Protein kinase A-dependent plasticity of local inhibitory synapses from hilar somatostatin-expressing neurons

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Protein kinase A-dependent plasticity of local inhibitory synapses from hilar somatostatin-expressing neurons.

Running title: Plasticity of local and distal hilar GABA synapses

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Conflict of Interest
No, authors report no conflict of interest

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Protein kinase A-dependent plasticity of local inhibitory synapses from hilar somatostatin-expressing neurons.

Running title: Plasticity of hilar inhibitory neuron synapses

Abstract

Hippocampal inhibitory neurons (INs) contact local targets and project to different brain areas to form synapses on distal neurons. Despite the importance of INs for hippocampal function and interregional brain communication, the impact of activity-dependent plasticity mechanisms on local and long-range GABAergic synapses formed by hippocampal INs remains to be fully elucidated. Here, we use optogenetic-coupled electrophysiology in mice to show that protein kinase A (PKA), a master regulator of GABAergic synapse plasticity, causes a form of long-term potentiation of inhibitory synapses (iLTP) in hippocampal granule cells (GCs). This form of iLTP is observed in GCs synapses originated in local INs expressing the marker somatostatin (SST) but not in those expressing parvalbumin (PV). Long-range synapses formed by SST INs onto medial septum neurons are unaffected by PKA activation. iLTP of local SST synapses on GCs is accompanied by changes in presynaptic probability of release and is occluded by pharmacological increase of synaptic activity in vivo. Our results suggest that PKA-dependent inhibitory synapse plasticity is expressed in local but not long-range targets of SST INs and selectively modifies inhibitory microcircuits essential for hippocampal function.

Significance statement

The hippocampus, a brain region essential for memory, is populated by a diverse group of inhibitory neurons (INs) that form synaptic contacts onto local principal cells and long-range targets. Changes in inhibitory synapse strength shape local and inter-regional brain communication. However, how synapse plasticity mechanisms are implemented in the rich assortment of hippocampal INs remains to be fully defined. Here we show that protein kinase A, a signaling protein involved in memory, enhances inhibitory neurotransmission from local but not long-range projecting hippocampal INs expressing the molecular marker somatostatin. The consequences of this plasticity are observed after increasing neuronal activity in vivo. Our work describes a form of activity-dependent synapse plasticity that regulates inhibitory microcircuits essential for hippocampal memory function.
Introduction

Inhibitory synapses in hippocampal excitatory neurons originate in a diverse array of presynaptic inhibitory neurons (INs) subtypes with specific roles in network activity, spatial navigation and memory (Klausberger and Somogyi, 2008). As their excitatory counterparts, inhibitory synapses that use γ-aminobutyric acid (GABA) as neurotransmitter undergo several forms of plasticity that modify their structure and function (Castillo et al., 2011; Flores and Méndez, 2014; Kullmann et al., 2012). Inhibitory synapses are endowed with a wide variety of plasticity mechanisms that parallels their molecular and functional heterogeneity (Mendez and Bacci, 2011). Defining the mechanisms of hippocampal GABAergic synapse plasticity in the context of IN subtype assortment is essential to refine our basic understanding of IN diversity and the role of inhibitory synapse plasticity in learning and memory.

In the hippocampal dentate gyrus (DG), INs expressing the molecular markers somatostatin (SST) and parvalbumin (PV) provide a large fraction of synaptic inhibition to local glutamatergic granule cells (GCs) (Hosp et al., 2014; Houser, 2007). Although they share common molecular features and developmental origin (Lim et al., 2018), SST and PV INs are heterogenous classes and comprise several subtypes with different target specificities at the level of subcellular compartment and target regions (Harris et al., 2018). Dentate SST INs form synaptic contacts onto local GC dendrites (Hosp et al., 2014) and send long-range axon collaterals to the medial septum (MS) (Jinno, 2009). Different types of PV INs provide perisomatic inhibition by impinging on the soma, proximal dendrites and axon initial segments of target neurons (Hu et al., 2014). This synapse specific features have been proposed to determine the functional impact of inhibitory synapse plasticity on principal excitatory neurons (Mendez and Bacci, 2011).

Protein kinase A (PKA) is a master regulator of synaptic plasticity of GABAergic synapses across multiple brain regions (Castillo et al., 2011). PKA regulates postsynaptic receptors acting on inhibitory synapse scaffolding proteins and receptors (Nugent et al., 2009; Poisbeau et al., 1999). In addition, PKA has presynaptic mechanisms of action, including the regulation of neurotransmitter release (Monday et al., 2018). The activity of PKA is tightly linked to neuronal activity through different neuromodulators, glutamate receptors and intracellular calcium levels (Greengard, 2001; Halls and Cooper, 2011).

Here we tested the existence of IN subtype-specific mechanisms of PKA-dependent GABAergic synapse plasticity. For this, we performed electrophysiological recordings of GABAergic synapses formed by two major types of DG INs (PV and SST INs) in local (i.e. GCs) and long-range (i.e. medial septum) target neurons. We found that PKA activity induced by pharmacological rise of...
cyclic adenosine monophosphate (cAMP) levels triggers a form of Long-Term Potentiation that affects local but not long-range synapses formed by SST DG INs. In addition, we tested the dependency of this form of plasticity on previous increases in neuronal activity using in vivo treatment with kainic acid. Our results suggest that the induction of PKA-dependent plasticity of GABAergic synapses interacts with neuronal activity and could be part of experience-dependent modifications of DG inhibitory circuits.

Materials and methods

Animals

Group housed adult male and female C57BL/6J wild-type, SST-Cre (Ssttm2.1(cre)Zjh/J), and PV-Cre (Pvalb tm1(cre)Arbr/J) mice maintained in a 12 h light/dark cycle with unlimited access to food and water were used for the study (45% males, 55% females). Mice were sacrificed at 8-12 weeks for electrophysiological recordings. All procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee of the Cajal Institute and approved by local veterinary office (Comunidad de Madrid).

