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Elfn1 regulates pyr → SOM release probability

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ABSTRACT

Excitatory synapses onto somatostatin (SOM) interneurons show robust short-term facilitation. This hallmark feature of SOM interneurons arises from a low initial release probability that regulates the recruitment of interneurons in response to trains of action potentials. Previous work has shown that Elfn1 (extracellular leucine rich repeat and fibronectin type III domain containing 1) is necessary to generate facilitating synapses onto SOM neurons by recruitment of two separate presynaptic components: mGluR7 (metabotropic glutamate receptor 7), and GluK2-KARs (kainate receptors containing glutamate receptor, ionotropic, kainate 2). Here, we identify how a transsynaptic interaction between Elfn1 and mGluR7 constitutively reduces initial release probability onto mouse cortical SOM neurons. Elfn1 produces glutamate-independent activation of mGluR7 via presynaptic clustering, resulting in a divergence from the canonical “autoreceptor” role of Type III mGluRs, and substantially altering synaptic pharmacology. This structurally-induced determination of initial release probability is present at both layer 2/3 and layer 5 synapses. In layer 2/3 SOM neurons synaptic facilitation in response to spike trains is also dependent on presynaptic GluK2-KARs. In contrast, layer 5 SOM neurons do not exhibit presynaptic GluK2-KAR activity at baseline and show reduced facilitation. GluK2-KAR engagement at synapses onto layer 5 SOM neurons can be induced by calmodulin activation, suggesting that synaptic function can be dynamically regulated. Thus, synaptic facilitation onto SOM interneurons is mediated both by constitutive mGluR7 recruitment by Elfn1 and regulated GluK2-KAR recruitment, which determines the extent of interneuron recruitment in different cortical layers.

SIGNIFICANCE STATEMENT

This study identifies a novel mechanism for generating constitutive G protein coupled receptor (GPCR) activity through a transynaptic Elfn1/mGluR7 structural interaction. The resulting tonic suppression of synaptic release probability deviates from canonical autoreceptor function. Constitutive suppression delays the activation of somatostatin interneurons in circuits, necessitating high frequency activity for somatostatin interneuron recruitment. Furthermore, variations in the synaptic proteome generate layer specific differences in facilitation at pyr → SOM synapses. The presence of GluK2 kainate receptors in L2/3 enhances synaptic transmission during prolonged activity. Thus, layer

specific synaptic properties onto somatostatin interneurons are mediated by both constitutive mGluR7 recruitment and regulated GluK2 kainate receptor recruitment, revealing a mechanism that generates diversity in physiological responses of interneurons.

INTRODUCTION

Nervous system function is derived from both stable, genetically encoded neural circuit architecture, and dynamic, activity-dependent synaptic and cellular physiology. Synaptic proteins play a role in altering synaptic structure and regulating synaptic activity to generate neural circuit physiology. The leucine rich repeat (LRR) containing superfamily of synaptic proteins conveys synaptic structure to pairs of connected neurons by regulating transsynaptic interactions with molecular specificity (de Wit and Ghosh, 2015). These synaptic proteins can not only selectively generate connectivity between specific cell types (Cao et al., 2015) or even subcellular regions (DeNardo et al., 2012), but can also determine the nature of the synaptic connection that is produced. Elfn1 (extracellular leucine-rich repeat fibronectin containing 1), a postsynaptic LRR-containing protein, is necessary to induce the electrophysiological properties of a synaptic connection between pyramidal neurons and somatostatin type interneurons (pyr → SOM) in the hippocampus (Sylwestrak and Ghosh, 2012). This strongly facilitating synapse regulates the recruitment of SOM interneurons by introducing a selective delay in spike generation as a result of the low initial release probability of incoming excitatory inputs, which in turn generates the distinct timing properties of these inhibitory interneurons (Pouille and Scanziani, 2004; Sylwestrak and Ghosh, 2012; Pala and Petersen, 2015). Elfn1 alters the proteomic constitution of these synapses, as shown by the identification of mGluR7 as a presynaptic binding partner for Elfn1 (Tomioka et al., 2014), as well as the demonstration that Elfn1 is necessary for the functional engagement of GluK2 subunit containing kainate receptors (GluK2-KARs aka GluR6) at pyr → SOM synapses (Sylwestrak and Ghosh, 2012). The synaptic specificity of these receptors has long been recognized, along with the ability of these presynaptic components to change synaptic release properties (Shigemoto et al., 1996; Sun and Dobrunz, 2006; Pelkey and McBain, 2008; Sun et al., 2009; Tomioka et al., 2014). Elfn1 has been shown to play a structural role critical for synapse formation in retina (Cao et al., 2015). However, it is unclear whether Elfn1 plays a passive structural role at pyr → SOM synapses by simply recruiting active presynaptic components, or whether Elfn1 may actively alter receptor function to produce the characteristic physiological properties of pyr → SOM synapses. Target cell specific synaptic release has also been described for L2/3 interneurons, both in cortical slices and *in vivo* (Reyes et al., 1998; Pala and Petersen, 2015). Here we examine how the target cell specific proteomic substrates of a synapse may be regulated to give rise to layer specific synaptic properties of pyr → SOM synapses.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All experimental protocols were conducted according to Swiss Laboratory Animal Science Association guidelines for animal research and were approved by the Roche Animal Care Committee and the Basel Cantonal Veterinary Committee.

Animals were housed on a 12 hr light (06:00) / dark (18:00) cycle with ad libitum access to water and mouse chow. For characterizing synaptic responses, male and female mice (11–19 days old) were used in cortical brain slice physiology experiments. Some off-target label in parvalbumin neurons has been reported (Hu et al., 2013) for the somatostatin-cre line used (Jackson Labs, Ssttm2.1(Cre)Zjh/J). Thus, following the initial characterization of WT vs KO synaptic phenotypes, which included all cells, the subsequent pharmacological experiments were not conducted on cells that carried a stereotypical parvalbumin signature (depressing synapse (Pouille and Scanziani, 2004; Tan et al., 2008)). Elfn1 KO was from the Davis KOMP mouse repository (Elfn1tm1(KOMP)Vlcg), and the Ai14, lox-stop-lox-tdTomato was from Jackson Labs (B6.Cg-Gt(ROSA)26Sor<tm14(CAG-tdTomato)Hze/J).

METHODS

Electrophysiology

Coronal brain slices from sensory cortex (approximately bregma -0.5 to -2.0 mm) from SOM-cre x tdTom or Elfn1 KO x SOM-cre x tdTom mice were prepared in cold cutting solution containing (in mM): 75 sucrose, 87 NaCl, 25 $NaHCO_3$, 25 D-glucose, 2.5 KCl, 7 $MgCl_2$, and 1.25 NaH_2PO_4, aerated with 95% O_2 / 5% CO_2. Slices were transferred to artificial cerebrospinal fluid (aCSF) containing (in mM): 120 NaCl, 26 $NaHCO_3$, 10 D-glucose, 3 KCl, 2 $MgCl_2$, 2 $CaCl_2$ and 1.25 NaH_2PO_4, aerated with 95% O_2 / 5% CO_2. The intracellular solution contained (in mM): 125 K gluconate, 2 KCl, 10 HEPES, 10 phosphocreatine, 4 $MgCl_2$, 1 EGTA, 0.1 $CaCl_2$, 4 ATP, 0.4 GTP, pH 7.35, 290 mOsm.

Human Embryonic Kidney (HEK) cell recordings were in HEPES buffered saline containing (in mM): 135 NaCl, 5 HEPES, 10 D-glucose, 5 KCl, 1.2 $MgCl_2$, and 2.6 $CaCl_2$, pH 7.2-7.4.

Bath applied compounds NS102 (20 mM, Sigma, N179, CAS: 136623-01-3) and AMN082 (1 mM, VWR, Ab120011, CAS: 97075-46-2) were dissolved as stock solutions in DMSO; MSOP (50 mM, Tocris, 0803, CAS: 66515-29-5) and L-AP4 (Enzo, ALX-550-026; CAS: 23052-81-5, pH 7.4) were

dissolved in water. HEK cell recordings were done in HEPES buffered saline, and Elfn1-Fc (~10 μM) and MAb 1/28 (2.5 mg/mL, ~20 μM) were applied directly to cells (2.5 μL into ~2.5 mL for ~1000x dilution).

