



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Postsynaptic GABABRs Inhibit L-Type Calcium Channels and Abolish Long-Term Potentiation in Hippocampal Somatostatin Interneurons

Citation for published version:

Booker, SA, Loreth, D, Gee, AL, Watanabe, M, Kind, PC, Wyllie, DJA, Kulik, Á & Vida, I 2018, 'Postsynaptic GABABRs Inhibit L-Type Calcium Channels and Abolish Long-Term Potentiation in Hippocampal Somatostatin Interneurons' Cell Reports, vol. 22, no. 1, pp. 36-43. DOI: 10.1016/j.celrep.2017.12.021

Digital Object Identifier (DOI):

[10.1016/j.celrep.2017.12.021](https://doi.org/10.1016/j.celrep.2017.12.021)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Cell Reports

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

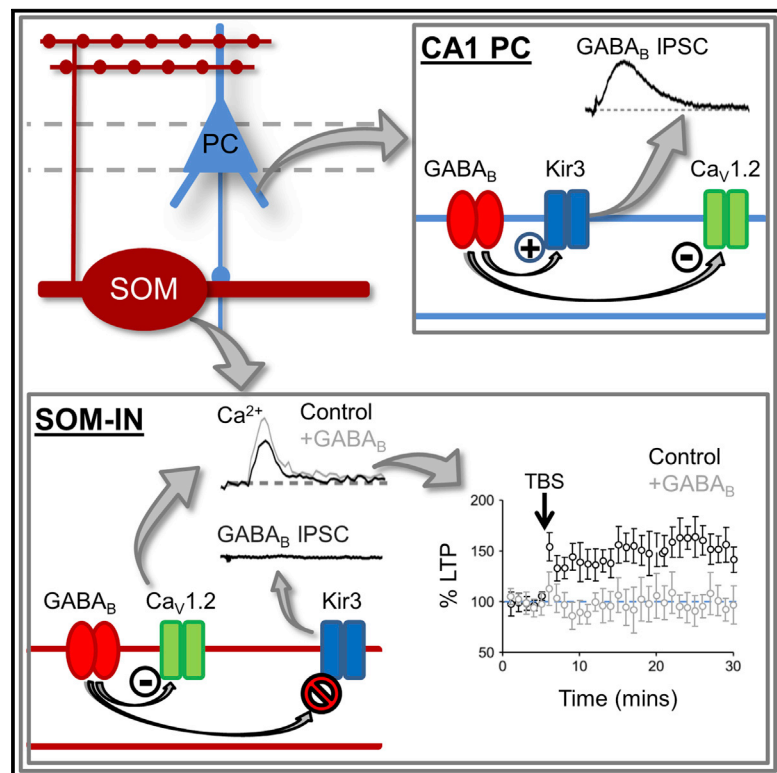
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Cell Reports

Postsynaptic GABA_BRs Inhibit L-Type Calcium Channels and Abolish Long-Term Potentiation in Hippocampal Somatostatin Interneurons

Graphical Abstract



Authors

Sam A. Booker, Desiree Loreth, Annabelle L. Gee, ..., David J.A. Wyllie, Ákos Kulik, Imre Vida

Correspondence

akos.kulik@physiologie.uni-freiburg.de (Á.K.),
imre.vida@charite.de (I.V.)

In Brief

Booker et al. show that GABA_B receptors are highly expressed on somatostatin interneuron dendrites. Rather than activating Kir3 channels, they preferentially co-cluster with, and negatively couple to, L-type calcium channels inhibiting long-term potentiation at excitatory inputs.

Highlights

- GABA_B receptors do not activate Kir3-currents in CA1 somatostatin interneurons
- In somatostatin interneurons, GABA_B receptors inhibit dendritic L-type Ca²⁺ channels
- Ca_v1.2 channels co-cluster with GABA_{B1} on somatostatin interneuron dendrites
- GABA_B activation abolishes long-term potentiation in somatostatin interneurons



Postsynaptic GABA_BRs Inhibit L-Type Calcium Channels and Abolish Long-Term Potentiation in Hippocampal Somatostatin Interneurons

Sam A. Booker,^{1,3,4,5,7} Desiree Loreth,² Annabelle L. Gee,⁷ Masahiko Watanabe,⁶ Peter C. Kind,^{3,4,5} David J.A. Wyllie,^{3,4,5} Ákos Kulik,^{2,8,*} and Imre Vida^{1,7,9,*}

¹Institute for Integrative Neuroanatomy, NeuroCure Cluster of Excellence, Charité - Universitätsmedizin Berlin, 10117 Berlin, Germany

²Institute of Physiology, Faculty of Medicine, University of Freiburg, Hermann-Herder-Str. 7, 79104 Freiburg, Germany

³Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh, UK

⁴Simons Initiative for the Developing Brain, University of Edinburgh, Edinburgh, UK

⁵Patrick Wild Centre for Autism Research, University of Edinburgh, Edinburgh, UK

⁶Department of Anatomy, Graduate School of Medicine, Hokkaido University, Sapporo 0608638, Japan

⁷Institute for Neuroscience, Glasgow University, Glasgow G12 8QQ, UK

⁸Centre for Biological Signalling Studies (BIOS), University of Freiburg, Schänzle-Str. 18, 79104 Freiburg, Germany

⁹Lead Contact

*Correspondence: akos.kulik@physiologie.uni-freiburg.de (Á.K.), imre.vida@charite.de (I.V.)

<https://doi.org/10.1016/j.celrep.2017.12.021>

SUMMARY

Inhibition provided by local GABAergic interneurons (INs) activates ionotropic GABA_A and metabotropic GABA_B receptors (GABA_BRs). Despite GABA_BRs representing a major source of inhibition, little is known of their function in distinct IN subtypes. Here, we show that, while the archetypal dendritic-inhibitory somatostatin-expressing INs (SOM-INs) possess high levels of GABA_BR on their somatodendritic surface, they fail to produce significant postsynaptic inhibitory currents. Instead, GABA_BRs selectively inhibit dendritic Ca_v1.2 (L-type) Ca²⁺ channels on SOM-IN dendrites, leading to reduced calcium influx and loss of long-term potentiation at excitatory input synapses onto these INs. These data provide a mechanism by which GABA_BRs can contribute to disinhibition and control the efficacy of extrinsic inputs to hippocampal networks.

