GsAHP ACTIVATION DURING STDP DETERMINES THE DIRECTION OF PLASTICITY IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS OF ADULT RATS

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Símbolos y unidades de medida

Conductance	Siemens	**nS**
Resistance	ohm	**MΩ**
Voltage	volts	**mV**
Current	ampere	**mA, pA**
Longitude	meters	**µm**
Temperature	Celsius	**°C**
Time	seconds	**ms**
Acidity	pH	
Volume	liters	**mL**
Concentration	moles/L	**mM, µM**
Osmolarity	osmoles	**mOsm**
Frequency	Hertz	**kHz, Hz**
Period	seconds	**s**

Abreviaturas estándar y convenciones

sAHP	slow after-hyperpolarization
GsAHP	slow after-hyperpolarization increment in membrane conductance
CA1	Cornu Ammonis 1, hippocampal region
NA	Noradrenergic
ISOP	Isoproterenol
APs	Action potentials
bAP	back-propagated action potential
RMP	resting membrane potential-dependent
Rm	membrane resistance
GPCR	G protein-coupled receptor
G_s	G protein S
cAMP	3',5'-cyclic adenosine monophosphate
PKA	Protein kinase A
eEPSP	evoked excitatory post-synaptic potential
eEPSC	evoked excitatory post-synaptic current
NMDAR	N-methyl-D-aspartate receptor

AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
VGCC	voltage-gated calcium channel
mGluR	metabotropic glutamate receptor
p30	postnatal day 30
CC	current-clamp
VC	voltage-clamp
T4sec	4 second period
fT4sec	forced 4 second period
T1sec	1 second period
fT1sec	forced 1 second period
V_m	membrane voltage
Vi	passive voltage amplitude
Ip	current pulse
SS_i	steady state voltage
R_i	internal resistance
R_a	access resistance
PPi	paired pulse index
DSI	depolarization induced suppression of inhibition
Pr	release probability
LTP	long term potentiation
LTD	long term depression
HFS	high frequency stimulation
LFS	low frequency stimulation
TBS	theta burst stimulation
ITDP	input timing dependent plasticity
STDP	spike timing dependent plasticity
CV	coefficient of variation

ABSTRACT

The slow after-hyperpolarization (sAHP) is a Ca^{2+}-dependent K^{+} current, which is implicated in the control of neuronal excitability as well as in the regulation of dendritic integration. In young rats (<p30), sAHP impairs LTP when is activated during plasticity induced by spike timing dependent plasticity (STDP) protocol and it is upregulated during development. However, the effect of the sAHP in synaptic plasticity of adult animals still has not been explored. Using whole-cell patch-clamp configuration in hippocampal CA1 pyramidal neurons of adult rats, we regulated the conductance of sAHP during causal STDP protocols to assess its effect on plasticity. To activate the sAHP we varied the spike frequency of STDP protocols and to attenuate sAHP we bath applied the noradrenergic (NA) agonist isoproterenol (ISOP). Our results demonstrate that the sAHP increment in membrane conductance (GsAHP) alone and not the spiking frequency determines the direction of plasticity using a single-AP pairing STDP protocol. Regenerative activation of GsAHP during a pre-post STDP protocol resulted in 3 outcomes: LTP, no change in EPSC efficacy (NON) and LTD. ISOP GsAHP-attenuation resulted exclusively in LTP. The corresponding GsAHP levels during STDP were: 2.39±0.53 nS for LTP, 4.40±0.66 nS for NON, 4.44±0.68 nS for LTD and 2.27±0.78 nS for ISOP. These results demonstrate the regulatory effect of GsAHP over the direction of plasticity and how the NA GsAHP regulation might subordinate hippocampal plasticity to attentional and emotional states.

INTRODUCTION

The GsAHP is one of a family of Ca^{2+}-dependent K^+ conductances activated by action potentials (APs) (Andrade et al., 2012; Faber and Sah, 2003; Storm, 1990) and it is observed as negative deflection of the resting membrane potential (RMP), due to a reduction of the membrane resistance (**Rm, Fig.1 and Fig.7C**) (Fernández de Sevilla et al., 2007; Gulledge et al., 2013; Hotson and Prince, 1980; Marrion and Tavalin, 1998; Pulver and Griffith, 2010; Velumian and Carlen, 1999). Despite being discovered more than four decades ago, the channels that generate the GsAHP are still unknown (Andrade et al., 2012; Gulledge et al., 2013; Kim et al., 2012; Krnjević et al., 1975; Wang et al., 2016). However, it was assumed that it could be a SK channel variant, but no evidence supported that hypothesis for several different reasons like pharmacological properties and unitary conductance of the putative underlying channels (Larsson, 2013; Lima and Marrion, 2007; Marrion and Tavalin, 1998; Sah and Faber, 2002; Velumian and Carlen, 1999).