Cell culture

Human embryonic kidney (HEK 293) cells were grown to 90-95% confluency in 6 well plates (with DMEM, 10% horse serum, penicillin/streptomycin), and transfected with 2 ug DNA/well using Lipofectamine 2000 (Life Technologies). Cells were washed, collected with trypsin/EDTA, pelleted, re-suspended in 1 mL DMEM, and re-plated at a 1:3 dilution onto poly-l-lysine coated coverslips. Co-plated cells were incubated 15-48 hours, then fixed in 4% paraformaldehyde for 30 minutes, stained with anti-ZO1 (mouse 1:100 Life Technologies #339100; secondary goat anti-mouse 647) and mounted onto slides with Prolong Gold medium. Surface fluorescence at cell-cell contact sites was quantified at ZO1 labeled tight junctions and was normalized within the same cell to surface fluorescence at junctions contacting unlabeled cells. FLIPR (fluorometric imaging plate reader) assay was performed with the FLIPR Calcium 6 Assay on a Hamamatsu FDSS 7000 using a mGluR7 HEK cell line provided by L. Lindemann (Lindemann et al., 2011). For FLIPR, Elfn-Fc concentration was diluted 100x.

Elfn1-Fc DNA (10 μg) was transfected into HEK 293 cells plated at 80% confluency on T75 culture flasks with Lipofectamine 2000. Extracellular culture medium was collected at 48-72 hours and run through a NAb protein A spin column. The column eluate was adjusted to pH 7 and then concentrated in Amicon Centrifugal 4ml 30KDa filters to generate concentrated Elfn1 Fc protein.

Primary cortical neurons were cultured from C57/Bl6 E16.5 mouse embryos. Neurons were maintained in Neurobasal-A medium, supplemented with B27, glucose, glutamax, and penicillin/streptomycin at a cell density of 15K/well in 96 well plates (441cells/mm^2). Cells were fixed on DIV14 or DIV20 with 4%PFA for 60 min.

Histochemistry

Immunostaining of 50 µm coronal brain sections was as follows: following transcardial perfusion with 4% paraformaldehyde and overnight post fixation, 1 hr blocking in normal donkey serum with 0.2% TritonX-100 was followed by overnight incubation in primary antibody, followed by 2 hrs in secondary antibody. Primary antibodies were: Mouse Monoclonal anti-ZO-1 (Life Technologies, ZO1-1A12), rabbit anti-Elfn (anti-Irrc62, Prestige Antibodies-Sigma, HPA000781), rabbit anti-GluR6/7 (anti-GluK2/3, Millipore, 04-921), anti-mGluR7 rabbit polyclonal antibody (Upstate/Millipore, 07-239), anti-mGluR7 mouse monoclonal antibody (MAB1/28, custom Roche antibody, (Ullmer et al., 2012)), control antibody, (donkey anti-chicken 488, Jackson Immuno, 703-545-155), rat anti-somatostatin (Santa Cruz, SC-7819).

In situ hybridization for GRIK2 mRNA and Elfn1 mRNA were performed using the protocol from the RNAScope multiplex in situ hybridization kit (ACDBio, 320850).

Western blot

Dissection of hippocampus from 6-week-old mice was followed by tissue homogenization in lysis buffer (containing 50 mM Tris, pH 7.5; 150 mM NaCl; 5 mM EDTA; 1% TritonX-100; 10% glycerol; 1mM PMSF; Roche protease inhibitors), with 20s shaking using Precellys ceramic bead system. MAB1/28 immunoprecipitation of mGluR7 was done from 500 µL of cortical lysates (~100 mg tissue), incubated overnight in a NAb protein A spin column prebound (1hr) to 1 µg of MAB1/28. The column was washed twice with lysis buffer, once with PBS, then eluted with 500 µL 2x SDS buffer. Sample eluate (20 µL) was run beside sample input (2 µL) with reducing agent on a 4-12% Bis-Tris gel in MOPS buffer for 2.5 hrs. Gels were blotted overnight onto PVDF membranes in transfer buffer with 0% methanol. Incubation overnight with primary then 2 hrs in anti-rabbit IR800 secondary was followed by exposure on Licor Odyssey IR scanner. Representative blot was verified with at least 2 biological replicates and at least 3 technical replicates.

Co-IP of mGluR7 was done from 500 µL of cortical lysates (~50 mg tissue) in lysis buffer using a Dounce homogenizer. GluK2-GFP/mGluR7-GFP expressing HEK cells were collected with trypsin/EDTA, pelleted, washed in cold PBS, and lysed in lysis buffer. Lysates were spun at 100 000 x g for 1 hr at 4°C, then stored at -20°C. Samples incubated overnight with 2.5 µg of MAB1/28 or 1 µg of rabbit anti mGlur7 and spun at 14000 rpm for 10 min. Protein A sepharose beads (30 µl) was added, with end over end mixing at 4°C (4hrs). Beads were pelleted at 3000 rpm for 2 minutes, the

unbound fraction saved for analysis, and then washed 3x with 500 µl lysis buffer, 1x with PBS, then pelleted at 14000 rpm for 1 min. Beads were resuspended in 30 µl of 2x SDS buffer (200 mM DTT, 100 mM Tris-HCl pH 6.8, 20% glycerol, 4% SDS, Bromophenol blue 0.025%), boiled at 95 C for 5 min, spun down at 14000 rpm for 5 min, then analyzed by western blot.

Elfn1-tdTomato expressing HEK cells were collected with trypsin/EDTA, pelleted, and washed in cold PBS. Crosslinking was performed with bis[sulfosuccinimidyl]suberate (BS3), applied either to cell suspensions at 0.1-3 mM in 500 µL PBS, or to 0.5 µg purified Elfn-Fc protein at 3-100 µM in 20 µL PBS for 30 min at room temperature, quenched with 20 µL 0.5M Tris-HCl pH 6.8 for 15 min. Cells were lysed in 50 mM Tris, pH 7.5; 150 mM NaCl; 5 mM EDTA; 1% TritonX-100; 0.1% SDS; 0.1 mM beta mercaptoethanol; protease inhibitors and were incubated on ice for 1 hour, spun at 14000 rpm for 10 min, then stored at -20°C . Western blotting was done on a 4-20% mini-PROTEAN TGX gel (Biorad 456-1093) gel, run in SDS buffer. Protein was transferred to a nitrocellulose membrane in 10% propranolol in SDS buffer, blocking in TBS tween 1% with 5% milk powder. Incubation overnight with primary then 2 hrs in HRP secondary was followed by exposure using Advansta WesternBright Sirius detection reagent (K-12043-D20) on a Biorad ChemiDoc MP imaging system. Representative blots were verified with at least 2 biological replicates and at least 3 technical replicates.

Image Acquisition and Analysis

Images were captured on the Operetta High-Content Imaging System (PerkinElmer) with 20X WD objective in non-confocal mode, a Leica SP5 confocal microscope (Leica Microsystems, Bannockburn,IL) or an Olympus FV1000 confocal microscope. Z-stacks were collapsed in a maximum projection and analyzed using LAS software (Leica) or ImageJ.

Plasmids

The Elfn1-tdTomato plasmid was created by swapping tdTomato for the GFP label in Efln1-GFP (Sylwestrak and Ghosh, 2012), containing the pEGFPN1 backbone. Elfn1-Fc plasmid was created by swapping Elfn1 ectodomain for the CASPR ectodomain in 3CPro backbone from Davide Comoletti (Rubio-Marrero et al., 2016). The ΔLRR-Elfn1-GFP plasmid was created by subcloning ΔLRR-Elfn1 into pEGFPN1 backbone. The Δecto-mGluR7-GFP plasmid was created by custom synthesis of