INTRODUCTION

Maintained balance of excitation and inhibition controlled by feedforward and feedback interneurons (INs) is essential for appropriate function of cortical networks. Despite recruitment of local INs being critical to this balance, the contributing cellular mechanisms remain largely unexplored. Somatostatin (SOM) expressing INs constitute a dominant feedback inhibitory element in cortical circuits. In hippocampal CA1, SOM-INs are characterized by a somato-dendritic domain confined to *stratum* (*str.*) *oriens* and an axon providing inhibition to distal dendrites of pyramidal cells (PCs) in *str. lacunosum-moleculare*, as such they are referred to as O-LM cells (McBain et al., 1994; Katona et al., 1999; Müller and Remy, 2014). SOM-INs gate extrinsic cortical inputs into CA1 (Leão et al., 2012) and contribute to

the generation of network oscillations at theta frequencies (Gloveli et al., 2005; Klausberger and Somogyi, 2008), with known roles in neuropathology (de Lanerolle et al., 1989; Dugladze et al., 2007; Wang et al., 2011). SOM-INs are recruited by recurrent input from CA1 PCs, involving ionotropic α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (Topolnik et al., 2005; Lamsa et al., 2007), N-methyl-D-aspartate (NMDA) (Standaert et al., 1996), and group 1 metabotropic glutamate receptors (mGluRs), particularly mGluR1 α (Baude et al., 1993; McBain et al., 1994; Topolnik et al., 2006). Group I mGluRs on SOM-INs activate Ca_v1.2 (L-type) high voltage-gated Ca²⁺ channels (VGCCs), promoting synaptic plasticity at excitatory inputs (Topolnik et al., 2009; Nicholson and Kullmann, 2014).

While glutamatergic mechanisms have been well characterized, inhibitory control of SOM-INs is less well understood (Tyan et al., 2014). In particular, little is known regarding the effects of metabotropic GABA_BRs, despite GABA_B1 subunits being highly expressed at SOM-IN somata (Sloviter et al., 1999). In this study, we used whole-cell recording, 2-photon Ca²⁺-imaging, and high-resolution quantitative SDS-digested freeze-fracture replica (SDS-FRL) immunoelectron microscopy to examine postsynaptic GABA_BR function and localization in SOM-INs.

RESULTS

To determine whether SOM-INs possess functional GABA_BRs, we performed whole-cell recordings from rat acute hippocampal slices. CA1 SOM-INs were located in *str. oriens/alveus* with horizontal dendrites (Figure 1A) and responded with a large voltage “sag” to hyperpolarizing currents and minimally adapting action potential (AP) trains to depolarizing currents (Figure 1A, inset). All INs tested were immunoreactive for SOM (155 INs), of which 64 (41.3%) were identified as O-LM cells and 3 (1.9%) were bistratified INs. The remaining 88 (56.8%) were not morphologically identified due to the axon being cut, but were included in further



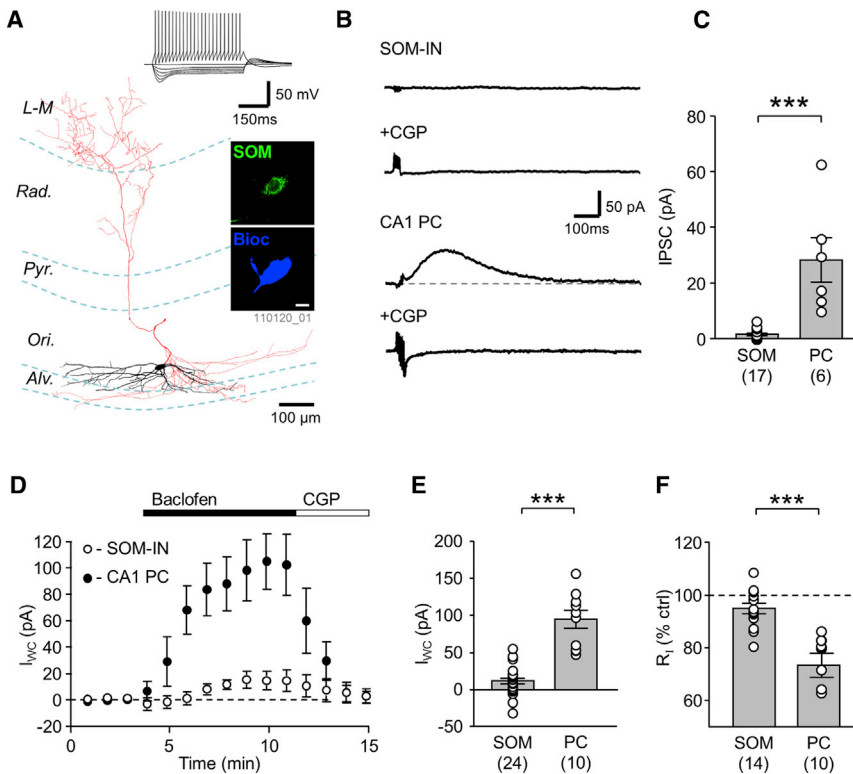


Figure 1. Postsynaptic GABA_BR-Mediated sIPSCs and Whole-Cell Currents Are Small in SOM-INs

(A) Reconstruction of a CA1 SOM-IN (black, dendrites; red, axon). Right inset: immunoreactivity for SOM (green) at the IN soma (blue; scale bar, 10 μ m). Top inset: voltage response of the cell to hyper- to depolarizing current pulses (-500 to 500 pA, 500 ms).

(B) Pharmacologically isolated sIPSCs evoked by stimulation to *str. oriens* in a SOM-IN (top) and CA1 PC (bottom), blocked by CGP-55,845 (CGP; 5 μ M, bottom traces).

(C) Bar chart of GABA_BR sIPSC amplitudes in SOM-INs and CA1 PCs.

(D) Time course of I_{WC} following application of baclofen (10 μ M) and CGP in SOM-INs (open circles) and CA1 PCs (filled circles).

(E and F) Summary charts of the changes in I_{WC} (E) and normalized input resistance (R_i) (F) during baclofen application in SOM-INs and CA1 PCs.

Bar charts show mean \pm SEM. Open circles correspond to individual data; cell numbers are in parentheses. *** $p < 0.001$.

See also Figures S1 and S2.

analysis. CA1 PCs were recorded as controls, given their well described functional GABA_BR expression (Lüscher et al., 1997).

GABA_BR-Mediated IPSCs Are Small in SOM-INs

Hippocampal neurons possess slow GABA_BR-mediated inhibitory postsynaptic currents (sIPSCs), elicited by extracellular stimulation (Degro et al., 2015). In SOM-INs, trains of stimuli (5 at 200 Hz) to *str. oriens* in the presence of GABA_A, NMDA, and AMPA receptor blockers produced very small or no sIPSC (Figures 1B, upper, and 1C). The mean sIPSC amplitude was 1.8 ± 0.5 pA (17 cells) and when present was blocked by the GABA_BR antagonist CGP-55,845 (CGP, 10 cells). In CA1 PCs, sIPSCs were markedly larger with a mean amplitude of 28.3 ± 8.8 pA (6 cells, $U_{(17,6)} = 0$; $p < 0.0001$, Mann-Whitney test) (Figures 1B and 1C) and were also blocked by CGP (Figure 1B, bottom), excluding technical limitations affecting SOM-IN recordings. A subset of recordings were performed in adult rats (9 cells, P50–P60) that confirmed that GABA_BR minimally activate K⁺ currents in SOM-INs (Figure S1).

