendritic spines could be secondary to the effects on GluA1 expression.
miR-137 and mGluR-LTD

In this paper, we provide evidence that miR-137 is necessary for mGluR-LTD, a form of protein-synthesis-dependent synaptic plasticity (Huber et al., 2000; Kinn and Dever, 2004; Snyder et al., 2001). Our experiments in organotypic hippocampal slices revealed that miR-137 levels were upregulated 15 min following LTD induction with DHPG, whereas pre-miR-137 expression decreased within the same time span, suggesting possibly increased miR-137 maturation upon DHPG-LTD. These data are in line with a previous study in which more than 50 miRNAs were found to be induced immediately after mGluR-LTD stimuli in vitro (Park and Tang, 2009). This activity-dependent regulation of miRNAs therefore suggests that they play a general role in regulating protein synthesis during LTD expression. However, to the best of our knowledge, only for miR-146a-5p and miR-125a the requirement for mGluR-LTD has been demonstrated directly (Chen and Shen, 2013; Muddashetty et al., 2011).

For miR-137, we addressed this question by using an LNA-anti-miR-137 oligonucleotide that efficiently sequestered endogenous miR-137. Using simultaneous recordings in organotypic brain slices, we found that acute inhibition of miR-137 during LTD induction blocked LTD expression, suggesting that this synaptically enriched miRNA indeed is necessary for mGluR-LTD. Of interest is the time dependency of miR-137 up-regulation. This has been observed for multiple miRNAs induced after mGluR-LTD (Park and Tang, 2009), and it might represent a general mechanism by which miRNAs may play a role in restoring the repression state of mRNA translation after the transient activation (Ashraf and Kunes, 2006). Because mGluR-induced miR-137 upregulation is transient, it may be required for only a particular phase of LTD, for example, during the consolidation phase. In line with this idea, we found that only the later phase of LTD was affected and not the initial phase when miR-137 function was blocked. Rapid upregulation of miR-137 following LTD induction is thus possibly part of a precisely controlled mechanism to maintain a defined pool of synaptic proteins involved in synaptic AMPAR expression.

In the present study, we found several lines of evidence for a key role for miR-137 in controlling synaptic efficacy and plasticity. Our study suggests that endogenous miR-137 functions as a synaptic break during development and regulates excitatory synaptic strength through direct modulation of GluA1 levels. In conclusion, we link genetic deficits in MIR137 to glutamatergic dysfunction, which may contribute to our understanding of the pathogenesis of miR-137-linked cognitive impairments.

EXPERIMENTAL PROCEDURES

Virus production, luciferase assay, western blot, immunofluorescence, and image analysis are described in the Supplemental Experimental Procedures.

miRNA Constructs

The sponge-miR-137 sequence was designed based on the rat miR-137-3p sequence according to the original publication describing the application of miRNA-sponge (Ebert et al., 2007; Klüver et al., 2012). Briefly, the sponge-miR-137-3p sequence contains four consecutive miR-137-3p binding sites, each separated by four nucleotides. The miR-137 binding site contains bulged sites that are non-complementary to the miR-137 positions 11–14, in order to enhance sponge efficacy (Ebert et al., 2007; Gentner et al., 2009). Sponge-miR-137 oligonucleotides contained recognition sequences for the restriction enzymes BsrGI and EcoRI. Oligonucleotides (Sigma) were annealed in annealing buffer (containing 10 mM Tris [pH 7.5], 50 mM NaCl, and 1 mM EDTA) through a 5-min incubation at 90°C, followed by a slow cooldown to room temperature. Annealed sponge-miR-137 oligonucleotides were ligated into the FUGW vector (Plasmid 14883, Addgene) directly following the stop codon of the eGFP cDNA. To create a lentiviral vector construct carrying the rat pre-miR-137 cDNA, we PCR-amplified the precursor miR-137 containing flanking 70 nucleotides for both the 3’ and 5’ regions of the rat miR-137 from total rat brain RNA, and we ligated the PCR product into the FUGW vector using the BsrGI and EcoRI restriction sites.

Assessment of Sponge-miR-137 Function

The direct coupling of the concatemeric sponge-miR-137 sequence to the 3’ site of the GFP cDNA allows efficient binding of miR-137 to the GFP-sponge-miR-137-encoding transcript, ultimately resulting in an attenuation of GFP translation. Reduced GFP fluorescent signal intensity therefore can be used as a measure of sponge-miR-137 functional efficacy (Figure S1). For GFP quantification, the sponge-miR-137-containing vector was transfected into B35 rat neuroblastoma cells together with either a non-targeting (nt) mimic or a mimic-miR-137 oligonucleotide using Lipofectamine 2000 (Life Technologies), according to the manufacturer’s instructions. Then, 48 hr post-transfection, cells were fixed in 4% paraformaldehyde (PFA) in sucrose in PBS and visualized with a Leica DFC 420C fluorescence microscope using a 20x magnification. GFP quantification was performed using the ImageJ software. We also detected significantly reduced endogenous miR-137 levels in sponge-miR-137-infected neurons compared to GFP-infected neurons, as measured by qPCR, suggesting that sponge-137 not only sequesters miR-137 but also results in an enhanced turnover rate of this miRNA.

Electrophysiology

Whole-cell recordings in cultured slices were obtained with Multichamp 700B amplifiers (Axon Instruments). To study the effects of miR-137 on synaptic transmission, cultured slices from P6–8 rats (Wistar, Harlan Laboratories) were infected with control, sponge-miR-137, or miR-137 lentiviruses directly upon plating. Then, 8 to 10 days later, whole-cell recordings were obtained simultaneously from an infected and an adjacent uninfected neuron in the CA1 region under visual guidance using epifluorescence and transmitted light illumination. See the Supplemental Experimental Procedures for details.

Statistical Analysis

Statistical analyses were performed using Prism software (GraphPad). Statistical tests were used with alpha level at 0.05. Two group comparisons were evaluated using non-paired two-tailed t test. Three or more groups were evaluated using one-way ANOVA followed by post hoc Bonferroni correction for multiple testing. Data are plotted as mean ± SEM. Quantification was performed in a blinded fashion.

Animals

All experiments involving animals were evaluated and approved by the Committee for Animal Experiments of the Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and two figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.05.040.

AUTHOR CONTRIBUTIONS

N.F.M.O.L., N.N.K., G.J.M.M., H.v.B., and A.A. conceived the experiments and wrote the manuscript. N.F.M.O.L., A.K., A.A., and A.J. performed all experiments except electrophysiology. N.N.K. and W.B. performed and analyzed all electrophysiology experiments. P.H.S. and G.S. contributed biochemical and molecular biology data.
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