206 Δecto-mGluR7 and subcloning into pCMV6-AC-GFP. mGluR7-GFP and GRIK2-GFP plasmids were
207 purchased from Origene (RG217402, RG222369).
208
209 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS
210 Potassium reversal potential for HEK cell recordings was -82 mV. The holding potential for neuronal
211 voltage clamp recordings was -70 mV (Cl^- E_{rev}). Hippocampal recordings used a bipolar stimulating
212 electrode positioned in the alveus. Cortical recordings used a monopolar stimulation through a glass
213 pipette positioned in L2/3 or L5 of sensory cortex in acute slices from SOM-cre x tdTomato (Ai14)
214 mice. The stimulating electrode was placed typically 50 - 150 µm from the recorded cell, moving
215 parallel to the pial surface. Stimulation within a cortical layer was thus presumed to preferentially
216 recruit activity from local neurons. However, the extensive nature of cortical connectivity makes it
217 impossible to rule out the possibility of excitation of synaptic inputs from distal sites. Representative
218 traces are an average of 12 sweeps (2 minutes of data).
219 For synaptically-induced SOM firing, action potentials were recorded with loose-seal patch in
220 response to synaptic stimulation (10 x 50 Hz stim, 50- 100 µs, 30-100 µA, set +10 µA above synaptic
221 spike threshold). Stimulation intensities were: L2/3 WT, 50 ± 5 µA, L2/3 KO 49 ± 3 µA, L5 WT, 52 ± 3
222 µA. Time to half max spiking was calculated by fitting the average spike probability from 10 sweeps of
223 10 x 50 Hz stimuli using the Matlab dose_response script (Evangelista, 2014). For Elfn1 KO, the first
224 5 of the 10 x 50 Hz events were fit, as these made better fits than when including the late events
225 where the maximum spiking probabilities were not maintained. Plots of spike probability show the
226 average of the first 5 of 10 events, for clarity.
227 Quantal amplitude in WT and Elfn1 KO were calculated by fitting spontaneous EPSC amplitude
228 distribution histograms with the multiple gaussian curve fitting function in Clampfit.
229 Synaptic release probability was calculated using a similar method to the variance-mean analysis
230 described by Oleskevich et al. (Oleskevich et al., 2000). Briefly, the variance from each of 5x 50 Hz
231 synaptic currents from 10 or more consecutive sweeps were plotted as a function of mean amplitude.
232 The rapidly changing release probability for synaptic currents 1 through 5 resulted in a curve that for
233 most cells could be fit with the equation:
234 $y = Ax - Bx^2$

y is the variance, x is the mean amplitude, and coefficients A and B were then used to calculate release probability for a given amplitude "x" as follows:

release probability = $x(B/A)(1 + \text{coefficient of variation}^2)$

The coefficient of variation (standard deviation / mean) was estimated to be 0.5 on average, based on asynchronous release events sampled from a subset of the data. For cells that could not be fit with $y = Ax - Bx^2$ with an $r^2 > 0.3$, the release probability calculation was excluded. The number of excluded cells for L2/3 WT was 1/24, for L2/3 KO was 2/24 for L5 WT was 4/24, for L5 KO was 8/24.

Values are represented as mean ± SEM. Number of measurements n/N represents n cells recorded from N animals, typically using 1 cell per slice. For HEK cell experiments n/N represents n cells from N biological replicates. Statistical testing was done in Matlab. Comparisons within conditions were made by two tailed paired Student's t test, treatment versus baseline. Comparisons across conditions or between genotypes were done with an unpaired t test assuming unequal variance. For multiple comparisons, a one-way or two-way ANOVA was done with a Bonferroni post hoc test. Holm's correction for multiple comparisons has been applied to p values (listed as "corrected p") wherever L2/3 WT data was compared first to L2/3 KO data and then later to L5 data. Statistical outcomes are represented in figures as: n.s. $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, whereas full p values and test statistics are reported throughout the text.

RESULTS

Interneurons in Elfn1 knockout mice show reduced synaptic facilitation

We had previously reported that shRNA-mediated knockdown of Elfn1 in oriens-lacunosum molecular (OLM) cells (SOM interneurons) in hippocampus leads to a marked reduction in synaptic facilitation of pyr → SOM synapses, whereas introduction of Elfn1 into parvalbumin interneurons is sufficient for the cell-autonomous induction of facilitation at those synapses (Sylwestrak and Ghosh, 2012). Synaptic facilitation arising from high frequency stimulation results in the preferential recruitment of dendritic targeting OLM cells rather than soma targeting interneurons (Pouille and Scanziani, 2004). To determine if cortical SOM neurons are likewise recruited by high frequency stimulation, we examined synaptic facilitation at pyr → SOM synapses in L2/3 of sensory cortex in

264 acute slices from SOM-cre x tdTomato (Ai14) mice. Labelled neurons in L2/3 showed a strong short-
265 term facilitation of excitatory transmission in response to high frequency stimulation (50 Hz, Fig 1A)
266 that occurred in conjunction with a delay of synaptic induced spiking, as seen previously for
267 hippocampal SOM interneurons (Pouille and Scanziani, 2004; Sylwestrak and Ghosh, 2012). Genetic
268 deletion of Elfn1 reduced the facilitation ratio (EPSC5/EPSC1 ratio: 3.1 ± 0.2 in KO vs. 7.7 ± 0.9 in
269 WT, corrected p = 0.00006, t = 5.02, t test, n/N = 24/6 and 24/9 respectively, 5 x 50 Hz, Fig 1B) and
270 the recruitment delay for L2/3 SOM neurons compared to WT (stimuli to half-max spiking: 1.1 ± 0.3 in
271 KO, vs. 2.8 ± 0.2 in WT, corrected p = 1 x 10^{-6}, t = 6.05, t test, n/N = 16/2 and 24/4 respectively, 5 x
272 50 Hz, Fig 1C,D). L2/3 pyr → SOM synapses showed an increased initial release probability in Elfn1
273 KO animals (Fig 1E, calculated from mean-variance analysis (Oleskevich et al., 2000)). Conversely,
274 Elfn1 KO produced no change in quantal amplitude, as calculated from spontaneous EPSC amplitude
275 distributions (quantal amplitude: 12.1 ± 0.5 in KO vs. 11.7 ± 0.7 in WT, p = 0.7, t = 0.42, t test, n/N =
276 6/1 and 8/3 respectively, Fig 1F). As shown previously for miniature EPSC amplitudes during shRNA
277 knockdown of Elfn1 (Sylwestrak and Ghosh, 2012), the similar synaptic amplitudes in WT and KO
278 indicate that Elfn1 removal does not affect postsynaptic properties. Thus, a prominent feature of pyr
279 → SOM synaptic facilitation and delay-to-spiking is the low initial release probability.
280
281 *Elfn1 recruitment of mGluR7 constitutively reduces synaptic release probability*
282 Elfn1 KO mice exhibit hyperactivity and handling induced seizures (Dolan and Mitchell, 2013;
283 Tomioka et al., 2014). A similar phenotype is observed in mGluR7 KO mice (Sansig et al., 2001),
284 which predicted the subsequent identification of mGluR7 as a presynaptic binding partner of Elfn1
285 (Tomioka et al., 2014). Elfn1 KO substantially reduces mGluR7 immunoreactivity at synapses onto
286 somatostatin neurons in hippocampus (Tomioka et al., 2014). Constitutive activity of mGluRs is
287 thought to contribute to the properties of this synapse (Losonczy et al., 2003) and mGluR7
288 homodimerization has been proposed as a mechanism by which such constitutive activity may be
289 generated (Kammermeier, 2015; Levitz et al., 2016). We therefore hypothesized that Elfn1 may
290 influence release probability through promoting constitutive activity of mGluR7. In cortical cultures,
291 mGluR7 is selectively clustered along the dendritic arbor of SOM neurons where it colocalizes with
292 Elfn1 at synapses (Fig 2A). In brain slices, mGluR7 dendritic clustering is also visible on SOM
293 interneurons in L2/3 of cortex (Fig 2B), and is disrupted by Elfn1 deletion (Fig 2C). Elfn1 co-

294 immunoprecipitates (co-IP) mGluR7, as well as other Type III mGluRs, indicating that these proteins
295 physically interact (Tomioka et al., 2014; Dunn et al., 2018). We assessed the effects of mGluR7 on
296 synaptic transmission at pyr → SOM synapses by antagonizing mGluR7 with the mGluR (Type III) -
297 selective antagonist MSOP (100 μM). MSOP increased synaptic transmission at pyr → SOM
298 synapses in L2/3 (EPSC amplitude: 63 ± 10 pA in MSOP vs. 55 ± 10 pA baseline, p = 0.002, t = 3.82,
299 paired t test, n/N = 14/8, Fig 2D). Surprisingly, the change in synaptic transmission was primarily
300 restricted to a change in the amplitude of the first EPSC. The effects of MSOP were reversible, with
301 washout of compound restoring first EPSC amplitude to baseline. The MSOP-mediated increase in
302 initial release was absent in Elfn1 knockout animals (EPSC amplitude, L2/3 KO: 150 ± 49 pA in
303 MSOP vs. 160 ± 54 pA at baseline, p = 0.3, t = 1.25, paired t test, n/N = 6/2, Fig 2E). The ability of the
304 mGluR7 antagonist MSOP to increase initial release probability in L2/3 suggests a novel mechanism
305 of action for Elfn1/mGluR7 signaling. Contrary to the expectation that mGluR7 is responding to
306 synaptic glutamate, the constitutive reduction in synaptic release probability is consistent with an
307 agonist independent constitutive mGluR7 activity and is not found in Elfn1 KO (Fig 2F). The loss of
308 constitutive activity in Elfn1 KO was not due to a reduction in total mGluR7 protein levels in cortex (Fig
309 2G), but rather due to a redistribution mGluR7 protein localization away from the discrete dendritic
310 clustering that is visible in WT (Fig 2H-I). These results are consistent with previous findings in the
311 hippocampus (Tomioka et al., 2014).