Endogenous release of GABA activates only a proportion of the cells' GABA_BR complement (Lüscher et al., 1997; Degro et al., 2015). Therefore, we next measured whole-cell currents (I_{WC}) produced by the GABA_BR agonist baclofen. Bath-applied baclofen (10 μ M) produced only a small outward I_{WC} in SOM-INs (12.1 ± 4.0 pA, 24 cells), which was fully reversed by subsequent CGP application (Figure 1D). The same activation in CA1 PCs produced a robust I_{WC} of 94.9 ± 12.7 pA (10 cells), ~ 8 -fold larger than SOM-INs ($U_{(24,10)} = 3.0$; $p < 0.0001$, Mann-Whitney test) (Figures 1D and 1E). This I_{WC} was accompanied

by a marked reduction in input resistance from 130 ± 13 M Ω to 95 ± 11 M Ω in CA1 PCs (10 cells) consistent with channel opening. In contrast, only a small change

from 207 ± 14 M Ω to 195 ± 13 M Ω was observed in SOM-INs (14 cells; $U_{(14,10)} = 4.0$; $p < 0.0001$, Mann-Whitney test) (Figure 1F). These findings were further validated by briefly activating GABA_BR through uncaging of GABA over the dendrites of SOM-INs and CA1 PCs (Figure S2A), which resulted in currents of 6.4 ± 2.6 pA (5 cells) and 93.7 ± 23.4 pA (6 cells), respectively ($U_{(5,6)} = 0$; $p = 0.0043$, Mann-Whitney test) (Figures S2B and S2C). In summary, the GABA_BR-mediated inhibitory conductance in SOM-INs is an order of magnitude lower than CA1 PCs, indicating that GABA_BR/Kir3 signaling does not significantly contribute to SOM-IN inhibition.

GABA_BRs Strongly Inhibit Dendritic L-Type VGCCs

The absence of GABA_BR-mediated currents in SOM-INs suggests that the receptors may signal through an alternative effector, such as high voltage-gated Ca_v1.2 (L-type) Ca²⁺ channels (Chalifoux and Carter, 2011) known to contribute to signaling and plasticity in SOM-INs (Topolnik et al., 2006). To determine whether GABA_BR inhibit Ca_v1.2 in SOM-INs, we performed 2-photon imaging of IN dendrites filled with a morphometric and a Ca²⁺-indicator dye and evoked short trains of back-propagating APs (bAPs, $4 \times$ at 200 Hz) (Figure 2A). Imaging a primary dendrite with rapid line-scans, we observed large Ca²⁺-transients in response to bAPs (Figures 2B and 2C), which had a $\Delta F/F$ of $31.5\% \pm 3.7\%$ (17 cells). These transients were stable for the 20-min recording (4 cells) (Figure S3A) and blocked by CdCl₂ (5 mM, 3 cells) (Figure S3B) and did not differ during baseline for any test group ($R^2_{(5,4,6)} = 0.04$; $p = 0.78$, one-way ANOVA). Baclofen applied to SOM-INs

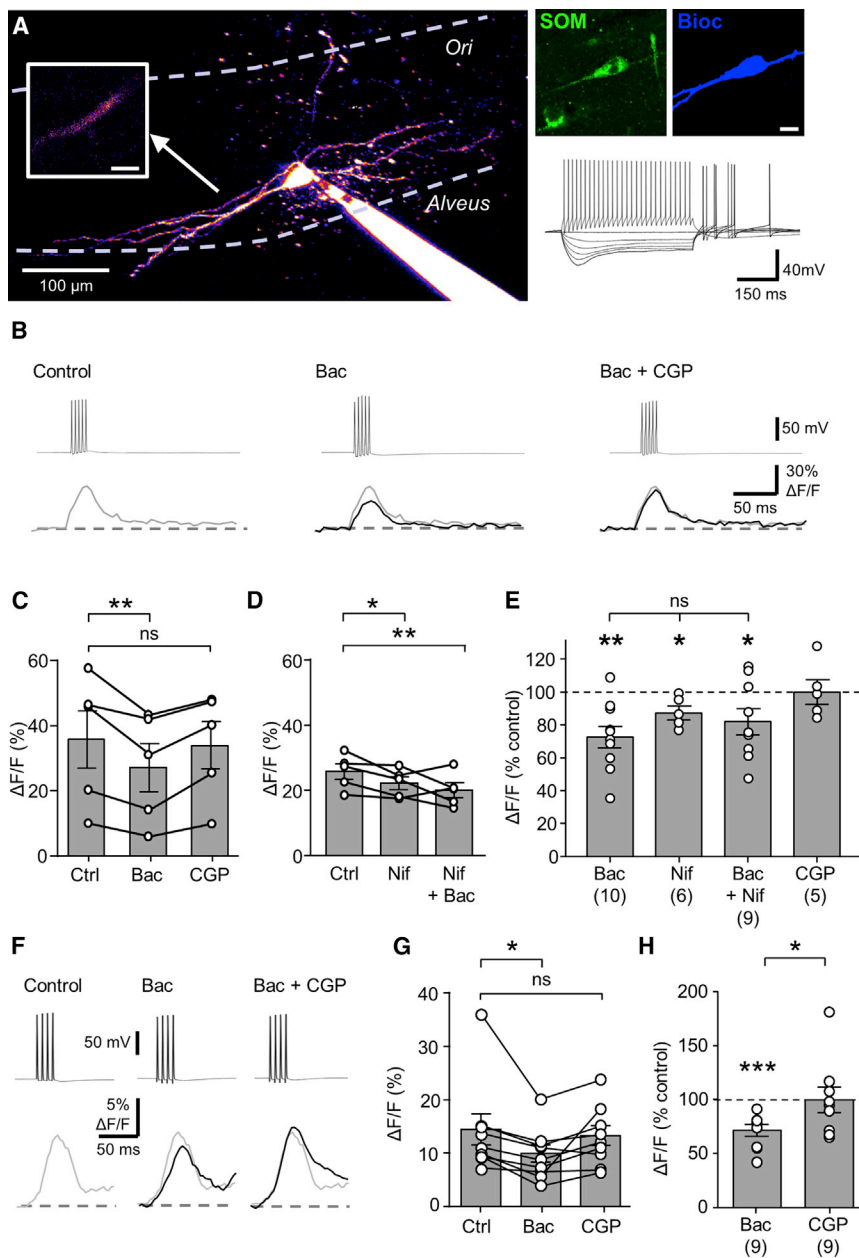


Figure 2. GABA_BRs Inhibit L-Type VGCC-Mediated Ca²⁺-Transients in SOM-IN Dendrites

(A) A 2-photon image of a SOM-IN filled with Alexa Fluor 594 (morphometric dye) and BAPTA-OG1 (100 μ M for Ca²⁺-imaging) and the imaged proximal dendritic segment (inset). Top right: confocal images confirm immunolabeling for SOM (green, left) at the soma (blue, right). Bottom right: voltage response of the cell to hyper- to depolarizing current pulses. Scale bars, 5 μ m (left inset); 10 μ m (right insets).

(B) Trains of bAPs (top) evoked by brief pulses (1 nA, 1 ms, 200 Hz) in the SOM-IN resulted in Ca²⁺-transients (bottom) under control conditions (left, gray), in baclofen (10 μ M, middle, in black) and CGP (5 μ M, right, in black).