Reagents and AAVs

Forskolin (FSK) and PKI14-22 (both from Tocris) were dissolved in DMSO (FSK: 10 mM, PKI14-22: 1 mM). H89 (Tocris) was dissolved in distilled water. Previous to use, the compounds were further dissolved in artificial cerebrospinal fluid (ACSF) to final concentration (FSK, H89: 10 µM; PKI14-22: 1 µM). Kynurenic acid (Sigma) and SR-95531 (Gabazine, Tocris) were dissolved in ACSF at a final concentration of 2 mM and 10 µM, respectively. Kainic acid (KA, Sigma) was dissolved in saline solution to 2 mg / ml and used at a final dose of 5 mg / kg. Adeno-associated viruses (AAVs) were used with serotype 5 and purchased from the University of North Carolina Vector Core (AAV-EF1a-DIO-ChETA-EYFP).

Surgery

Analgesic (paracetamol 0.2 g/kg) treatment was administered for 4 days around surgery. Under anesthesia (isoflurane, induction 5%, maintenance 1.5 - 2.0%), mice were placed in a stereotaxic frame (RWD) and one craniotomy (left hemisphere) was performed using stereotaxic coordinates adopted to target the dorsal DG: -2.2 anterior–posterior; -1.45 medial–lateral; -1.9 dorsal–ventral. Injections of AAV (0.5-0.6 μl) were performed using graduated pipettes (Drummond Scientific Company), pulled and broken back to a tip diameter of 10–15 μm, at an infusion rate of ~0.05 μl min⁻¹. Micropipettes were left in place 5 min following microinjection and slowly retracted (0.4 mm/minute) to avoid reflux of the viral solution. Experiments involving AAVs started on the 3rd week after the viral injection.
Kainate treatment

Mice were treated with 2-3 intraperitoneal injections of kainate at low dose (5 mg/kg) at 30 min intervals. The behavior of the animals was monitored between injections and rated according to Racine (Racine, 1972) until behavior compatible with stages 2 and 3 (head nodding and forelimb clonus) was observed. Mice were processed for immunohistochemistry or electrophysiological recordings 90 minutes after the last KA injection.

Electrophysiology

Acute slices for electrophysiological recordings were prepared from 8-10 weeks old male and female mice. Brains were quickly removed and coronal slices containing the dorsal hippocampus (300 μm) or the medial septum (250 μm) were cut at 4°C with a vibratome in a solution containing: 234 mM sucrose, 11 mM glucose, 26 mM NaHCO3, 2.5 mM KCl, 1.25 mM NaH2PO4, 10 mM MgSO4, and mM 0.5 CaCl2 (equilibrated with 95% O2 – 5% CO2). Recordings were obtained at 30-32°C from DG granular cells (GCs) visually identified using infrared video microscopy. Recordings were performed in 95% O2 – 5% CO2 equilibrated ACSF containing 126 mM NaCl, 26 mM NaHCO3, 2.5 mM KCl, 1.25 mM NaH2PO4, 2 mM MgSO4, 2 mM CaCl2 and 10 mM glucose (pH 7.4). In order to isolate GABAergic currents resulting from electrical and optogenetic stimuli, the wide spectrum glutamate receptor blocker kynurenic acid (2 mM) was added to the recording solution in all experiments. Patch-clamp electrodes contained a "high chloride" intracellular solution composed of: 60 mM Cs Methane Sulfonate, 70 mM CsCl, 10 mM HEPES, 5 mM EGTA, 4 mM QX-314 and 4 mM Mg ATP, pH 7.3, corrected with CsOH (290 mOsm). In a set of experiments (Fig. 1E-F), we used a “low chloride” solution composed of 127 mM Cs Methane Sulfonate, 2 mM CsCl, 10 mM HEPES, 5 mM EGTA, 4 mM QX-314 and 4 mM Mg ATP, pH 7.3, corrected with CsOH (290 mOsm). The reversal potential for chloride (ECl) is approximately 16mV in “high chloride” and -80 mV in “low chloride” solutions based on the Nernst equation, without correction for liquid junction potential. All recordings were performed in the voltage clamp configuration at a holding potential of – 70 mV, resulting in inward and outward responses to GABA A receptor activation in “high” and “low chloride” conditions respectively. Biocytin (Sigma, 2 mg/ml) was added to a set of experiments to recover the morphology of the recorded neurons (Figs. 1A, 2C and 4C). All drugs were dissolved to final concentration in ACSF and applied through a gravity-driven bath perfusion system at flow rate of approximately 2-3 ml/min. Electrically evoked postsynaptic currents were elicited with a bipolar theta-glass pipette filled with ACSF positioned in the outer portion of the molecular layer. For optogenetic stimulation, we used a LED source (CoolLED’s pE-300white) filtered at 490 nM and directed to the ChR2 expressing neurons through the objective of the microscope. Average LED power (% full LED light source) was 38% in hippocampal slices from SST-Cre mice, 46 % in hippocampal slices from PV-Cre mice and 2% in Medial Septum slices from SST-Cre mice. Signals were amplified using a Multiclamp 700B patch-clamp amplifier.
(Molecular Devices), sampled at 20 kHz, filtered at 10 kHz, and stored on a PC. Data were analyzed using pClamp (Molecular Devices) software. For illustration purposes, we used the average of 5 consecutive sweeps.