312 To further characterize this novel mode of action for synaptic mGluR7, we activated mGluR7
313 directly with the Type III mGluR agonist L-AP4. L-AP4 caused no further suppression of initial release
314 in WT slices but suppressed initial release in L2/3 in Elfn1 KO slices (EPSC amplitude, L2/3 WT: 16 ±
315 3 pA in 30 μM L-AP4 vs. 18 ± 3 pA at baseline, p = 0.3, t = 1.06, paired t test, n/N = 11/5, L2/3 KO: 54
316 ± 12 in 30 μM L-AP4 vs. 77 ± 16 at baseline, p = 0.007, t = 4.86, paired t test, n/N = 9/2, Fig 2J). The
317 lack of suppression in WT slices was not likely due to a "floor effect" or to a reduction in total receptor
318 expression levels, as high dose L-AP4 (300 μM) still produced strong suppression of initial release in
319 both Elfn1 WT and KO slices (EPSC amplitude, L2/3 WT: 28 ± 8 pA in 300 μM L-AP4 vs. 51 ± 11 pA
320 at baseline, p = 0.0004, t = 4.90, paired t test, n/N = 13/6, L2/3 KO: 37 ± 8 in 300 μM L-AP4 vs. 65 ±
321 18 at baseline, p = 0.02, t = 2.82, paired t test, n/N = 9/4, Fig 2K). Comparing responses across doses
322 and genotypes, we find that initial release in WT, L-AP4 (300 μM), KO, L-AP4 (300 μM), and KO, L-
323 AP4 (30 μM) differ from baseline (p = 0.000001, p = 0.0009, and p = 0.03 respectively, F = 4.4, 2-way

ANOVA, Bonferroni post-hoc). In contrast to antagonism with MSOP, later EPSCs in both WT and KO slices showed synaptic suppression in the presence of exogenous agonist (change vs baseline, WT: EPSC1 p = 1, EPSC2 p = 0.02, EPSC3 p = 0.006, EPSC4 p = 0.06, EPSC5 p = 0.03, F = 44.31, two-way ANOVA, post hoc Bonferroni, n = 11/5; change vs baseline, KO: EPSC1 p = 9 $x10^{-7}$, EPSC2 p = 3 $x10^{-5}$, EPSC3 p = 3 $x10^{-5}$, EPSC4 p = 0.0004, EPSC5 p = 0.0004, F = 140.18, two-way ANOVA, post hoc Bonferroni, n = 9/2). Thus, although mGluR7 can act as an "autoreceptor" in response to exogenous application of L-AP4, we find no evidence for autoreceptor activity in the generation of synaptic facilitation at pyr → SOM synapses (Fig 2L).

Elfn1 recruitment of mGluR7 generates constitutive mGluR7 activity

We tested the sufficiency of Elfn1 to generate constitutive mGluR7 activity in a heterologous cell assay. Human embryonic kidney (HEK) cell tight junctions consist of transcellular interactions between proteins, with the close proximity of the interacting membranes affording an opportunity for protein-protein interactions to preferentially localize interacting partners to junctions (Williams et al., 2011). By transfecting mGluR7-GFP into one population of cells and Elfn1-tdTomato into another population of cells, then co-plating the cells, we were able to assess the transcellular interaction between Elfn1 and mGluR7 at tight junctions by measuring GFP fluorescence intensity (Fig 3A). Cells expressing mGluR7-GFP displayed an approximately 6-fold greater fluorescence intensity at tight junctions (defined by ZO1 label) of juxtaposed cells expressing Elfn1-tdTomato than at tight junctions formed with Elfn1-tdTomato negative cells, indicating that Elfn1 is sufficient to cluster mGluR7 via a transcellular interaction (mGluR7-GFP fluorescence intensity: 6.1 ± 1.1 fold increase at Elfn1 junctions versus non-Elfn1 junctions, p = $3x10^{-9}$, t = 7.22, paired t test, n/N = 50/8). Removal of the extracellular ligand binding domain of mGluR7 prevented the Elfn1-induced recruitment (Δecto-mGluR7-GFP intensity: 1.0 ± 0.2 fold increase at Elfn1 junctions, p = 0.1, t = 1.74, paired t test, n/N = 21/3), as did removal of the LRR binding domain for Elfn1 (mGluR7-GFP intensity: 1.1 ± 0.1 fold increase at Elfn1-ΔLRR junctions, p = 0.3, t = 1.18, paired t test, n/N = 19/3; mGluR7-GFP/Elfn1conditionvs Δecto-mGluR7-GFP/Elfn1 condition, p=0.003, vs mGluR7-GFP/ΔLRR-Elfn1 condition, p=0.006, F = 8.38, one way ANOVA, post hoc Bonferroni, Fig 3B). The absence of mGluR7 enrichment in the deletion constructs was not due to mislocalization of the mutant proteins (data not shown).

We next assessed whether transcellular clustering of mGluR7 might have functional consequences for receptor activation. Dimerization of G_i/G_o coupled metabotropic glutamate receptors induces a weak, constitutive activation of inwardly rectifying potassium channel (GIRK) conductance through a conformational change in the ligand binding domain that bears similarities to agonist binding (Levitz et al., 2016). Noting that Elfn1 is present in a multimeric form (Fig 3C), we hypothesized that the clustering induced by Elfn1 could constitutively activate mGluR7 by stabilizing dimerized mGluR7 in an active conformation. As a sensitive assay for mGluR7 activation we measured changes in potassium conductance in HEK cells, co-transfected with mGluR7-GFP and GIRK1/2, in response to application of two soluble putative dimerizing agents: MAB1/28 and Elfn-Fc. We used MAB1/28, an antibody directed against the extracellular domain of mGluR7 that immunoprecipitates (IP, Fig 3D) and selectively binds to dimeric mGluR7 (Ullmer et al., 2012), to determine whether receptor dimer stabilization alone is sufficient to induce GIRK activation. We used Elfn-Fc, a soluble Elfn dimer composed of the extracellular domain of Elfn1 fused to an Fc domain, in order to mimic Elfn1-induced dimer stabilization (Fig 3E). Focal application of MAB1/28 to mGluR7-expressing HEK cells produced a reversible increase in GIRK (Fig 3F). Conductance was calculated from the current response to a series of voltage steps between -125 and +15 mV, which enabled intrinsic verification that the dimerization-activated conductance was an inwardly rectifying potassium conductance (GIRK, Fig 3G). In contrast, application of a control antibody had no effect (conductance: 1.34 ± .38 nS with control ab. vs. 1.35 ± .39 nS baseline, $p = 0.4$, $t = 0.92$, paired t test, n/N = 5/1; conductance: 1.01 ± .38 nS with MAB1/28 vs. 0.92 ± .38 nS baseline, $p = 0.02$, $t = 3.27$, paired t test, n/N = 7/4, Fig 3H). Focal application of Elfn-Fc likewise produced an increase in GIRK, suggesting that Elfn1-mediated dimer stabilization of mGluR7 is sufficient to increase receptor activity. In contrast, control Fc protein had no effect (conductance: 0.98 ± 0.25 nS with control Fc vs. 0.98 ± 0.25 nS baseline, $p = 0.5$, $t = 0.70$, paired t test, n/N = 10/4; conductance: 1.26 ± 0.45 nS with Elfn1-Fc vs. 1.11 ± 0.39 nS baseline, $p = 0.04$, $t = 2.42$, paired t test, n/N = 11/6, Fig 3I). Notably, MAB1/28 reportedly does not induce detectable G_i/G_o activation, as assessed by suppression of forskolin induced cAMP activation, nor by calcium mobilization with promiscuous G protein coupling in a fluorometric imaging plate reader (FLIPR) assay (Ullmer et al., 2012). To test whether Elfn1 likewise had biased signaling properties regarding G_i/G_o activation, we assessed Gα-mediated calcium mobilization with a FLIPR assay validated with a canonical mGluR7 activator, L-AP4 (Fig 3J)