(C) Summary of peak Ca²⁺-transients during control (Ctrl), baclofen (Bac), and CGP in 5 SOM-INs from juvenile rats (P17–P25).

(D) Ca²⁺-transients amplitudes during control condition, application of nifedipine (Nif) and co-application of nifedipine and baclofen (Bac+Nif) from 5 SOM-INs.

(E) Bar chart of normalized Ca²⁺-transients during application of Bac, Nif, Nif+Bac, and CGP in juvenile rats.

(F) Ca²⁺-transients (bottom) evoked by bAP trains (top) in a SOM-IN from an adult rat under control conditions (left, gray) in baclofen (middle, in black) and CGP (right, in black).

(G) Summary of peak Ca²⁺-transients during control (Ctrl), in baclofen (Bac) and CGP in 9 SOM-INs from adult rats (P50–P60).

(H) Bar chart of normalized Ca²⁺-transient amplitudes in Bac and CGP in juvenile rats.

Bar charts show mean \pm SEM. Data from individual cells is superimposed on bars (open circles); numbers of tested cells are in parentheses. ns, $p > 0.05$; * $p < 0.05$; ** $p < 0.01$. See also Figures S3 and S4.

(10 cells) resulted in a $27.8\% \pm 6.5\%$ reduction in the Ca²⁺ response ($t_{(9)} = 4.25$; $p = 0.002$, Wilcoxon test), which recovered in CGP (5 μ M; $t_{(4)} = 0.08$; $p = 0.94$, Wilcoxon test) (Figures 2B and 2C).

To confirm that L-type VGCCs contribute to Ca²⁺-transients, we applied the selective blocker nifedipine (10 μ M), resulting in a $13.2\% \pm 4.2\%$ reduction in the signal ($t_{(4)} = 3.20$; $p = 0.033$, Wilcoxon test) (Figure 2D), comparable to baclofen effect ($t_{(10,5)} = 1.35$; $p = 0.57$, one-way ANOVA). Moreover, co-application of baclofen and nifedipine did not further reduce the Ca²⁺ signal ($t_{(10,9)} = 1.02$; $p = 0.68$) (Figures 2D and 2E), independent of whether the co-application followed an initial baclofen ($t_{(4,4)} = 1.05$; $p = 0.62$, Holm-Sidak's post-test) or nifedipine application

(Figure S4). Furthermore, GABA_BR inhibition of Ca²⁺-transients was maintained into adulthood (Figures 2F–2H).

GABA_BRs and Ca_v1.2 Channels Preferentially Cluster on Dendritic Shafts of mGluR1 α -Expressing Cells

Our data indicate that GABA_BRs modulate L-type VGCCs but not Kir3 channels in SOM-INs. Therefore, we next examined the distribution, density, and spatial relationship of GABA_BR, Kir3, and Ca_v1.2 channel on SOM-IN dendrites by quantitative SDS-FRL electron microscopy, using mGluR1 α as a surface marker for SOM-INs (Baude et al., 1993). Immunoreactivity for the GABA_{B1} subunit was consistently observed at mGluR1 α -positive dendrites (Figure 3A), with a density of 49.1 ± 4.5 particles/ μ m²

($t_{(6,6)} = 1.39$; $p = 0.22$, Holm-Sidak's post-test). This mutual occlusion indicates that GABA_BRs predominantly inhibit L-type VGCCs in SOM-IN dendrites. This result was verified using 1-photon imaging, using the same pharmacological treatment

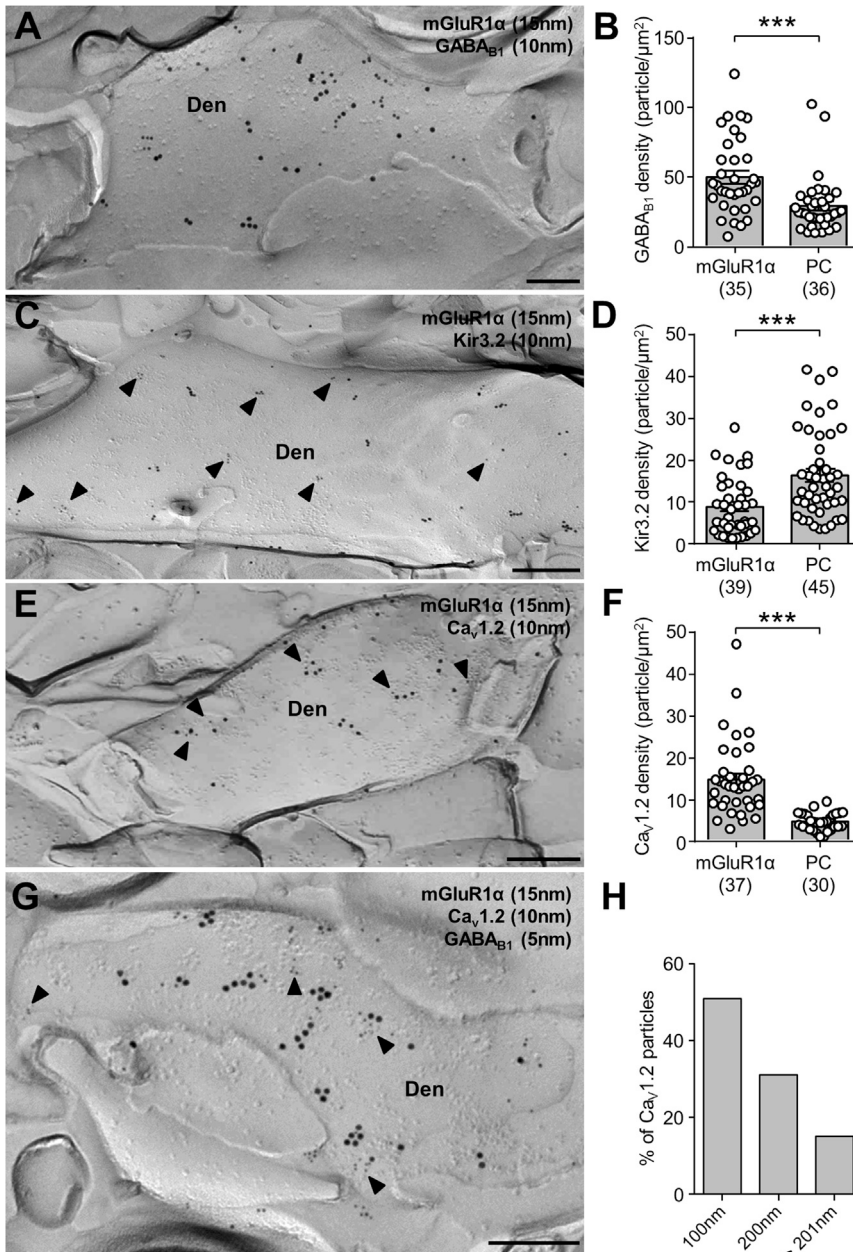


Figure 3. Differential Surface Densities of GABA_{B1}, Kir3.2, and Ca_v1.2 on Putative SOM-IN Dendrites

(A, C, and E) SDS-FRL electron micrographs illustrating the distribution of GABA_{B1} (A), Kir3.2 (C), and Ca_v1.2 (E) (all 10 nm gold particles, arrowheads in C and E) on mGluR1 α -expressing (15 nm) dendrites (Den).