sAHP pharmacology

Several monoaminergic neurotransmitters regulate the GsAHP, sharing a common biochemical pathway through GPCR (Pedarzani and Storm, 1993; Preininger and Hamm, 2004; Zhang et al., 2013). The GsAHP reduction begins with monoaminergic binding to GPCRs, G_s protein activation and an increase of cAMP synthesis by Adenylyl cyclase enzyme. cAMP activates PKA that reduces GsAHP by phosphorylation. However, the final effector of this

phosphorylation is still unknown (Ireland et al., 2004; Nouranifar et al., 1998; Oh et al., 2009; Storm, 1990). Recently, the sAHP calcium sensor has been identified, named *Hippocalcin*, which binds to the cell membrane to activate the sAHP, but its interaction has not been elucidated (Andrade et al., 2012; Kim et al., 2012; Tzingounis et al., 2007).

sAHP activation increases membrane conductance (GsAHP), therefore lowering neuronal excitability, concomitantly with membrane hyperpolarization of the RMP, in this way augmenting the depolarization needed to reach the AP threshold. sAHP regulates dendritic integration, through the increment of membrane conductance that reduces the amplitude and temporal course of EPSPs in what is called *shunting* (Fernández de Sevilla et al., 2007; Hausser, 2001; Staley and Mody, 1992), that consequently restrict the conditions necessary to generate synaptic plasticity (Fernández de Sevilla et al., 2007; Fuenzalida et al., 2007). Also, the sAHP can control the rhythm of a successive burst of APs, being the disruption of this mechanism a candidate to hippocampal epileptogenesis (Fernández de Sevilla et al., 2006; Kaczorowski, 2011; Kaczorowski et al., 2007).

Synaptic plasticity

Glutamatergic synaptic plasticity is given by a modification in the efficacy of the EPSP/EPSC. The efficacy corresponds to the average amplitude of the summed postsynaptic responses (del Castillo and Katz, 1954a; Jonas et al., 1993; Stevens, 1993). Changes in synaptic efficacy can be by modifications of the Pr, the number of release sites (n), the number of postsynaptic AMPA receptors (q) or the unitary conductance of AMPA receptors (γ) (Andrásfalvy and Magee, 2004; Arendt et al., 2015; Banerjee et al., 2014; Benke et al., 1998; Chater and Goda, 2014; Feldman, 2000; Henley and Wilkinson, 2016; Isaac et al., 1996; Poncer et al., 2002). The eEPSC amplitudes are drawn from a binomial or sum of binomial distributions (Faber and Korn, 1991; Korn et al., 1981).

There are several models of synaptic plasticity induction, where plastic changes in efficacy last from milliseconds to minutes (short term plasticity, STP) and minutes to hours (long term plasticity). These forms of plasticity can result in an increased or decreased efficacy (potentiation or depression respectively).

Two widely known forms of short term plasticity are PPF/PPD (del Castillo and Katz, 1954b; Zucker and Regehr, 2002) and DSI (Chevaleyre and Castillo, 2004; Vincent et al., 1992), but we will focus on the former: PPF (paired pulse facilitation) and PPD (paired pulse depression). These are the result of a transient accumulation of $[Ca^{2+}]_i$ in the presynaptic terminal that alters the Pr (Low Pr $R_2>R_1$, PPF; high Pr $R_2<=R_1$, PPD) (Blitz et al., 2004; Catterall et al., 2013; de Jong and Fioravante, 2014; Dobrunz et al., 1997; Zucker and Regehr, 2002).

Long term models of plasticity induction depend in the stimulation frequency and the timing of activation between the presynaptic (pre) and postsynaptic (post) compartments. Some protocols are independent of the activation timing between pre and postsynaptic compartments: HFS, LFS, and TBS (Albensi et al., 2007). Sometimes these protocols are not physiologically relevant, because they require conditions of intensive axonal stimulation (HFS and TBS) (Andrásfalvy and Magee, 2004; Benke et al., 1998), or in some cases sustained postsynaptic depolarization (LFS) (Isaac et al., 1995, 1996), that are not present in physiological conditions. On the other hand, there are protocols that depend in the timing between the pre and postsynaptic compartments: ITDP (Basu et al., 2016; Leroy et al., 2017) and STDP (Feldman, 2012; Fuenzalida et al., 2007, 2010; Markram, 1997), we will focus on STDP that has also been demonstrated in-vivo (Cassenaer and Laurent, 2007; Jacob et al., 2007; Richards, 2010).