383 (Lindemann et al., 2011). As with MAB1/28, Elfn1-Fc protein did not induce detectable Gα signaling
384 as measured by FLIPR (Fig 3K), suggesting a divergence from the diffusible second messenger
385 signaling cascades typically evoked by agonist activation. In fact, a recent study demonstrates that
386 Elfn1 interactions produce negative allosteric modulation of Type III mGluRs by suppressing G protein
387 coupling efficiency for $G\alpha_i$ and $G\alpha_o$ (Dunn et al., 2018). This series of observations is instead
388 consistent with the fast, membrane delimited, voltage- sensitive suppression of calcium channels by
389 G protein coupled receptors (GPCRs) described by Hille and colleagues (Beech et al., 1992).
390 Evidence suggests that mGluR7 homodimerization likewise induces a constitutive, voltage-sensitive
391 suppression of calcium channel activity (Kammermeier, 2015).
392
393 *Constitutive mGluR7 activity permits allosteric modulation of spike timing*
394 A voltage-dependent suppression of calcium channel activity predicts that synaptic
395 suppression is transient within a train of action potentials, which may modulate SOM interneuron
396 spike timing and thus impact circuit function. The constitutive mGluR7 activity present at pyr → SOM
397 synapses could therefore allow for the use of allosteric modulators to impact the spike output of SOM
398 neurons. Positive allosteric modulators enhance ongoing receptor activity, without generating the
399 exogenous receptor activation and subsequent synaptic suppression produced by canonical agonists
400 such as L-AP4. We applied the mGluR7-selective mixed agonist/positive allosteric modulator,
401 AMN082, at a low dose (30 nM, Fig 4A), as at higher doses, AMN082 has been found to have a
402 number of off-target effects (Mitsukawa et al., 2005; Sukoff Rizzo et al., 2011). We confirmed that low
403 dose AMN082 had effects on first EPSC amplitude, which we attribute to allosteric modulation of
404 constitutively active mGluR7 by contrasting the effects of AMN082 with that of low dose L-AP4
405 agonism (i.e. early suppression vs. late suppression). The effect on initial release was absent in Elfn1
406 KO. AMN082 selectively impaired first EPSC amplitude at L2/3 synapses (EPSC amplitude: 33 ± 6 pA
407 in AMN082 vs. 37 ± 6 pA baseline, $p = 0.002$, $t = 4.65$, paired t test, n/N = 8/4, Fig 4B). At L2/3
408 synapses in Elfn1 KO slices, 30 nM AMN082 failed to alter first EPSC amplitude (EPSC amplitude:
409 142 ± 32 pA in AMN082 vs. 134 ± 25 pA baseline, $p = 0.5$, $t = 0.79$, paired t test, n/N = 9/2, Fig 4C).
410 We next assessed the effect of mGluR7 on synaptically-induced SOM firing. Application of 30
411 nM AMN082 reduced average spike probability, and increased the number of stimuli required to reach
412 half-maximal spiking in L2/3 neurons (spike probability, L2/3: 27 ± 3% in AMN082 vs. 31 ± 3% at

baseline, p = 0.04, t = 2.14, paired t test; stimuli to half max spiking, L2/3: 3.1 ± 0.3 in AMN082 vs. 2.8 ± 0.2 at baseline, p = 0.03, t = 2.29, paired t test, n/N = 24/4, Fig 4D). L2/3 KO neurons did not show impairments (spike probability, L2/3 KO: 65 ± 15% in AMN082 vs. 63 ± 13% at baseline, p = 0.7, t = 0.45, paired t test; stimuli to half max spiking, L2/3 KO: 1.1 ± 0.3 in AMN082 vs. 1.0 ± 0.2 at baseline, p = 0.6, t = 0.52, paired t test, n/N = 7/2, Fig 4E). Thus, Elfn1 induction of constitutive mGluR7 activity suppresses initial release probability, resulting in delayed recruitment of SOM interneurons in response to circuit activity. Positive allosteric modulation of mGluR7 can further delay SOM interneuron recruitment.

Constitutive mGluR7 activity generates frequency dependent SOM synapses

Delayed recruitment of SOM interneurons in response to incoming synaptic input provides a possible explanation for the observation that the recruitment of hippocampal SOM neurons is frequency dependent (Sun and Dobrunz, 2006). Elfn1 immunolabeling is visible in hippocampus and throughout the cortex (Fig 5A). Initial release probability at pyr → SOM synapses were similar across brain regions and was similarly increased in Elfn1 KO animals across regions (L2/3 release probability, as in Fig 1E: 0.19 ± 0.02 in KO vs. 0.08 ± 0.01 in WT, $p = 6 \times 10^{-5}$, n/N = 22/5 and 23/9 respectively, L5 release probability: 0.17 ± 0.02 in KO vs. 0.10 ± 0.01 in WT, p = 0.03, n/N = 20/11 and 23/13 respectively, HC release probability: 0.22 ± 0.07 in KO vs. 0.07 ± 0.01 in WT, p = 0.007, n/N = 6/4 and 11/5 respectively, F = 41.84, two way ANOVA, post hoc Bonferroni, Fig 5B). To assess frequency dependence of SOM synapses, we recorded synaptic events from SOM neurons in L2/3 in response to 5, 10, 20, 40, and 50 Hz synaptic stimulation (50 Hz facilitation as in Fig 1B, shown again for comparison in Fig 5C). Short term facilitation was impacted across a range of stimulus frequencies, with the most prominent disparity between WT and KO occurring at higher frequencies (L2/3 WT: 20 Hz, p = 0.001, 40 Hz, $p = 2 \times 10^{-10}$, 50 Hz, $p = 3 \times 10^{-17}$ vs. KO, F = 69.77, two-way ANOVA, post hoc Bonferroni, n/N = 21/4 WT and 8/2 KO, Fig 5D). L5 pyr → SOM synapses facilitation was also impacted by Elfn1 KO (EPSC5/EPSC1 ratio: 3.4 ± 0.3 vs. 4.6 ± 0.4 in WT, p = 0.02, t = 2.46, t test, n = 31/13 and 25/10 respectively, Fig 5E). Recordings from L2/3 and L5 of cortex, as well as the CA1 region of the hippocampus, verified that release probability and frequency dependence generalize across SOM neurons (L5 EPSC5/EPSC1 ratio: 40 Hz, p = 0.002, 50 Hz, $p = 2 \times 10^{-6}$ vs. KO, F = 17.68, two-way ANOVA, post hoc Bonferroni, n/N = 15-17/7 WT and 11/3 KO, Fig

5F; CA1 EPSC5/EPSC1 ratio: 2.0 ± 0.3 in KO vs. 4.8 ± 0.6 in WT, p = 0.0007, t = 4.21, t test, n/N = 6/4 and 13/5 respectively, Fig 5G, CA1: 40 Hz stim, p = 0.02, F = 24.30, two-way ANOVA, post hoc Bonferroni, 50 Hz was not acquired, n/N = 9-15/5 WT and 6-7/4 KO, Fig 5H). Notably, L5 pyr → SOM synaptic facilitation was less impacted by Elfn1 KO than L2/3 (EPSC5/EPSC1 ratio at 50 Hz: layer x genotype interaction p = 0.002, F = 10.62, two-way ANOVA).

Constitutive mGluR7 activity at pyr → SOM synapses is similar in L2/3 and L5

SOM neurons in both L2/3 and L5 showed a strong short-term facilitation of excitatory transmission in response to high frequency stimulation (50 Hz, Fig 6A), as seen previously for hippocampal SOM interneurons. However, L5 pyr → SOM synapses facilitated to a lesser extent (EPSC5/EPSC1 ratio: 4.6 ± 0.4 vs. 7.7 ± 0.9 in L2/3, p = 5 $x10^{-6}$, EPSC4/EPSC1 ratio: 3.9 ± 0.4 vs. 6.6 ± 0.7 in L2/3, p = 0.0002, F = 40.15, two-way ANOVA, post hoc Bonferroni, n = 31/13 and 24/10 respectively, L2/3 data as in Fig 1B, L5 data as in Fig 5E, replotted for comparison in Fig 6B). L5 pyr → SOM synapses also showed longer delays in the synaptic induction of SOM interneuron spiking (stimuli to half-max spiking: 4.7 ± 0.3 in L5 vs. 2.8 ± 0.2 in L2/3, corrected p= 4 $x10^{-6}$, t = 5.31, t test, n/N = 24/4 and 24/5 respectively, L2/3 data as in Fig 1C,D, replotted for comparison in Fig 6C,D).