(B, D, and F) Summary bar charts of the surface densities of GABA_{B1} (B), Kir3.2 (D), and Ca_v1.2 (F) particles on mGluR1 α -positive dendrites, compared to CA1 PCs. Numbers of dendrites are in parentheses.

(G) Immunogold labeling for GABA_{B1} (5 nm particles, arrowheads) and Ca_v1.2 (10 nm) on a mGluR1 α -immunoreactive (15 nm) dendrite.

(H) Distribution of Ca_v1.2 relative to GABA_{B1} particles, plotted as percentage against their distance to nearest GABA_{B1} particles in 100-nm bins.

Bar charts show mean \pm SEM. Data from individual cells is shown (open circles); numbers of tested cells are in parentheses. *** $p < 0.001$. Scale bars, 200 nm (A, E, and G) and 100 nm (C).

(35 dendrites from 3 animals), higher than that of CA1 PCs in the same replicas (28.5 ± 3.2 particles/ μm^2 , 36 dendrites, $U_{(34,35)} = 287.0$; $p < 0.0001$, Mann-Whitney test) (Figure 3B). In contrast, Kir3 channel subunit density was 8.6 ± 1.1 particles/ μm^2 on mGluR1 α dendrites (39 dendrites from 3 animals) (Figure 3C), $\sim 50\%$ lower than on neighboring PCs (16.2 ± 1.6 particles/ μm^2 , 45 dendrites, $U_{(38,44)} = 459.0$; $p = 0.0001$, Mann-Whitney test) (Figure 3D), explaining the small GABA_BR-mediated currents in SOM-INs.

Next, we determined the surface expression of Ca_v1.2, which was observed on mGluR1 α -positive dendrites (Figure 3E) with a density of 14.3 ± 1.5 particles/ μm^2 (37 dendrites from 3 animals) (Figure 3F), over 3-fold higher than on PCs (4.3 ± 0.4 par-

ticles/ μm^2 , 30 dendrites, $U_{(36,29)} = 77.0$; $p < 0.0001$). Finally, to examine the spatial relationship between GABA_{B1} and Ca_v1.2 we performed triple labeling for mGluR1 α , GABA_{B1} and Ca_v1.2 (Figure 3G) and measured the proximity of Ca_v1.2 particles to the closest GABA_{B1} particle. This analysis revealed that 51% of Ca_v1.2 subunit-containing channels were located within 100 nm of a GABA_{B1} subunit (Figure 3H). Thus, GABA_{B1} and Ca_v1.2 subunits are present at high density and colocalize on SOM-IN dendrites.

Postsynaptic GABA_BR Activation Inhibits Synaptic Plasticity in SOM-INs

Long-term potentiation (LTP) at excitatory synapses onto SOM-INs critically depends on L-type VGCC activation (Topolnik et al., 2009). Therefore, we asked if associative LTP in SOM-INs is sensitive

to GABA_BR activation. EPSC amplitudes were potentiated to $163.8\% \pm 17.3\%$ (measured at 20–25 min; $t_{(6)} = 3.99$; $p = 0.007$, t test, 7 cells) (Figure 4A) following LTP induction in SOM-INs. When baclofen was pre-applied, the same stimulus did not potentiate EPSCs (mean EPSC amplitude: $95.8\% \pm 10.8\%$ of baseline; $t_{(5)} = 0.47$; $p = 0.66$, t test, Wilcoxon test, 6 cells) (Figures 4B and 4F). A comparable GABA_BR-mediated inhibition of LTP was observed in adult rats (Figures 4C, 4D, and 4F).

To confirm that the LTP observed was dependent on L-type VGCCs, as previously shown (Topolnik et al., 2009), we pre-applied nifedipine (10 μM) to 6 SOM-INs. As expected, this manipulation fully abolished LTP (EPSC: $89.2 \pm 11.0\%$ of baseline $t_{(5)} = 1.07$; $p = 0.33$, t test) (Figures 4E and 4F). These data,