**Immunohistochemistry and imaging**

Mice were injected with a lethal dose of pentobarbital (150 mg/kg) and perfused transcardiacaally with cold PBS and 4% paraformaldehyde (PFA) solution. Brains were extracted and submerged in fixative for 2 h at 4°C. Coronal 50 μm-thick sections were blocked in PBS with 10% BSA and 0.3% Triton X-100 followed by overnight incubation in with the primary antibody raised against cFos (1:5000, Synaptic Systems). After 3 × 15 min wash in PBS at room temperature, brain sections were incubated in PBS and 0.3% Triton X-100 with Alexa-Fluor 488 conjugated anti-rabbit secondary antibody (Abcam, 1:500). After 3 more steps of washing in PBS, the samples were mounted and covered on microscope slides using DAPI containing mounting medium. Slices used for electrophysiological experiments containing biocytin-loaded neurons were fixed in 4% PFA for 24 h at 4°C, blocked as described above and incubated overnight with Alexa 633-Streptavidin (Abcam, 1:500). After washing, slices were incubated with DAPI and mounted using mounting medium. Images were obtained with a Leica SP5 confocal microscope (LEICA LAS AF software) using 10x or 20x objectives and 405, 488, 561 and 633 nm laser excitation wavelengths. 1024x1024 images with a resolution of 1.3-2.6 pixel/μm, at 3-4 μm step size were collected. Microscopy images in figures 1, 2 and 5 were obtained by maximal intensity projections of 3 - 6 optical sections.

**Statistical analysis**

Values are given in mean ± SEM. In addition, we show individual measurements in each experiment. We used recorded neurons as "n" in electrophysiological experiments. Criteria for significant increase of electrically-evoked or optogenetically-evoked Inhibitory Post-Synaptic Currents (eIPSCs or oeIPSCs) amplitude was established as z-score > 2 standard deviation (SD) of 8-10 min baseline recorded normalized eIPSCs or oeIPSCs. Values of IPSC amplitude and PPR used for statistical analysis were obtained averaging the 5 last min of baseline and post treatment condition in each experiment. Standard two-tailed t tests were performed to compare normally distributed data points, otherwise we used the non-parametric Mann-Whitney test. Two-way ANOVA followed by Bonferroni’s post hoc comparisons were used when noted. Fisher’s exact test was used to compare proportions. For all tests, we adopted an alpha level of 0.05 to assess statistical significance. Statistical analysis was performed using Prism (GraphPad software). Experimenters were not blind to condition during data acquisition and analysis.
Results

PKA activation induces long-term potentiation of inhibitory synapses (iLTP) on hippocampal GCs.

Cyclic AMP (cAMP) and protein kinase A (PKA) regulate synaptic plasticity in multiple brain regions. However, its impact on plasticity of GABAergic synapses in hippocampal dentate gyrus (DG) granule cells (GCs) remains unresolved. To address this question, we pharmacologically manipulated cAMP levels and PKA activity during whole cell voltage clamp recordings of GABAergic synapse activity in GCs in acutely prepared slices from young adult male and female mice (Fig. 1A). We recorded GABA A receptor-mediated transmission using a “high chloride” internal solution in the presence of a glutamatergic receptor blocker (kynurenic acid, 2 mM). The addition of biocytin to the internal solution allowed the morphological reconstruction of the recorded GCs. Reconstructed GCs had well developed dendritic arborization with numerous ramifications and dendrites covered with dendritic spines (Fig. 1A), suggesting that recordings were performed from mature GCs.

GABA A receptor-mediated inhibitory postsynaptic currents (eIPSCs) were evoked every 30 s using a bipolar electric stimulation electrode placed in the outer third part of the molecular layer (ML, Fig. 1A). After 8-10 min baseline period of stable amplitude eIPSCs recordings, the adenylate cyclase activator forskolin (FSK, 10 µM) was applied for 10 minutes through the perfusion bath and recordings were maintained for 20 additional minutes. Transient application of FSK induced an increase in the amplitude of eIPSCs (Fig. 1B, FSK), in 18 of 24 recorded GCs (Fig. 1C, FSK). The normalized eIPSC amplitude changed from 102 ± 2 % (Baseline, Bsl) to 144 ± 7 % after FSK application ($p < 0.0001$, Fig. 1D). To test the dependency of FSK-induced effects on PKA activity, we performed recordings in the presence of the PKA inhibitors H89 (10 µM) and myristoylated PKI14-22. In these conditions, FSK failed to alter eIPSC amplitude (H89: Bsl 99 ± 1 %, FSK 80 ± 6 %, $p = 0.27$, PKI14-22: Bsl 102 ± 2 %, FSK 95 ± 13 %, $p > 0.99$) in all recorded GCs ($n = 8$ and $n = 5$, H89 and PKI14-22, respectively, Fig. 1C, D). To test the potential effect of PKA inhibition on basal synaptic activity, we recorded eIPSCS after transient application of H89 (10 µM, 10 min). We failed to detect a significant effect of H89 in the amplitude of eIPSCs in GCs (Bsl 99 ± 2 %, H89 91 ± 4 %, Mann-Whitney test, $U = 6$, $p = 0.22$, $n = 5$).

We performed additional recordings using an internal solution with “low chloride” concentration imposing a physiological driving force for chloride currents (see methods). In this condition, FSK application induced iLTP of comparable magnitude (Bsl 96 ± 3 %, FSK 146 ± 17 %, $p = 0.02$) in a similar proportion of GCs tested (6 of the 7 recorded GCs, Fig. 1E, F, G) compared with the “high chloride” condition.
Altogether, these results suggest that increasing cAMP production induces a form of inhibitory long-term potentiation (iLTP) on GABAergic synapses of GCs through PKA activation. This form of iLTP is observed at physiological chloride concentration.

PKA-induced iLTP is observed in GABA synapses originated in SST but not in PV INs.