STDP paradigm

STDP consists in the coincidence between an EPSP and an AP (pairing) during a brief time window (window of coincidence). Normally in hippocampal and cortical glutamatergic

synapses, the pairing order results in the following outcomes: pre-post results in LTP (causal) and post-pre in LTD (D'amour and Froemke, 2015; Feldman, 2012; Froemke and Dan, 2002; Fuenzalida et al., 2010; Larsen, 2010; Markram, 1997). Only correlated responses with windows of coincidence between -50 ms (post-pre LTD) to +50 ms (pre-post LTP) result in significant changes of efficacy, being the maximum change in efficacy near 0 ms and minimum as the window widens (Bi and Poo, 1998; D'amour and Froemke, 2015; Edelmann et al., 2015), however, this rules are brain region dependent (Feldman, 2012). In pre-post STDP, normally we have the EPSP (pre) and then the AP (post) with a 10 ms window of coincidence, which results in LTP in young rats (<p30). The back-propagated AP (bAP) goes into the dendrite to produce non-linear depolarization in the dendritic spines (Balbi et al., 2015; Buzsáki et al., 1996; Stuart et al., 1997a, 1997b; Stuart and Spruston, 2015), removing the NMDAR Mg^{2+}-blocking. NMDAR acts as a coincidence detector of presynaptic glutamate release and postsynaptic depolarization. Ca^{2+} entrance through NMDAR initiates the synaptic potentiation process by activation of kinases like CAMKII (Feldman, 2012; Fuenzalida et al., 2010; Hoerndli et al., 2015; Lisman and Spruston, 2005; Magee and Johnston, 1997; Nevian and Sakmann, 2006; Poncer et al., 2002). Also, in post-pre STDP the activation of voltage-dependent Ca^{2+} channels followed by mGluR resulted in PLC dependent release of eCB acting as retrograde messenger generating LTD by lowering the Pr (Feldman, 2012; Nevian and Sakmann, 2006). However, NMDAR-dependent LTD has been reported in several works (Banerjee et al., 2014; Christie et al., 1996), as well as NMDA-independent LTD (Froemke et al., 2005; Nishiyama et al., 2000), demonstrating the heterogeneity of LTD mechanisms compared to LTP. Whether is LTP or LTD, postsynaptic $[Ca^{2+}]_i$ transients are present for both outcomes, therefore is the source and the kinetics the relevant determinants for the direction of plasticity; strong and rapid NMDAR activation results in LTP, low and slow activation of NMDAR or VGCC results in LTD (Aihara et al., 2007; Magee and Johnston, 1997; Nevian and Sakmann, 2006). STDP is heterogeneous

throughout the neuronal dendrite, where LTP is stronger near the soma and basal dendrites and LTD has a wider window of coincidence at distal portions of the dendrite (Froemke et al., 2005; Magee and Johnston, 1997; Yuste, 2013). Also, STDP influences dendritic integration where pre-post LTP facilitates dendritic integration and post-pre LTD depresses dendritic integration (Branco and Häusser, 2011; Campanac and Debanne, 2008).

GsAHP regulation of synaptic plasticity

To assess the effect of the GsAHP over synaptic plasticity, causal STDP single-AP protocols can be used (Bi and Poo, 1998; Feldman, 2012; Fuenzalida et al., 2007; Magee and Johnston, 1997; Markram, 1997). In young rats (<p30), postsynaptic regenerative GsAHP activation (sustained activation) during a pre-post STDP protocol impairs plasticity due to a change in the temporal course of the EPSPs, rendering necessary a reduction in the temporal window of coincidence (Fernández de Sevilla et al., 2007; Fuenzalida et al., 2007). However, a reduction of the pairing frequency could also be a suitable candidate to reduce the GsAHP regenerative activation during the STDP protocols and in this way avoiding the shunting. The work of Zaitsev and Anwyl, 2011 shows that causal STDP in layer 2/3 of prefrontal cortex in young rats (<p20) produces a presynaptically expressed LTD that is dependent in presynaptic NMDAR and that inhibition of sAHP restores the classical STDP producing postsynaptically expressed LTP. However, the site of sAHP regulation (whether pre or postsynaptic) was not clear. In the work of Wittenberg and Wang, 2006 a methanesulfonate based intracellular solution was employed that preserves the sAHP. They reported that single-AP causal STDP resulted in LTD in young rats (<p20), for pairing frequencies between 0.1 to 5.0 Hz and windows of coincidence between 5 to 20 ms, however, no GsAHP estimation was made.

sAHP increases through development and it is reduced after learning (Cohen-Matsliah et al., 2010; Matthews et al., 2009; Oh et al., 2009; Power et al., 2002; Saar et al., 1998). The role of

the sAHP over synaptic plasticity in adult rats has not been equally explored. Also, the single-AP causal STDP protocol was supposedly impossible to apply or incapable in generating plasticity at low frequency pairings, even in the absence of the sAHP in adult rats (Meredith et al., 2003; Pike et al., 1999).