Similar to L2/3, L5 shows a punctate immunostaining pattern for mGluR7, concentrated at the dendrites of SOM neurons labelled with tdTomato (Fig 6E). Further, MSOP increased synaptic transmission at pyr → SOM synapses in L5 (EPSC amplitude: 27 ± 5 pA in MSOP vs. 21 ± 3 pA baseline, p = 0.04, t = -2.46, paired t test, n/N = 9/5, Fig 6F). Positive allosteric modulation of mGluR7 at L5 synapses reduced first EPSC amplitude (EPSC amplitude: 68 ± 6 pA in AMN082 vs. 74 ± 7 pA baseline, p = 0.009, t = 3.31, paired t test, n/N = 10/3, Fig 6G). No difference in mGluR7 activity was detected, as measured by the change in synaptic release probability for L2/3 and L5 in response to MSOP or in response to AMN082 (Fig 6H), although baseline facilitation ratios were different between L2/3 and L5 for those conditions (p= 1 $x10^{-5}$ for L2/3 vs. L5 at baseline, F = 25.68, two-way ANOVA). As an internal control for effects of synaptic rundown over time, comparison of MSOP effects against AMN082 effects on release probability significantly diverged from one another over the same time window (L2/3: p = 0.003; L5: p= 0.02 for MSOP vs. AMN082, F = 24.69, by two-way ANOVA, Bonferroni post hoc). As with L2/3 neurons, L5 neurons also showed reduced and delayed synaptic spiking in response to AMN082 (spike probability, L5: 23 ± 4% in AMN082 vs. 26 ± 3% at baseline, p

= 0.0005, t = 4.07, paired t test; stimuli to half max spiking, L5: 5.4 ± 0.5 in AMN082 vs. 4.7 ± 0.3 at baseline, p = 0.02, t = 2.43, paired t test, n/N = 24/5, Fig 6I). Since mGluR7 activity and protein were detected at both L2/3 and L5 synapses, mGluR7 activity cannot explain the layer-specific differences in facilitation ratio.

Elfn1 recruitment of GluK2-KARs is non-constitutive but generates a strongly facilitating synapse

The strong facilitation at hippocampal pyr → SOM synapses during natural stimulus patterns is attributed, in part, to the presence of calcium permeable presynaptic kainate receptors (responsive to the GluK2-KAR selective antagonist, NS102, Fig 7A) (Sun et al., 2009). Elfn1 is necessary for recruitment of presynaptic GluK2-KARs, as shown by the loss of NS102 sensitivity following shRNA knockdown of Elfn1 (Sylwestrak and Ghosh, 2012). The presence of constitutive mGluR7 activity at both L2/3 and L5 synapses left us with the hypothesis that the layer specific differences in facilitation ratio at these 2 synapses was the result of differences in presynaptic GluK2-KARs. We first verified that GluK2-KARs are present at cortical synapses by looking for cell type specific localization on dissociated cortical neuronal cultures. Using an antibody that recognizes GluK2, but also GluK3, we found a strong colocalization with synaptic mGluR7 (Fig 7B). However, the GluK2/3 staining was not as discretely localized as mGluR7, labelling other neurons and cell bodies as well. The presence of presynaptic GluK2-KARs generates a late, strong facilitation of glutamatergic synaptic transmission onto SOM cells in L2/3 cortex that is reduced by the GluK2-KAR antagonist NS102 (20 μM, EPSC5/EPSC1, L2/3: 5.4 ± 0.7 in NS102 vs. 6.7 ± 0.9 at baseline, p = 0.007, t = 4.03, paired t test, n/N = 7/5, Fig 7C). The reversible change in synaptic transmission was primarily restricted to EPSCs evoked later in the train (EPSC5 amplitude: 318 ± 68 pA in NS102 vs. 376 ± 72 pA at baseline, p = 0.001, t = 5.89, paired t test, n/N = 7/5). In contrast to L2/3, we found that presynaptic GluK2-KARs do not play a role in the facilitation of excitatory synaptic inputs onto L5 SOM neurons, as these synapses facilitate to a lesser extent and are NS-102 insensitive (EPSC5/EPSC1, L5: 3.4 ± 0.7 in NS102 vs. 3.4 ± 1.3 at baseline, p = 0.9, t = 0.11, paired t test, n/N = 7/5, Fig 7D). L2/3 SOM neurons from Elfn1 KO animals are also NS-102 insensitive (EPSC5/EPSC1, L2/3 KO: 0.8 ± 0.1 in NS102 vs. 0.8 ± 0.3 at baseline, p = 0.5, t = 0.65, paired t test, n/N = 6/1, Fig 7E). Coefficient of variation analysis (Faber and Korn, 1991) indicates that the NS102 sensitive receptors alter presynaptic release probability (EPSC5, Fig 7F), consistent with previous studies indicating that for excitatory synapses

503 onto SOM neurons, GluK2-KARs are presynaptic and calcium permeable (Sun and Dobrunz, 2006;
504 Sun et al., 2009). The presence of GluK2 protein and mRNA in both L2/3 and L5 suggests that
505 differences in GluK2-KARs are not due to an absence of GluK2 in L5 (Fig 7G-J). Likewise, no
506 reduction in GluK2 expression levels were observed in KO animals compared to WT by western blot
507 or immunostaining (Fig 7K-M), although the strong postsynaptic labelling and the lack of specificity of
508 the GluK2/3 antibody limits interpretability of these data. Since L5 differences could not be explained
509 simply by absence of GluK2, we hypothesized that local protein interactions might regulate GluK2-
510 KAR presynaptic engagement.
511
512 *mGluR7 acts a scaffolding structure for presynaptic GluK2-KAR recruitment*

513 The ubiquitous presence of mGluR7 activity at Elfn1 synapses suggested that mGluR7 might
514 act as a protein scaffold for GluK2 recruitment. We found that mGluR7 could co-immunoprecipitate
515 GluK2 when co-transfected into HEK cells (Fig 8A). Likewise, mGluR7 IP from cortical lysates of both
516 WT and Elfn1 KO animals pulled down GluK2 (Fig 8B), which indicate that this interaction occurs
517 even in the absence of Elfn1. Control IP with mGluR1α did not pull down GluK2. Speculating that
518 protein-protein interactions between mGluR7 and GluK2 at pyr → SOM synapses must somehow be
519 regulated, we tested whether activation of calmodulin, a protein previously shown to both regulate
520 protein interactions for the c-terminal of mGluR7 ((O'Connor et al., 1999; Suh et al., 2008)) and to
521 bind GluK2 (Coussen et al., 2005), could alter GluK2 recruitment. Indeed, we discovered that
522 activating calmodulin altered L5 → SOM synaptic responses. Calmodulin activation (3 min application
523 of 100 μM CALP3, calcium like peptide 3, plus an additional 20 minutes for baseline stabilization)
524 rendered synaptic facilitation NS-102 sensitive, indicating an induction of GluK2-KAR recruitment to
525 L5 → SOM synapses (EPSC5/EPSC1, L5, CALP3 treated: 4.5 ± 0.7 in NS102 vs. 5.0 ± 0.9 at
526 baseline, $p = 0.03$, $t = 2.63$, paired t test, n/N = 8/5, Fig 8C). Thus, in contrast to the Elfn1-induced
527 constitutive synaptic suppression conveyed by mGluR7, a differential recruitment of GluK2 to L2/3 or
528 L5 pyr → SOM synapses accounts for the observed layer-specific difference in synaptic facilitation,
529 with calmodulin activation facilitating the engagement of GluK2 at the presynaptic terminal (Fig 8D).
530
531 DISCUSSION

The characteristic facilitation of pyr → SOM synapses is generated by the presence of Elfn1, which is expressed by the postsynaptic SOM neurons (Sylwestrak and Ghosh, 2012; Tomioka et al., 2014). In hippocampal SOM neurons, Elfn1 dependent synaptic facilitation is strongly influenced by synaptic frequency, where high frequency stimulation preferentially facilitates pyr → SOM synapses in WT compared to Elfn1 KO animals. Strongly facilitating pyr → SOM synapses are also found in L2/3 of cortex and this facilitation is greatly reduced in KO animals. At pyr → SOM synapses, Elfn1 interacts with at least 2 presynaptic partners, mGluR7 and GluK2-KARs, to produce a strong short-term synaptic facilitation. Our data indicates that the mechanism of facilitation is two-fold. First, the actions of mGluR7 reduce initial release probability, resulting in greater synaptic facilitation. After several consecutive action potentials, the strong facilitation in L2/3 is primarily GluK2 dependent, as application of the GluK2 selective antagonist NS-102 reduces late facilitation. Notably, synapses onto L5 cortical SOM interneurons facilitate less, take longer to drive spikes, are less affected in Elfn1 knockout, and are insensitive to GluK2 antagonists. Thus, the synaptic proteome dictates functional features of synaptic transmission in a synapse specific and laminar specific fashion.