GABAergic synapses in GCs originate from two major inhibitory neuron (IN) types, the somatostatin (SST) and the parvalbumin (PV) expressing INs (Hosp et al., 2014). We investigated the impact of PKA on GC GABA synapses originated in PV INs (PVGC) and SST INs (SSTGC). We used optogenetics to evoke GABA release in acutely prepared slices from PV-Cre and SST-Cre transgenic mice previously infected with AAVs carrying a Cre-dependent modified channelrhodopsin (ChETA-EYFP). After establishing a recording of a GC, we used a LED to deliver a brief (2 ms) 490nm light pulse to activate ChETA-expressing SSTGC and PVGC inhibitory synapses (Fig. 2A).

We detected time-locked responses in GCs to light stimulation of SSTGC and PVGC synapses, which we named optogenetic-evoked IPSCs (oeIPSCs). Analysis of the kinetics showed that the rise slope of oeIPSCs was higher in PVGC synapses compared to SSTGC synapses (Fig. 2B, PVGC 0.35 ± 0.03; SSTGC 0.27 ± 0.02 pA/ms, Two-sided t-test, t (78) = 2.11, p = 0.04; n = 44 and 36 GCs from PV-Cre and SST-Cre mice respectively). Reconstruction of the recorded GCs and visualization of the EYFP-tagged ChETA showed that the majority of the axonal arborization from PV INs accumulated around GC soma, in the granule cell layer (GCL). In contrast, axonal arborization from SST INs concentrated in the outer part of the molecular layer (ML) where GCs extend their dendrites (Fig. 2C). Thus, this optogenetic-based approach allows the selective stimulation of different sets of GABA synapses of GCs with different presynaptic origin, different axonal distribution and different synaptic current kinetics.

We next tested the effect of PKA activation on SSTGC and PVGC synapses. We observed that the amplitude of oeIPSC of PVGC synapses was significantly increased after transient application of the PKA activator FSK in only 1 of the 12 recorded GCs (Fig. 2D, E). At the population level, FSK failed to increase oeIPSC amplitude of PVGC synapses compared with the baseline period (Baseline 103 ± 2 %, FSK 90 ± 10 %, p = 0.73, Fig. 2F). In contrast, oeIPSCs amplitude of SSTGC synapses increased in 8 of 10 recorded GCs after transient FSK application (Fig. 2D, E). The amplitude of oeIPSC of SSTGC synapses changed from 99 ± 2 % during baseline period to 192 ± 23 %.
20 % after FSK application \((p < 0.0001, \text{Fig. 2F})\). FSK failed to significantly increase the amplitude of oeIPSC of SST\(_{GC}\) synapses in slices treated with PKA inhibitor H89 \((\text{SST}_{GC} + \text{H89}, \text{Fig. 2D, E, F})\)

These results suggest that PKA-induced iLTP in GCs preferentially affects GABA synapses originated in SST INs but not in PV INs.

**PKA-induced iLTP is accompanied by changes in PPR**

We next investigated changes in presynaptic function associated to PKA-dependent iLTP observed in GABAergic synapses in GCs. We calculated the Paired Pulse Ratio (PPR) of the response amplitude (eIPSCs or oeIPSCs) to a pair of stimuli delivered 50 ms apart (Fig. 3A). Changes in the PPR are assumed to reflect changes in the probability of neurotransmitter release. The PPR of eIPSCs on GCs was significantly decreased after transient application of FSK \((p = 0.003, \text{Fig. 3B})\). The FSK-induced change in PPR was not observed in the experiments performed in the presence of the PKA inhibitor H89 \((p = 0.99, \text{Fig. 3B})\). We performed similar analysis in oeIPSCs from GCs after optogenetic stimulation of SST\(_{GC}\) and PV\(_{GC}\) synapses. We observed that baseline PPR of oeIPSCs from PV\(_{GC}\) synapses was significantly smaller with respect to the PPR of oeIPSCs from SST\(_{GC}\) synapses \((\text{PPR, mean ± SEM, PV}_{GC} 0.34 ± 0.05, \text{SST}_{GC} 0.74 ± 0.09, \text{Mann-Whitney test, } U = 48.5, p < 0.0001, n = 22, 16)\). Transient application of FSK significantly reduced the PPR of oeIPSC from SST\(_{GC}\) synapses \((p = 0.03, \text{Fig. 3C})\), but not the PPR of oeIPSC from PV\(_{GC}\) synapses \((p = 0.29, \text{Fig. 3C})\).

These data suggest that altered presynaptic release probability is involved in PKA-dependent iLTP of GABAergic synapses from SST INs.

**Synaptic properties and PKA-dependent plasticity of DG SST INs distal synapses on medial septal neurons (SST\(_{MS}\)).**

In addition to local axons that contact with GCs, DG SST INs extend collaterals through the fimbria that form synapses with medial septum (MS) neurons (Jinno, 2009). We sought to compare synaptic properties and PKA-dependent plasticity of long-range (SST\(_{MS}\)) and local (SST\(_{GC}\)) synapses established by DG SST INs in MS neurons and GCs, respectively. We used bilateral injections of AAVs ChETA-EYFP in the DG of SST-Cre mice to anterogradely label and manipulate DG SST INs projections to the MS (Fig. 4A). Consistent with previous reports, fibers with abundant varicosities were observed in the MS of injected mice (Fig. 4B). We used acutely
prepared slices and patch-clamp recordings of MS neurons coupled to optogenetics to determine
the properties and study PKA-dependent plasticity of SST$_{MS}$ synapses, as we did previously with
SST$_{GC}$ synapses (Fig. 4C).