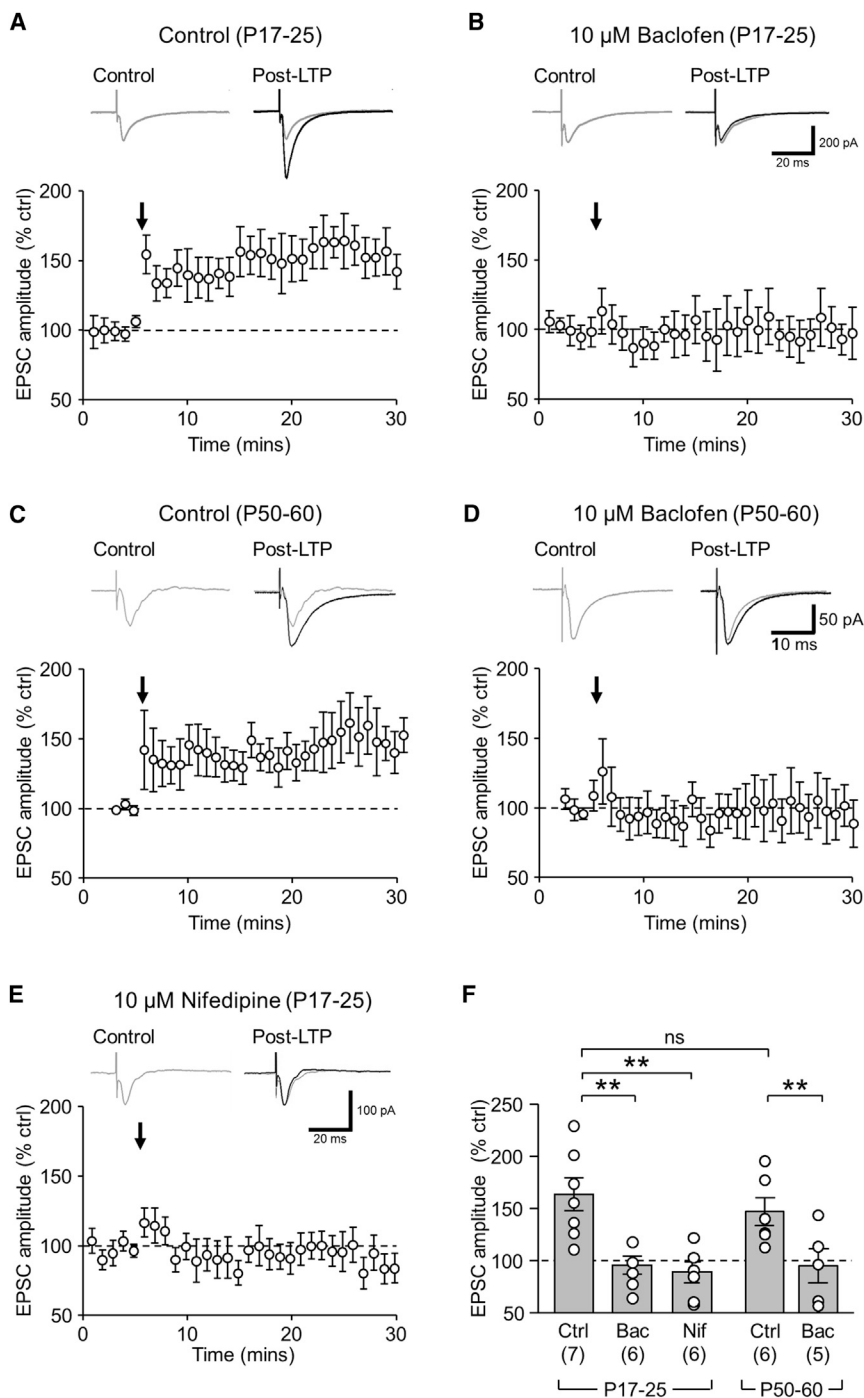


Figure 4. Postsynaptic GABA_B Activation Abolishes LTP in SOM-INs

(A and C) Time course plots of normalized EPSC amplitude in SOM-INs before and after LTP induction (TBS, arrow at 5 min) in slices from juvenile (P17–P25) (A, 7 cells) and adult rats (P50–P60) (C, 6 cells). Example traces corresponding to control (in gray) and LTP (25–30 min, in black) are shown above. (B and D) Time course of the normalized EPSC amplitudes following LTP induction in SOM-INs in the presence of baclofen (10 μ M) in juvenile (B, 6 cells) and adult animals (D, 5 cells). (E) Time course of EPSC amplitudes following LTP induction in the presence of nifedipine (10 μ M) in juvenile animals (6 cells). (F) Summary of normalized EPSC amplitudes following LTP induction under control (Ctrl) and in the presence of baclofen (Bac, 10 μ M) or nifedipine (Nif, 10 μ M) in SOM-INs from juvenile and adult rats. Dashed line indicates baseline; cell numbers are in parentheses. Bar charts show mean \pm SEM. **p < 0.01.

INs, but do not activate the canonical Kir3 signaling cascade. Rather, GABA_BRs cluster with and inhibit L-type VGCCs, reducing dendritic calcium influx and blocking LTP at excitatory synapses onto SOM-INs. This effect will preclude synaptic strengthening during network activation, a mechanism by which GABA_BRs can contribute to a long-term alteration of excitation and inhibition balance in the network.

Small GABA_BR-Mediated Inhibitory Currents in SOM-INs

The major GABA_BR signaling in postsynaptic compartments has long been considered to involve Kir3 channels (Lüscher et al., 1997; Kaupmann et al., 1998; Degro et al., 2015). In contrast, while we find that GABA_BRs and Kir3 channels are present on SOM-IN dendrites, only very small K⁺ currents were produced, partially explained by a lower Kir3 channel expression. In a network context, the small GABA_BR currents in SOM-INs are consistent with observations in other dendritic-targeting IN types: parvalbumin bistratified cells (Booker et al., 2013) and cholecystokinin INs (Booker et al., 2017) and may be a common principle for dendritic-targeting INs.

thus, demonstrate that activation of postsynaptic GABA_BRs, via inhibition of Ca_v1.2 Ca²⁺ channels, abolishes postsynaptic LTP induction at excitatory synapses onto SOM-INs.

DISCUSSION

In the present study, we provide compelling evidence that GABA_BRs are present on dendritic membranes of CA1 SOM-

INs. This divergence between perisomatic and dendritic inhibitory INs implies that GABA_BR activation shifts inhibition between the two target compartments.

Colocalization and Negative Coupling of GABA_BRs and VGCCs in SOM-INs

We provide evidence that postsynaptic GABA_BRs preferentially signal through and inhibit VGCCs in SOM-INs. The high

expression of $Ca_v1.2$ and nifedipine-sensitive Ca^{2+} -transients further indicate that these channels substantially contribute to Ca^{2+} influx in SOM-IN dendrites. In fact, GABA_BRs have been shown to inhibit VGCCs, as an alternative postsynaptic effector in PC dendrites (Sabatini and Svoboda, 2000; Chalifoux and Carter, 2011; Pérez-Garci et al., 2013). The co-clustering of the GABA_{B1} subunits with the $Ca_v1.2$ (L-type) VGCCs subunit is a structural correlate of this interaction in SOM-IN dendrites and may reflect a tight functional coupling through a membrane delimited $G_{i/o}$ - $\beta\gamma$ interaction (Pérez-Garci et al., 2013). Whether these mechanisms also apply to dendritic spines remains an open question.

GABA_BR Signaling Abolishes Synaptic Plasticity in SOM-INs

By negatively coupling to L-type VGCCs, GABA_BRs block the induction of LTP in SOM-INs, adding to the wide repertoire of molecular mechanisms involved in synaptic plasticity in these INs (Topolnik et al., 2006; Nicholson and Kullmann, 2014; Vasuta et al., 2015). The form of plasticity is dependent on the activity pattern (Lamsa et al., 2007) that is plausibly translated into a differential activation of glutamate receptors and downstream signaling cascades (Topolnik et al., 2005, 2006, 2009; Oren et al., 2009). Indeed, L-type VGCCs potentiation by group I mGluRs promotes LTP in SOM-INs (Topolnik et al., 2009). In cerebellar Purkinje cells, GABA_BRs facilitate mGluR1 activation (Hirono et al., 2001). In SOM-INs, the two receptors converge on L-type VGCCs, but exert opposing actions: GABA_BRs intercept mGluR-mediated signaling by inhibiting L-type channels and thereby abolish LTP induction.

Inhibition of LTP in SOM-INs by GABA_BRs is in stark contrast to the facilitation of LTP by GABA_BR activation observed in PCs (Davies et al., 1991; Mott and Lewis, 1991). In fact, despite their inhibitory nature, GABA_BRs can produce disinhibitory effects in cortical networks due to a preferential inhibition of INs and their output synapses (Foster et al., 2013; Papatheodoropoulos, 2015). In SOM-INs, GABA_BRs do not produce hyperpolarization, but prevent the induction of LTP and thereby preclude an enhanced recruitment of the feedback circuit. Considering that the main output of SOM-INs is onto PC distal dendrites, this reduced recruitment will allow increased synaptic transmission onto CA1 PCs and may lead to a breakdown of the specificity of spatial information carried by entorhinal inputs onto CA1 PCs (Leão et al., 2012) via activation of GABA_BRs on nearby pre- and postsynaptic elements, as previously described in the neocortex (Urban-Ciecko et al., 2015). Indeed, prior studies have shown that GABA_BR activation is capable of impairing hippocampal-dependent spatial learning (McNamara and Skelton, 1996; Arolfo et al., 1998), consistent with the importance of this circuit.