Elfn1 transcellular recruitment of mGluR7 clusters the receptor at HEK cell tight junction interfaces and at terminals presynaptic to SOM neurons where it generates constitutive suppression of synaptic transmission. Dimerization-induced mGluR activation is due to a conformational change in the ligand binding domain, which can be antagonized with orthosteric antagonists (Levitz et al., 2016). Elfn1-mGluR7 interactions generate a structurally-induced constitutive GPCR activation, tonically suppressing pyr → SOM transmission. A previous report indicates that Elfn1 induces negative allosteric modulation of G protein signaling in response to glutamate (Dunn et al., 2018). Although Elfn1-induced negative allosteric modulation is not explicitly tested here, a reduction in L-AP4 mediated synaptic suppression for WT versus Elfn1 KO provides support for these *in vitro* findings. Elfn1 interactions would thus serve to bias mGluR7 activity towards a tonic signaling mode rather than a glutamate-induced signaling mode, which may help explain observations that mGluR7 are present and functional at GABAergic presynaptic terminals, where high levels of glutamate are unlikely to be found (Somogyi et al., 2003; Summa et al., 2013). In addition, we find a divergence in the synaptic outcome of allosteric modulation of the mGluR7 receptor with 30 nM AMN082 compared to agonism with L-AP4. Allosteric modulation enhances the “delay” function of mGluR7 by further suppressing

561 initial release, whereas agonism suppresses late release, resulting in inverse pharmacological profiles
562 of synaptic modulation.

563 Within a train of high frequency activity, synaptic suppression is transient, resulting in delayed
564 recruitment of SOM interneurons. Thus Elfn1 activation of mGluR7 generates a synapse that
565 responds robustly to stimulation when it occurs at high frequency, a hallmark feature of SOM neurons
566 in hippocampus and cortex (Pouille and Scanziani, 2004; Sylwestrak and Ghosh, 2012; Hu and
567 Agmon, 2016). Excitatory transmission onto SOM neurons initially fails (median *in vivo* failure rate of
568 unitary synaptic potentials = 80%), but rapidly facilitates from the initial suppressed state during high
569 frequency spike trains (Pala and Petersen, 2015). Interestingly, this suggests that high frequency
570 (gamma band or high beta band) cortical activity may strongly drive the recruitment of somatostatin
571 neuron-mediated inhibition. Indeed, gamma frequency activation of SOM interneurons in lateral
572 septum controls food seeking behavior (Carus-Cadavieco et al., 2017).

573 During a spike train, transient, early, mGluR7-dependent suppression of synaptic
574 transmission is followed by a late, sustained GluK2-KAR-dependent facilitation. Delayed recruitment
575 of GluK2-KAR dependent synaptic release may be related to glutamate spillover in the synaptic cleft,
576 as has been proposed previously (Sun et al., 2009), or may also relate to the voltage-dependent
577 activation delay of these receptors induced by GluK2-KAR sensitivity to intracellular polyamine block
578 (Bowie and Mayer, 1995). Delayed recruitment of presynaptic GluK2-KARs substantially contributes
579 to synaptic facilitation at late EPSCs at pyr → SOM synapses (Sylwestrak and Ghosh, 2012), and
580 these receptors have been proposed as spike timing dependent ‘‘conditional amplifiers” of spike
581 transmission during natural stimulus trains at hippocampal mossy fiber synapses (Sachidhanandam
582 et al., 2009). Presynaptic GluK2-KARs thus represent a means of enhancing SOM interneuron
583 recruitment, as evidenced by the reduced number of stimuli required to recruitment SOM spiking for
584 L2/3 neurons than for L5 neurons. The ability to modify GluK2-KAR engagement with the activation of
585 calmodulin thus provides a potential mechanism for regulating the strength of SOM interneuron
586 recruitment.

587 Elfn1 generates strongly facilitating synapses through the contribution of early, constitutive
588 mGluR7 activity, and late, conditional kainate receptor recruitment, with both components contributing
589 to the distinctive timing of pyr → SOM synapses. This stepwise construction of synaptic function

provides for both a “hardwired” genetic structure and for a tunable structure to allow for both stability and flexibility in the synaptic recruitment of SOM neuron mediated inhibition.

AUTHOR CONTRIBUTIONS AND DISCLOSURE

T.J.S, E.L.S., P.S., B.J.H., and A.G. designed experiments; T.J.S. and E.L.S. performed the experiments; T.J.S. and A.G. wrote the paper. T.J.S, E.L.S., B.J.H., and A.G. were full time employees of F. Hoffmann-La Roche Ltd. A.G. is a full time employee of Biogen. T.J.S. was a Biogen postdoctoral fellow.

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740 FIGURE LEGENDS

741 Figure 1: Elfn1 generates facilitating pyr → SOM synapses in cortex. A) Recording from Layer 2/3
742 SOM neurons, high frequency stimulation results in substantial facilitation of excitatory transmission,
743 which is reduced in Elfn1 KO. B) In L2/3 of cortex, Elfn1 KO has a reduced synaptic facilitation ratio at
744 50 Hz. ESPC amplitude is normalized to first EPSC amplitude. C) Within a 50 Hz train of synaptic-
745 driven SOM activation, spiking onset is delayed in L2/3 neurons. Spiking onset is rapid in L2/3 Elfn1
746 KO. D) Probability of spiking takes longer to reach half-maximum in WT compared to KO neurons. E)
747 Initial release probabilities calculated for L2/3 differ between WT and KO. F) Quantal EPSC amplitude
748 does not differ from WT to KO. Values are represented as mean ± SEM. ***$p < 0.001$.
749
750 Figure 2: Elfn1 produces constitutive suppression of synaptic transmission at pyr → SOM synapses
751 through agonist-independent mGluR7 activation. A) mGluR7 (green) colocalizes with Elfn1 (red) at
752 synapses in cortical neuronal cultures. B) Selective synaptic distribution of mGluR7
753 immunofluorescence (green) onto the dendritic arbor of a L2/3 SOM interneuron (red) in cortical
754 slices. C) Colocalization of mGluR7 with dendritic arbors is disrupted in Elfn1 KO L2/3 SOM neurons.
755 D) mGluR7 inhibition with MSOP (100 μM) increases evoked EPSC amplitude onto L2/3 SOM
756 neurons for the first EPSC. Initial release probability is increased. E) MSOP has no effect at L2/3
757 synapses in Elfn1 KO. F) Summary of MSOP findings. Elfn1 induced recruitment of mGluR7 induces
758 constitutive activity, which suppresses synaptic release. This constitutive activity is antagonized by
759 MSOP, de-suppressing initial release. Surprisingly, no effect of blocking synaptic glutamate is
760 detected, as late events are not altered by MSOP blockade. Likewise, no effect is detected in the
761 absence of Elfn1. G) Absence of mGluR7 activity in Elfn1 KO is not due to reduction in total protein,
762 as shown by western blot of cortical lysates. H-I) Redistribution of mGluR7 protein. In Elfn1 KO,
763 mGluR7 no longer labels dendritic processes. J) Application of the mGluR agonist L-AP4 (30 μM) fails
764 to further suppress initial release in WT but not in Elfn1 KO animals. Late release events are still
765 affected. K) L-AP4 (300 μM) is still able to suppress initial release in both WT and KO animals. Partial
766 dose response profiles for L-AP4 diverge in WT and KO animals. L) Summary of L-AP4 findings.
767 Elfn1 induced constitutive activity occludes the suppression of initial release by L-AP4 in WT but not
768 KO animals. Agonism with exogenous L-AP4 unveils a canonical autoreceptor function not observed