We first determined the connectivity and synaptic properties of SST$_{MS}$ synapses. The proportion of
recorded MS neurons that showed time-locked responses to SST$_{MS}$ synapse activation was 45 %
(39 of 86 MS neurons tested). In contrast, the response to SST$_{GC}$ synapse activation was observed
in all GCs tested (36 of 36 GCs tested, Fig. 4D, Connectivity, $p < 0.0001$). The rise slope of
oeIPSCs from SST$_{MS}$ synapses was significantly higher compared to SST$_{GC}$ synapses (Fig. 4D,
Rise Slope, $p = 0.04$). The response to optogenetic activation of SST$_{MS}$ synapses was completely
abolished by the GABA A receptor blocker gabazine ($p < 0.0001$, Fig. 4E), suggesting that, similar
to SST$_{GC}$ synapses, responses to SST$_{MS}$ synapses are mediated by the release of GABA. The
PPR of oeIPSCs of SST$_{MS}$ synapses was similar to SST$_{GC}$ synapses (Fig. 4F, $p = 0.97$),
suggesting no differences in the presynaptic probability of release between these two groups of
synapses.

We next tested the role of PKA in regulating plasticity of SST$_{MS}$ synapses. The amplitude of
oeIPSC of SST$_{MS}$ synapses was not significantly altered in any of the 9 MS neurons tested after
transient (10 min) application of FSK (Fig. 4G, H). At the population level, FSK failed to increase
oeIPSC amplitude of SST$_{MS}$ synapses in all MS neurons tested (Baseline 93 ± 5 %, FSK 84 ± 9 %,
$p = 0.37$, Fig. 4I). FSK did not alter the PPR of oeIPSCs of SST$_{MS}$ synapses (Baseline 0.79 ± 0.13
%, FSK 0.65 ± 0.11 %, $p = 0.29$, $n = 9$ MS neurons).

These results show connectivity and functional differences between SST$_{GC}$ and SST$_{MS}$ synapses
and suggest that PKA-dependent iLTP affects local (SST$_{GC}$) but not long-range (SST$_{MS}$) synaptic
contacts established by DG SST INs.

Kainic acid-induced increases in neuronal activity prevents the induction of iLTP in GCs.

In the hippocampus, neuronal activity induces multiple forms of GABAergic synapse plasticity
(Castillo et al., 2011; Mendez and Bacci, 2011). For this reason, we tested the role of neuronal
activity in regulating PKA-dependent iLTP of GC inhibitory synapses in vivo. We pharmacologically
increased synaptic activity by injecting mice with kainic acid (KA, 5 mg / kg, 2-3 injections, Fig. 5A).
KA lead to the induction of the activity-regulated transcription factor cFos in virtually all GCs 90
minutes after the injection, in contrast with the sparse presence of cFos in GCs from vehicle
treated mice (Fig. 5B). These results indicate a widespread increase in neuronal activity in GCs
after KA injection. We determined the effect of in vivo neuronal activity enhancement on FSK-induced iLTP and inhibitory presynaptic function. For this, we recorded eIPSCs in GCs from brain slices acutely prepared 90 minutes after vehicle or KA injections.

We first tested the induction of iLTP with FSK in GCs from vehicle or KA-injected mice. Transient application of FSK failed to induce iLTP in slices from KA treated mice, in contrast with slices from vehicle injected control animals (Fig. 5C, D). FSK induced iLTP in 6 of 8 GCs recorded from vehicle-injected mice while none of the 11 GCs recorded from KA injected animals showed apparent increases in eIPSC amplitude (Fig. 5E). While the amplitude of eIPSC in vehicle-injected animals increased after FSK treatment (Baseline 101 ± 2 %, FSK 153 ± 20 %, \( p = 0.004 \), Fig. 4F), the amplitude of eIPSCs from KA injected animals was not significantly increased by FSK application (Baseline 100 ± 1 %, FSK 79 ± 7 %, \( p = 0.18 \), Fig. 5F).

We then tested the PPR of pairs of eIPSCs delivered 50 ms apart in GCs from vehicle and KA injected mice (Fig. 5A). We first compared the PPR of the eIPSCs in baseline conditions and found that eIPSC in slices from KA injected animals had reduced PPR compared with vehicle treated animals (\( p = 0.02 \), Fig. 5G). The PPR of eIPSCs of KA treated animals did not change in response to FSK treatment (\( p > 0.99 \), Fig. 5G) while the PPR of eIPSCs in GC from vehicle treated mice decreased after FSK application (\( p = 0.02 \), Fig. 5G).

These results show that increasing synaptic activity with KA treatment in vivo prevents FSK-induced iLTP. In addition, the reduced baseline PPR of eIPSCs in GCs from KA injected mice suggests that in vivo increases in neuronal activity occlude the induction of iLTP in GCs.

**Discussion**

The functional role of inhibitory synapse plasticity needs to be understood from the point of view of the heterogeneity of inhibitory cell types that establish GABAergic synapses. Our results reveal a novel plasticity mechanism of GC GABAergic synapses that may be linked to the identity and projection characteristics of presynaptic INs. Activation of PKA through cAMP production leads to the potentiation of a fraction of GABAergic synapses of GCs, those originated in local SST INs, but leaves GABAergic synapses from PV INs unaffected. In contrast to local GC synapses, long-range synapses formed by SST INs collaterals in MS are not affected by PKA activation. PKA-dependent iLTP reduces the PPR of SST INs GABAergic synapses in GCs, but it did not affect PPR of PV INs synapses onto GCs or the PPR of distal SST IN synapses onto MS neurons. This suggests that a change in the presynaptic release is part of the mechanism of PKA-dependent GABAergic synapse plasticity in GCs. Our experiments with systemic KA injections suggest that increasing
hippocampal network activity in vivo occludes iLTP of GABAergic synapses on GCs. This is in line with a fundamental role of activity in triggering GABAergic synapse plasticity in vivo and point to a potential role of GABAergic plasticity in experience-dependent plasticity mechanisms.