EXPERIMENTAL PROCEDURES

Electrophysiological Recordings

A full description of methods can be found in the Supplemental Information. In brief, 300- μ m acute hippocampal slices were prepared from juvenile (17- to 25-day-old) and adult (50- to 60-day-old) male Wistar rats (Booker et al., 2014, 2017). All experiments were performed in accordance with institutional, local governmental (LaGeSo, Berlin T 0215/11; LaGeSo, Freiburg X-14/11H)

and national guidelines (German Animal Welfare Act; ASPA, United Kingdom Home Office). Whole-cell recordings were made using pipettes filled with K-gluconate-based solution at $32^\circ\text{C} \pm 1^\circ\text{C}$. GABA_BR-mediated currents were measured in the presence of the ionotropic receptor blockers NBQX, CNQX or DNQX, DL-APV, and bicuculline, gabazine, or picrotoxin at a holding potential of -65 mV. Synaptic currents were elicited by a glass monopolar electrode in *str. oriens*.

For Ca^{2+} -imaging, we used 2-photon microscopy with pipettes filled with intracellular solution containing BAPTA-OGB1 and a morphometric dye. Ca^{2+} transients were measured in proximal dendrites following trains of 4 APs to evoke Ca^{2+} influx, line-scans were recorded at ~ 200 Hz. Baclofen or nifedipine was applied to the bath; CGP was applied following baclofen to confirm receptor specificity.

LTP was induced at inputs to SOM-INs with EPSCs elicited by a monopolar electrode placed in the alveus. Theta-burst stimulation was paired with a post-synaptic depolarization to -20 mV, repeated 3 times at 30-s intervals. In a subset of experiments, baclofen or nifedipine were pre-applied to the bath, and the EPSC was titrated to match control recordings. All neurons were filled with biocytin during recordings, fixed overnight, labeled with streptavidin and antibodies to SOM, and imaged with confocal microscopy.

Electron Microscopy

Electron microscopic analysis was performed on 60-day-old wild-type Wistar rats (Althof et al., 2015). Coronal hippocampal sections were cut, cryoprotected, and blocks of *str. oriens/alveus* of CA1 were dissected and frozen under high-pressure. Samples were fractured and coated with carbon and platinum in a freeze-fracture replica machine. Replicas were then digested, washed, blocked, and then incubated with subunit-specific primary antibodies followed by incubation with gold-coupled secondary antibodies. Strongly mGluR1 α -immunoreactive and CA1 PC dendrites in *str. oriens* were imaged and analyzed.

Statistics

All data are shown as mean \pm SEM. Analysis was performed in GraphPad Prism 3.0 (GraphPad Software, CAUSA). For all electrophysiology data, “n” refers to the number of recorded cells; for electron microscopy “n” refers to the number of dendrites tested from 3–4 rats. Group data were compared with one-way ANOVA test combined with Holm’s-Sidak post-test. Analysis of unpaired and paired data was performed with Mann-Whitney or Wilcoxon matched-pairs tests, respectively. Significance was assumed if $p < 0.05$.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at <https://doi.org/10.1016/j.celrep.2017.12.021>.

ACKNOWLEDGMENTS

We thank Natalie Wernet, Julia Bank, and Ina Wolter for excellent technical support. Funding was provided by DFG (FOR 2134 to I.V. and A.K.), BIOS-2 (to A.K.), McNaught Bequest (to I.V. and S.A.B.), Tenovus Scotland (to I.V.), MRC (to S.A.B., D.J.A.W., and P.C.K.), The Patrick Wild Centre for Autism Research (to S.A.B., D.J.A.W., and P.C.K.), and the Dame Stephanie Shirley Foundation (to S.A.B., D.J.A.W., and P.C.K.).

AUTHOR CONTRIBUTIONS

S.A.B., D.L., D.J.A.W., P.C.K., A.K., and I.V. designed experiments. S.A.B. and A.L.G. performed electrophysiological experiments and analyzed data. A.K. and D.L. performed SDS-FRL and analyzed data. M.W. provided antibodies. S.A.B., D.L., D.J.A.W., P.C.K., A.K., and I.V. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: July 12, 2017
Revised: October 14, 2017
Accepted: December 6, 2017
Published: January 2, 2018