769 for endogenous glutamate. Values are represented as mean ± SEM. Scale bars = A-C) 10 μm, H-I)
770 100 μm. * p < 0.05, **p < 0.01, ***p < 0.001.
771
772 Figure 3: Elfn1 induced clustering of mGluR7 produces constitutive receptor activity. A) In HEK cell
773 cultures, mGluR7-GFP intensity (green) is strongly enriched at ZO-1 (blue) labelled cell-cell interfaces
774 (white arrow) that are abutting Elfn1 containing cells (red), relative to interfaces abutting other cells
775 (black arrow). In the absence of the extracellular domain of mGluR7 (Δecto-mGluR7-GFP),
776 enrichment does not occur. B) Quantification of Elfn1 enrichment for mGluR7-GFP, Δecto-mGluR7-
777 GFP, and for mGluR7-GFP abutting LRR-deleted versions of Elfn1. C) Elfn1 forms homomultimers, as
778 evident from the increase in dimer band in lysates from Elfn1 transfected HEK cells treated with
779 increasing concentrations of BS3 crosslinking agent. D) The mGluR7 antibody MAB1/28 specifically
780 recognizes dimerized mGluR7, as immunoprecipitation (IP) with MAB1/28 preferentially pulls down
781 dimerized mGluR7. Blotting with polyclonal anti-mGluR7 recognizes both monomer and dimer bands.
782 E) Elfn1-Fc exists as a dimer, based on Western blot analysis with BS3 crosslinking of purified protein
783 samples. F) Application of MAB1/28 produces an increase in conductance recorded from mGluR7 /
784 GIRK 1/2 transfected HEK cells. An example trace shows the time-course in response to acute
785 application. G) Current-voltage analysis of the induced conductance increase verifies that the
786 activated channel is an inwardly rectifying (flattened I-V above -40 mV) potassium conductance
787 (reversal near -82 mV; K^+ E_{rev}). H-I) Quantitation shows the increase in GIRK induced by MAB1/28
788 (H) or Elfn-Fc (I) as a percentage of the whole cell conductance. The respective control treatments
789 produced no increase. J) L-AP4 control produces robust agonism of mGluR7, generating Gα -
790 mediated calcium fluorescence (ΔF/F) in a FLIPR assay. An example trace is shown. Each point
791 represents an individual ΔF/F measurement. K). Elfn1-Fc shows no detectable Gα - mediated
792 fluorescence signal in a FLIPR assay. Values are represented as mean ± SEM. Scale bars = 10 μm. *
793 p< 0.05, **p<0.01.
794
795 Figure 4: Constitutive mGluR7 activity permits allosteric modulation of spike timing at pyr → SOM
796 synapses. A) Schematic illustration of AMN082 modulation of Elfn1-mGluR7 signaling. Elfn1
797 stabilization of an mGluR7 dimer active state induces constitutive activity, which suppresses synaptic
798 release. AMN082 facilitates the constitutive activity of mGluR7, further suppressing release. B)

799 Activation of mGluR7 with 30 nM of the mixed agonist/positive allosteric modulator (PAM) AMN082
800 reduces early EPSC amplitude for L2/3 SOM neurons. AMN082 reduces initial release probability. C)
801 AMN082 has no effect in Elfn1 KO D) AMN082 reduces spike probability and increases the number of
802 stimuli needed to evoke to half-max spiking for synaptic evoked spikes in L2/3 SOM neurons. 10
803 overlaid spike trains before (black) and during AMN082 wash in (red). E) Synaptic evoked spiking
804 shows little delay in Elfn1 KO, and PAM activity of AMN082 is absent. Values are represented as
805 mean ± SEM. * $p < 0.05$.
806
807 Figure 5: Elfn1 mediates release probability and frequency dependence of facilitation at SOM
808 synapses. A) Elfn1 immunostaining is visible in hippocampus and across cortical layers. Although
809 anti-Elfn1/Elfn2 antibody is not specific for Elfn1, loss of staining on discrete dendritic processes in
810 Elfn1 KO suggests most of this signal is Elfn1. B) Release probability in WT (black) and KO (grey)
811 SOM neurons is comparable across L2/3, L5 and hippocampus. C) In L2/3 of cortex, Elfn1 KO
812 reduces facilitation ratio at 50 Hz. Replicated from Fig 1, for comparison. D) Frequency dependent
813 facilitation in L2/3 increases at higher frequencies for WT (black) but not Elfn1 KO (grey) neurons. E)
814 Elfn1 KO has a less dramatic effect on L5 synaptic facilitation. F) As in L2/3, frequency dependent
815 facilitation in L5 increases for WT (black) but not Elfn1 KO (grey) neurons. G) The presence of Elfn1
816 in SOM interneurons also produces a strongly facilitating synapse in hippocampus. Facilitation of
817 EPSCs evoked in CA1 SOM neuron by alveus stimulation is attenuated in Elfn1 KO. H) Elfn1 KO
818 neurons (grey) show a frequency dependent impairment in short term facilitation, with the difference
819 from WT (black), more pronounced for higher frequency stimulation. Values are represented as mean
820 ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
821
822 Figure 6: Constitutive mGluR7 activity at pyr → SOM synapses is similar in L2/3 and L5. A) Recording
823 setup for L2/3 SOM neurons or L5 SOM neurons; stimulation is within layers. B) In L2/3 and L5 of
824 cortex, facilitation ratios at 50 Hz differ in magnitude. ESPC amplitude is normalized to first EPSC
825 amplitude. C) Within a 50 Hz train of synaptic-driven SOM activation, spiking onset is delayed in both
826 L2/3 and L5 neurons. D) Probability of spiking takes more stimuli to reach half-maximum in L5
827 compared to L2/3 neurons. E) mGluR7 immunofluorescence (green) colocalizes with dendritic arbors
828 of L5 SOM interneurons (red) in cortical slices. F) mGluR7 inhibition with MSOP (100 μM) increases

829 evoked EPSC amplitude onto L5 SOM neurons for the first EPSC. Initial release probability is
830 increased. G) Activation of mGluR7 with 30 nM of the mixed agonist/positive allosteric modulator
831 (PAM) AMN082 reduces early EPSC amplitude for L5 SOM neurons. Initial release probability is
832 reduced. H) The change in release probability in response to MSOP or AMN082 does not differ
833 between L2/3 and L5. I) AMN082 reduces spike probability and increases the number of stimuli to
834 half-max spiking for synaptic evoked spikes in L5 SOM neurons. 10 overlaid spike trains before
835 (black) and during AMN082 wash in (red). Values are represented as mean ± SEM. Scale bar = 10
836 μm. * $p < 0.05$, ** $p < 0.01$.
837 .
838 Figure 7: Strongly facilitating synaptic transmission at pyr → SOM synapses is mediated by
839 recruitment of GluK2 containing kainate receptors (GluK2-KARs). A) Proposed organization of
840 excitatory pyr → SOM synapses in L2/3, with Elfn1-clustered mGluR7 and presynaptic GluK2-KARs.
841 B) GluK2/3 (aka GluR6/7) staining colocalizes with mGluR7 on dendrites of cortical neurons in culture
842 (arrows). C) Facilitation onto L2/3 neurons is suppressed by NS-102, implicating GluK2-KARs. D) L5
843 to L2/3 differences can be explained by absence of recruitment of GluK2-KARs in L5. E) NS-102 has
844 no effect in Elfn1 KO L2/3 neurons. Facilitation ratio in L2/3 WT, but not in L5 WT nor in L2/3 KO, is
845 altered by NS-102. F) Coefficient of variation analysis shows that the GluK2 selective antagonist NS-
846 102 primarily alters synaptic amplitude (EPSC5) by changing presynaptic release probability (region
847 in yellow), rather than postsynaptic amplitude (postsynaptic changes would lie along the horizontal,
848 red). G-H) GluK2/3 immunostaining (red) is not reduced for L5 compared to L2/3 SOM neurons (blue).
849 Staining is visible in pre-and postsynaptic cells. I-J) GRIK2 mRNA (red) labels an abundance of cells
850 in both L2/3 and L5. Elfn1 mRNA labels a smaller subset of cells in these layers. K) GluK2/3
851 immunoblotting does not show reductions in total protein in Elfn1 KO cortical lysates compared to WT.
852 L-M) GluK2/3 immunostaining does not show reductions in GluK2/3 in Elfn1 KO. Values are
853 represented as mean ± SEM. Scale bars = B,G,H) 10 μm I,J,L,M) 100 μm.. * $p < 0.05$.

854 Figure 8: Calmodulin can dynamically regulate GluK2-KAR dependent facilitation at SOM synapses.
855 A) From HEK cells co-expressing mGluR7 and GluK2, immunoprecipitation of mGluR7 co-precipitates
856 GluK2. The GluK2/3 immunoblot includes an input lane showing homodimers alongside the IP lane.
857 B) From cortical tissue lysates, mGluR7 IP also co-precipitates GluK2/3, while control IP with
858 mGluR1α did not. From cortical lysates of both WT and Elfn1 KO tissue, mGluR7 IP co-precipitates

GluK2/3. C) Application of the calmodulin activator calcium like peptide 3 (CALP3, 100 μM) renders L5 SOM neurons responsive to NS102, which now suppresses facilitation onto L5 neurons. D) Proposed mechanism underlying the layer-specific differences in the function of pyr → SOM synapses. For a train of stimuli onto L5 SOM neurons, Elfn1 mediated clustering and activation of constitutive mGluR7 generates a moderately facilitating synapse, with little to no kainate receptor activation. In L2/3 SOM neurons, early synaptic suppression with mGluR7 is followed by late synaptic facilitation with GluK2-KARs to generate a strongly facilitating synapse. Calmodulin activation can induce engagement of GluK2-KARs at L5 synapses, enabling dynamic regulation of pyr → SOM synaptic strength.