Cyclic AMP regulates the probability of glutamate release of GC output synapses onto CA3 pyramidal cells (Monday et al., 2018). The control over GABAergic synapse plasticity that we describe here suggests that PKA may be a central mediator of the mechanisms that regulate excitatory and inhibitory synapse strength and may in this way contribute to adjust the E/I balance in hippocampal circuits. Potentiation was observed in outward IPSC recorded with low chloride solution, suggesting than this form of plasticity is independent of the reversal potential for GABA A receptor currents in mature GCs (Woodin et al., 2003) and argues against the requirement of postsynaptic GABAergic synaptic responses in this form of plasticity. In addition to the control of synaptic function, activation of PKA induces the formation of GABAergic terminal boutons in the hippocampus (Liang et al., 2021), suggesting additional plasticity mechanisms involving structural remodeling of GABA synapses.

Several subcortical neuromodulatory systems targeting the hippocampus may activate, alone or in combination, receptors with potential to regulate cAMP synthesis and thus, PKA activity, including dopamine, adrenergic and cholinergic receptors (Greengard, 2001). Our results suggest a new mechanism by which neuromodulatory signaling from subcortical structures may regulate plasticity of inhibitory synapses and hippocampal information processing. In addition, INs are exquisitely sensitive to different forms of neuronal activity that activate multiple signaling pathways resulting in changes in gene expression, synapse structure and efficacy (Spiegel et al., 2014).

Different subsets of GABAergic synapses on GCs are endowed with specific plasticity mechanisms that are linked to the identity of the presynaptic INs. PV and SST INs are major contributors to synaptic inhibition in the DG (Hainmueller and Bartos, 2020). Despite their common developmental origin in the medial ganglionic eminence (Lim et al., 2018), the output synapses of these two types of INs are differently affected by plasticity-inducing stimuli in the DG (this study) and CA1 (Udakis et al., 2020). Along with postsynaptic calcium (Udakis et al., 2020), our results show that presynaptic mechanisms that change the probability of release participate in altering synaptic efficacy of SST INs in GCs, similar to PKA-dependent plasticity of synapses in other brain regions (Bocklisch et al., 2013; Melis et al., 2002; Nugent et al., 2009). PKA is widely expressed in excitatory and inhibitory hippocampal neurons what makes unlikely that the specificity of PKA-dependent plasticity of synapses originated in SST INs arises from selective expression of the catalytic or regulatory PKA subunits. It is conceivable however, that differences in the expression and subcellular localization of PKA-anchoring proteins (AKAPs) may differ in PV and...
SST INs. Of particular interest is AKAP7, abundantly expressed in the hilus and molecular layer, coinciding with the location SST cell bodies and axonal arborizations, since this protein is essential for PKA-induced presynaptic potentiation of GC glutamatergic synapses (Jones et al., 2016). Selective expression or axonal targeting of AKAP7 in SST INs may explain the preferential expression of PKA-dependent iLTP in GABAergic synapses of GC originated in this IN population. In addition to synapses from SST INs, electrically-evoked IPSCs (eIPSCs) reflect the activity of synapses originated in several types of DG INs. Some of these synapses may be also FSK sensitive and increase amplitude with a faster time course compared with those originated in SST INs. This is compatible with the fact that eIPSC amplitude continues to increase 10-20 minutes after FSK washout, when eIPSCs from SST INs (SSTGC) have the steepest increase in amplitude.

The connectivity and functional difference between SSTGC and SSTMS synapses point to differences in DG SST INs regulation of local hippocampal and extrahippocampal activity. The projection to the MS has been proposed to arise from a subset of DG SST INs that are different from Hilar-perforant-path-associated (HIPP) SST INs that exclusively target hippocampal GCs (Yuan et al., 2017). Our results extend this dichotomy between locally and distally projecting DG SST INs by showing that connectivity, postsynaptic response kinetics and plasticity mechanisms are also different between SSTGC and SSTMS synapses. Sparse connectivity of SSTMS synapses is probably the reflection of innervation specificity among MS neuronal types (Yuan et al., 2017). The rapid kinetics and high probability of release of SSTMS synapses suggest the relevance of these synapses in coordinating the timing of hippocampal activity paced by the MS, in particular for theta oscillations (Jinno, 2009). Our results suggest that plasticity mechanism can be of help to understand the functional diversity of SST INs, which is likely present in other hippocampal (CA1, CA3) and cortical brain regions (Hostetler et al., 2023; Pelkey et al., 2017).

Several reports have shown that hippocampal GABAergic synapses undergo activity-dependent plasticity. Our results suggest that the potentiation of GC synapses originated in local SST INs can be induced by pharmacological increases of neuronal activity in vivo. This observation suggests that physiological (i.e behaviorally induced) activity may lead to changes in GC GABA synapses and refine the important role of hippocampal INs in different stages of experience-dependent information processing and updating (Topolnik and Tamboli, 2022) by specifically modifying connectivity with excitatory neurons. Although our experiments do not directly demonstrate the involvement of PKA in kainate-induced potentiation of GABAergic, the specificity of PKA, an important regulator of memory processes, in regulating the activity of dendrite targeting SST INs is in line with the important role of these subtype of INs in regulating DG excitability and memory allocation and recall (Cummings and Clem, 2020; Stefanelli et al., 2016). The control over synaptic inhibition exerted by PKA-dependent iLTP could allow a limitation to disinhibitory mechanisms through strengthening feed-back, feed-forward and lateral inhibition onto GCs. In addition, SST INs...
have a critical role in controlling cFos expression and regulating plasticity of excitatory synapses and excitability of GCs. The control of GC function by synaptic inhibition regulated by PKA could have important consequences to control the fraction of active GC and excitatory synapse function required to adjust the tight balance between pattern separation and completion in hippocampal circuits.