REFERENCES

- Althof, D., Baehrens, D., Watanabe, M., Suzuki, N., Fakler, B., and Kulik, Á. (2015). Inhibitory and excitatory axon terminals share a common nano-architecture of their $\text{Ca}_v2.1$ (P/Q-type) Ca^{2+} channels. *Front. Cell. Neurosci.* 9, 315.
- Arofo, M.P., Zanutto, M.A., and Ramirez, O.A. (1998). Baclofen infused in rat hippocampal formation impairs spatial learning. *Hippocampus* 8, 109–113.
- Baude, A., Nusser, Z., Roberts, J.D.B., Mulvihill, E., McIlhinney, R.A., and Somogyi, P. (1993). The metabotropic glutamate receptor (mGluR1 α) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. *Neuron* 11, 771–787.
- Booker, S.A., Gross, A., Althof, D., Shigemoto, R., Bettler, B., Frotscher, M., Hearing, M., Wickman, K., Watanabe, M., Kulik, Á., and Vida, I. (2013). Differential GABAB-receptor-mediated effects in perisomatic- and dendrite-targeting parvalbumin interneurons. *J. Neurosci.* 33, 7961–7974.
- Booker, S.A., Song, J., and Vida, I. (2014). Whole-cell patch-clamp recordings from morphologically- and neurochemically-identified hippocampal interneurons. *J. Vis. Exp.* 2014, e51706.
- Booker, S.A., Althof, D., Gross, A., Loreth, D., Müller, J., Unger, A., Fakler, B., Varro, A., Watanabe, M., Gassmann, M., et al. (2017). KCTD12 auxiliary proteins modulate kinetics of GABAB receptor-mediated inhibition in cholecystokinin-containing interneurons. *Cereb. Cortex* 27, 2318–2334.
- Chalifoux, J.R., and Carter, A.G. (2011). GABAB receptor modulation of voltage-sensitive calcium channels in spines and dendrites. *J. Neurosci.* 31, 4221–4232.
- Davies, C.H., Starkey, S.J., Pozza, M.F., and Collingridge, G.L. (1991). GABA autoreceptors regulate the induction of LTP. *Nature* 349, 609–611.
- de Lanerolle, N.C., Kim, J.H., Robbins, R.J., and Spencer, D.D. (1989). Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. *Brain Res.* 495, 387–395.
- Degro, C.E., Kulik, Á., Booker, S.A., and Vida, I. (2015). Compartmental distribution of GABAB receptor-mediated currents along the somatodendritic axis of hippocampal principal cells. *Front. Synaptic Neurosci.* 7, 6.
- Dugladze, T., Vida, I., Tort, A.B., Gross, A., Otahal, J., Heinemann, U., Kopell, N.J., and Gloveli, T. (2007). Impaired hippocampal rhythmogenesis in a mouse model of mesial temporal lobe epilepsy. *Proc. Natl. Acad. Sci. USA* 104, 17530–17535.
- Foster, J.D., Kitchen, I., Bettler, B., and Chen, Y. (2013). GABAB receptor subtypes differentially modulate synaptic inhibition in the dentate gyrus to enhance granule cell output. *Br. J. Pharmacol.* 168, 1808–1819.
- Gloveli, T., Dugladze, T., Saha, S., Monyer, H., Heinemann, U., Traub, R.D., Whittington, M.A., and Buhl, E.H. (2005). Differential involvement of oriens/pyramidal interneurons in hippocampal network oscillations in vitro. *J. Physiol.* 562, 131–147.
- Hirono, M., Yoshioka, T., and Konishi, S. (2001). GABA(B) receptor activation enhances mGluR-mediated responses at cerebellar excitatory synapses. *Nat. Neurosci.* 4, 1207–1216.
- Katona, I., Acsády, L., and Freund, T.F. (1999). Postsynaptic targets of somatostatin-immunoreactive interneurons in the rat hippocampus. *Neuroscience* 88, 37–55.
- Kaupmann, K., Malitschek, B., Schuler, V., Heid, J., Froestl, W., Beck, P., Mosbacher, J., Bischoff, S., Kulik, Á., Shigemoto, R., et al. (1998). GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature* 396, 683–687.
- Klausberger, T., and Somogyi, P. (2008). Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* 321, 53–57.
- Lamsa, K.P., Heeroma, J.H., Somogyi, P., Rusakov, D.A., and Kullmann, D.M. (2007). Anti-Hebbian long-term potentiation in the hippocampal feedback inhibitory circuit. *Science* 315, 1262–1266.
- Leão, R.N., Mikulovic, S., Leão, K.E., Munguba, H., Gezelius, H., Enjin, A., Patra, K., Eriksson, A., Loew, L.M., Tort, A.B.L., and Kullander, K. (2012). OLM interneurons differentially modulate CA3 and entorhinal inputs to hippocampal CA1 neurons. *Nat. Neurosci.* 15, 1524–1530.
- Lüscher, C., Jan, L.Y., Stoffel, M., Malenka, R.C., and Nicoll, R.A. (1997). G protein-coupled inwardly rectifying K^+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron* 19, 687–695.
- McBain, C.J., DiChiara, T.J., and Kauer, J.A. (1994). Activation of metabotropic glutamate receptors differentially affects two classes of hippocampal interneurons and potentiates excitatory synaptic transmission. *J. Neurosci.* 14, 4433–4445.
- McNamara, R.K., and Skelton, R.W. (1996). Baclofen, a selective GABAB receptor agonist, dose-dependently impairs spatial learning in rats. *Pharmacol. Biochem. Behav.* 53, 303–308.
- Mott, D.D., and Lewis, D.V. (1991). Facilitation of the induction of long-term potentiation by GABAB receptors. *Science* 252, 1718–1720.
- Müller, C., and Remy, S. (2014). Dendritic inhibition mediated by O-LM and bis-tratified interneurons in the hippocampus. *Front. Synaptic Neurosci.* 6, 23.
- Nicholson, E., and Kullmann, D.M. (2014). Long-term potentiation in hippocampal oriens interneurons: postsynaptic induction, presynaptic expression and evaluation of candidate retrograde factors. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 369, 20130133.
- Oren, I., Nissen, W., Kullmann, D.M., Somogyi, P., and Lamsa, K.P. (2009). Role of ionotropic glutamate receptors in long-term potentiation in rat hippocampal CA1 oriens-lacunosum moleculare interneurons. *J. Neurosci.* 29, 939–950.
- Papatheodoropoulos, C. (2015). Higher intrinsic network excitability in ventral compared with the dorsal hippocampus is controlled less effectively by GABAB receptors. *BMC Neurosci.* 16, 75.
- Pérez-Garci, E., Larkum, M.E., and Nevian, T. (2013). Inhibition of dendritic Ca^{2+} spikes by GABAB receptors in cortical pyramidal neurons is mediated by a direct $\text{Gi}/\text{o}-\beta$ -subunit interaction with Cav1 channels. *J. Physiol.* 591, 1599–1612.
- Sabatini, B.L., and Svoboda, K. (2000). Analysis of calcium channels in single spines using optical fluctuation analysis. *Nature* 408, 589–593.
- Sloviter, R.S., Ali-Akbarian, L., Elliott, R.C., Bowery, B.J., and Bowery, N.G. (1999). Localization of GABA(B) (R1) receptors in the rat hippocampus by immunocytochemistry and high resolution autoradiography, with specific reference to its localization in identified hippocampal interneuron subpopulations. *Neuropharmacology* 38, 1707–1721.
- Standaert, D.G., Landwehrmeyer, G.B., Kerner, J.A., Penney, J.B., Jr., and Young, A.B. (1996). Expression of NMDAR2D glutamate receptor subunit mRNA in neurochemically identified interneurons in the rat neostriatum, neocortex and hippocampus. *Brain Res. Mol. Brain Res.* 42, 89–102.
- Topolnik, L., Congar, P., and Lacaille, J.-C. (2005). Differential regulation of metabotropic glutamate receptor- and AMPA receptor-mediated dendritic Ca^{2+} signals by presynaptic and postsynaptic activity in hippocampal interneurons. *J. Neurosci.* 25, 990–1001.
- Topolnik, L., Azzì, M., Morin, F., Kougioumoutzakis, A., and Lacaille, J.-C. (2006). mGluR1/5 subtype-specific calcium signalling and induction of long-term potentiation in rat hippocampal oriens/alveus interneurons. *J. Physiol.* 575, 115–131.
- Topolnik, L., Chamberland, S., Pelletier, J.-G., Ran, I., and Lacaille, J.-C. (2009). Activity-dependent compartmentalized regulation of dendritic Ca^{2+} signaling in hippocampal interneurons. *J. Neurosci.* 29, 4658–4663.
- Tyan, L., Chamberland, S., Magnin, E., Camiré, O., Francavilla, R., David, L.S., Deisseroth, K., and Topolnik, L. (2014). Dendritic inhibition provided by interneuron-specific cells controls the firing rate and timing of the hippocampal feedback inhibitory circuitry. *J. Neurosci.* 34, 4534–4547.

Urban-Ciecko, J., Fanselow, E.E., and Barth, A.L. (2015). Neocortical somatostatin neurons reversibly silence excitatory transmission via GABA_B receptors. *Curr. Biol.* *25*, 722–731.

Vasuta, C., Artinian, J., Laplante, I., Hébert-Seropian, S., Elayoubi, K., and Lacaille, J.-C. (2015). Metaplastic regulation of CA1 Schaffer collateral pathway plasticity by Hebbian mGluR1a-mediated plasticity at excitatory syn-

apses onto somatostatin-expressing interneurons. *eNeuro* *2*, ENEURO.0051-15.2015.

Wang, A.Y., Lohmann, K.M., Yang, C.K., Zimmerman, E.I., Pantazopoulos, H., Herring, N., Berretta, S., Heckers, S., and Konradi, C. (2011). Bipolar disorder type 1 and schizophrenia are accompanied by decreased density of parvalbumin- and somatostatin-positive interneurons in the parahippocampal region. *Acta Neuropathol.* *122*, 615–626.