In summary, our results show a form of activity-dependent inhibitory synapse plasticity that is selectively expressed in local but not in long-range targets of hilar SST INs and regulates inhibitory microcircuits essential for hippocampal function.

References


**Figure Legends**

**Figure 1.** Protein kinase A activation induces long-term potentiation of inhibitory synapses (iLTP) on hippocampal GCs.

**A.** Upper panel: Schematic of experimental procedure. Lower panel: representative example of a biocytin (green) filled GC recorded during the experiments. The boxed region is magnified in the right part of the panel and shows a dendritic spine covered dendritic segment. GCL, granule cell layer; ML, molecular layer. Scale bars 20 µm and 5 µm.

**B.** Representative traces of electrically-evoked Inhibitory Post-Synaptic Currents (eIPSCs) before (Baseline, Bsl) and after forskolin (FSK, 10 µM) bath application in the absence or presence of protein kinase A (PKA) inhibitor H89 (+ H89, 10 µM). Scale bar: 50 ms, 50 pA. The time course of FSK effects on normalised eIPSC amplitude in the absence (FSK) or presence of PKA inhibitors H89 (+ H89, 10 µM) and PKI14-22 (+ PKI, 1 µM) is shown in the right graph.

**C.** Parts of whole graphs represent the proportion of recorded GCs showing significant increase of eIPSC amplitude after FSK application in the absence (FSK) or presence of PKA inhibitors H89 (+ H89, 10 µM) and PKI14-22 (+ PKI, 1 µM). Two-sided Fisher’s exact test, FSK vs FSK+H89 \( p = 0.002; \) FSK vs FSK+PKI \( p = 0.002; \) \( n = 24, 8 \) and 5 GCs in FSK, FSK + H89, FSK + PKI respectively.

**D.** Population data of FSK effects on normalized eIPSC amplitude before (Bsl) and 20 after FSK application in the presence and absence of PKA inhibitors H89 and PKI14-22. Two-way ANOVA, Treatment (FSK, FSK + H89, FSK + PKI) \( F(2, 34) = 13.86, p = 0.0001, \) Bonferroni’s multiple comparisons test, Bsl vs FSK \( p < 0.0001, \) Bsl vs FSK + H89 \( p = 0.27, \) Bsl vs FSK + PKI \( p > 0.99; \) \( n = 24, 8 \) and 5 GCs in FSK, FSK + H89, FSK + PKI respectively.

**E.** Representative traces of eIPSC before (Baseline, Bsl) and after forskolin (FSK, 10 µM) bath application recorded with a “low chloride” internal solution. The time course of FSK effects is shown in the right graph.

**F.** Proportion of GCs recorded with “low chloride” showing significant increase of eIPSC amplitude after FSK application.

**G.** Population data of FSK effects on normalized eIPSC amplitude before (Bsl) and after FSK application in the “low chloride” condition. Two-sided t-test, \( t(12) = 2.772, p = 0.02; \) \( n = 7 \) GCs. * \( p < 0.05; \) n.s. non-significant. Dots and vertical bars in B and E and horizontal and vertical bars in D and G represent mean ± SEM. Dots in D and G represent individual measurements.

**Figure 2.** PKA-induced iLTP is observed in GABA synapses originated in SST but not in PV INs.

**A.** Schematic of experimental procedure.

**B.** Representative traces of normalized optogenetically-evoked IPSC (oeIPSCs) recorded in GCs from PV-Cre and SST-Cre mice infected with AAV-DIO-ChETA-EYFP. The blue light (490 nm) pulse used to elicit oeIPSCs is represented with a vertical blue bar. Scale bar: 10 ms.

**C.** Representative images of Cre-dependent ChETA expression (cyan) and biocytin filled GC (green) in the DG of slices prepared from PV-Cre and SST-Cre mice. Scale bar 25 µm. GCL, granule cell layer; ML, molecular layer.

**D.** Representative traces of oeIPSCs before (baseline, Bsl) and after forskolin (FSK) bath application in GCs from PV-Cre (PVGC) and SST-Cre (SSTGC) mice. Scale bar: 50 ms; 30pA (SST-Cre) and 50 pA (PV-Cre). The time course of FSK effects on normalised oeIPSC amplitude in both conditions is shown in the right graph. Open symbols correspond to FSK application in the presence of PKA inhibitor H89 in experiments performed in SST-Cre mice (SSTGC+H89).

**E.** Parts of whole graphs represent the proportion of recorded GCs showing significant increase of oeIPSC amplitude after FSK application in PV-Cre and SST-Cre mice in the absence and presence of PKA inhibitor H89. Two-sided Fisher’s exact test, \( p = 0.002 \) (SSTGC vs PVGC) and \( p = 0.0007 \) (SSTGC vs SSTGC+H89); \( n = 12, 10 \) and 8 GCs in PV-Cre and SST-Cre and SST-Cre + H89, respectively.
F. Population data of FSK effects on normalised oeIPSC amplitude before (Bsl) and after FSK application in GCs from PV-Cre and SST-Cre mice. Two-way ANOVA, Treatment/genotype $F(2, 570) = 20.64, p < 0.0001$, Bonferroni's multiple comparisons test, PV-Cre Bsl vs FSK $p = 0.99$, SST-Cre Bsl vs FSK $p < 0.0001$, SST-Cre +H89 Bsl vs FSK $p = 0.71$; $n = 12, 10$ and $8$ GCs in PV-Cre and SST-Cre mice and SST-Cre + H89, respectively. * p < 0.05; n.s. non-significant. Dots and vertical bars in D and horizontal and vertical bars in F represent mean ± SEM. Dots in F represent individual measurements.

Figure 3. PKA-induced iLTP is accompanied by changes in PPR.

A. Schematic of the method used to calculate Paired-Pulse Ratio (PPR) for eIPSCs and oeIPSCs.

ISI, Inter Stimulus Interval.

B. Population data for eIPSCs PPR calculated before (Bsl) and after FSK application in the presence and absence of PKA inhibitor H89. Two-way ANOVA, FSK vs FSK + H89 $F(1, 24) = 2.57, p = 0.12$, Bonferroni’s multiple comparisons test, Bsl vs FSK $p = 0.003$, Bsl vs FSK + H89 $p = 0.99$; $n = 18, 8$ GCs in FSK and FSK + H89, respectively.

C. Population data for oeIPSCs PPR calculated before (Bsl) and after FSK application in PVGC and SST GC synapses. Two way ANOVA, PVGC vs SSTGC $F(1, 20) = 4.71, p = 0.04$, Bonferroni’s multiple comparisons test, PVGC Bsl vs FSK $p = 0.29$, SSTGC Bsl vs FSK $p = 0.03$; $n = 12, 10$ GCs recorded from PV-Cre and SST-Cre mice, respectively.

* p < 0.05; n.s. non-significant. Dots in B and C represent individual measurements.

Figure 4. Synaptic properties and PKA-dependent plasticity of DG SST INs distal synapses on medial septal neurons (SSTMS).

A. Schematic of experimental procedure.

B. Representative images of AAV-dependent ChETA expression in SST-Cre mice injected in the DG. The upper panel shows the injection site of AAV in the DG (Scale bar 200 µm). The lower panel shows low (left, scale bar 500 µm) and high (right, scale bar 200 µm) magnification of DG SST IN long-range projections in the medial septum (MS). Anatomical diagrams approximately corresponding to the images shown are adapted from Paxinos.

C. Representative image of medial septum slice showing a biocytin filled MS neuron (green). Long-range axonal projections from hilar ChETA expressing SST IN are shown in cyan. Scale bar 50 µm.

D. Connectivity and rise slope comparison between distal SSTMS and local SSTGC synapses. Connectivity: Two-sided Fisher’s exact test, $p < 0.001$; rise slope: two-sided Mann-Whitney test, U = 89.5, $p = 0.04$; $n = 36, 9$ for SSTGC and SSTMS synapses.

E. The GABA A receptor blocker Gabazine (GBZ) blocks oeIPSCs responses in MS neurons to SSTMS synapses optogenetic stimulation. Two-sided Mann-Whitney test, U = 0, $p < 0.0001$. Scale bar: 100 pA; 50 ms.

F. The PPR of SSTMS and SSTGC oeIPSCs did not differ. Two-sided Mann-Whitney test, $U = 71$, $p = 0.97$; $n = 16, 9$ for SSTGC and SSTMS synapses.

G. Representative traces of SSTMS oeIPSCs before (baseline, Bsl) and after forskolin (FSK) bath application. Scale bar: 50 pA; 50 ms. The time course of FSK effect on normalised SSTMS oeIPSCs amplitude is shown in the right graph.

H. Proportion of recorded MS neurons showing significant increase of eIPSC amplitude after FSK application. I. Population data of FSK effects on normalised SSTMS oeIPSC amplitude before (Bsl) and after FSK application. Two-sided t-test, $t(16) = 0.93$, $p = 0.37$; $n = 9$ MS neurons.

* p < 0.05; n.s. non-significant. Dots in D, E, F and I represent individual measurements. Horizontal and vertical bars in D and I and circles and vertical bars in G represent mean ± SEM.
Figure 5. Kainic acid-induced increases in synaptic activity prevents the induction of iLTP in GCs.

A. Schematic of experimental procedure.

B. Representative example of cFos (green) expression in the dentate gyrus (DG) of vehicle and kainic acid (KA) injected mice. Boxed region in upper panels are showed in lower panels. Scale bars 500 µm and 50 µm, upper and lower panels, respectively.

C. Representative traces of eIPSCs before (Baseline, Bsl) and after Forskolin (FSK) bath application recorded from GCs in vehicle and KA treated mice. Scale bar: 25 ms, 50 pA.

D. Time course of FSK effects on normalised eIPSC amplitude in GCs from vehicle and KA treated mice. n = 8, 11 GCs from vehicle and KA injected mice, respectively.

E. Proportion of recorded GCs showing significant increase of eIPSC amplitude after FSK application in the vehicle and KA treated mouse. Two-sided Fisher’s exact test, p = 0.001; n = 8, 11 GCs from vehicle and KA injected mice, respectively.

F. Population data of FSK effects on normalised eIPSC amplitude before (Bsl) and after FSK application in GCs from vehicle and KA treated mice. Two-way ANOVA, Veh vs KA F (1, 17) = 15.85 p = 0.001, Bonferroni’s multiple comparisons test, Veh Bsl vs FSK p = 0.004, KA Bsl vs FSK p = 0.19; n = 8, 11 GCs from vehicle and KA injected mice, respectively.

G. Population data for eIPSCs PPR calculated before (Bsl) and after FSK application in GCs from vehicle and KA injected mice. Two-way ANOVA, Veh vs KA F (1, 17) = 2.43 p = 0.14, Bonferroni’s multiple comparisons test, Veh Bsl vs FSK p = 0.02, KA Bsl vs FSK p = 0.99; Bsl Veh vs KA p = 0.02; n = 8, 11 GCs from vehicle and KA injected mice, respectively.

* p < 0.05; n.s. non-significant. Dots and vertical bars in D and horizontal and vertical bars in F represent mean ± SEM. Dots in F and G represent individual measurements.
**Panel A**

PPR = P2/P1

**Panel B**

- Bsl
- FSK

- Bsl FSK + H89

**Panel C**

- Bsl
- FSK

- PVGC
- SSTGC

- n.s.
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