Experimental problem

Thus, we asked if was it possible to generate synaptic plasticity with single-AP pre-post STDP protocols that regeneratively activate the sAHP and what is the effect of this sAHP increment in membrane conductance (GsAHP) over synaptic plasticity in hippocampal CA1 pyramidal neurons of adult rats. Also, we asked whether the GsAHP in adult rats could generate new patterns of plasticity given its role in learning and memory and if their attenuation could rescue the impairment in plasticity observed in previous studies (Fuenzalida et al., 2007; Wittenberg and Wang, 2006; Zaitsev and Anwyl, 2011).

HYPOTHESIS

Our main hypothesis is that **the sAHP increment in membrane conductance controls the direction of plasticity in hippocampal glutamatergic synapses of pyramidal neurons of adult rats.**

AIM

Our general aim is to determine **if the sAHP increment in membrane conductance controls the direction of glutamatergic synaptic plasticity in pyramidal neurons from adult rats.**

Objectives

1. We will determine if is it possible to generate plasticity with a single-AP causal STDP.

2. We will determine the effect of the regenerative sAHP increment in membrane conductance during STDP over synaptic plasticity.
3. We will determine whether the sAHP conductance or the paring frequency are the primary regulators of synaptic plasticity.

METHODS

The procedures of animal care; surgery and recording were in accordance with the guidelines laid down by the Institutional Animal Care and Ethics Committee at the Faculty of Sciences, Universidad de Valparaíso (DIUV Law 20380, Chile) and NIH (USA).

Animals

We employed 69 male Sprague-Dawley rats of 57.0±0.9 post natal days (p57), weighing on average 350 grams. We used rats over p45 because the begin the reproductive phase at this age, therefore they could be considered young adults and also because sAHP is higher in adults. Females were not employed because estradiol reduces the sAHP (Kumar and Foster, 2002), in this way interfering with the experiments. All rats were fed *ad-libitum* and housed in pairs at 22°C with a 12/12 dark-light cycle. For the craniotomy, all rats were sedated with isoflurane (100% by air intake).

Slices

We made coronal slices of 400 μm thick, to preserve the hippocampal tri-synaptic circuit. The extracted brains were submerged in cold ACSF (6-10°C) bubbling with carbogene (95% O_2, 5% CO_2), with the following composition of Cutting-ACSF (mM) to increase the viability of the slices due the age of the animals: 248 Sucrose, 2.69 KCl, 1.25 KH_2PO_4, 10.0 $MgSO_4$, 26.0 $NaHCO_3$, 0.05 $CaCl_2$, 10.0 glucose at pH 7.4, 310mOsm. The slicing was conducted in a

Vibroslice microtome, every slice was submerged a few seconds in warm recording-ACSF at 27°C to remove the cutting-ACSF and posteriorly incubated at 22°C before recordings for at least 1 hour in recording-ACSF. The recording was made with a gasified perfusion system (2mL/min).

Electrophysiological recordings

The recordings were made in whole-cell configuration current and voltage-clamp mode, in the CA1 region of the hippocampus with the blind patch technique. Picrotoxin (PTX 10 μM) was bath applied to block $GABA_A$ receptors (inhibitory transmission). The recording-ACSF (mM) was: 124.0 NaCl, 2.7 KCl, 1.25 KH_2PO_4, 1.3 Mg_2SO_4, 26.0 $NaHCO_3$, 2.5 $CaCl_2$ and 10.0 glucose, pH 7.4, 310mOsm. The pipettes had a resistance between 3-5 MΩ used for AHP/I-AHP recordings the Zhang solution (Kaczorowski et al., 2007; Zhang et al., 1994) in mM: 130 $KMeSO_4$, 10 HEPES, 4 ATP-Na_2 and 20 KCl, adjusted to pH 7.3, 290mOsm. Once the seal was opened the RMP was measured in CC, to use CC-COM posteriorly. Neurons had a Ra of 22.6MΩ; neurons with an Ra variation >30% were discarded. Also, neurons with >5mV of RMP variation were discarded. For the sAHP attenuation experiments, isoproterenol 50 μM (ISOP) was bath applied 10 minutes before the STDP protocol.

A DAGAN PC-ONE amplifier was employed. For VC recordings were used a 20kHz sample rate with a low-pass 3 pole Bessel filter of 3kHz and for CC a 10kHz sample rate.

STDP protocols

The STDP consisted on pairings an EPSPs and a single bAPs (pre-post, **Fig.2A**), stimulating in stratum radiatum the Schaffer collaterall axons coming from CA3 to evoke the EPSPs with Theta (θ) borosilicate electrodes and injecting depolarizing current in the soma of CA1 pyramidal neurons to evoke a bAPs. These pairings were repeated 60 times (for all STDP

variants). To dissect the sAHP contribution of the pairing frequencies we employed 4 STDP protocols: two at 0.25Hz (T4sec and fT4sec) and two at 1.0Hz (T1sec and fT1sec). The protocols fT4sec and fT1sec (forced sAHP protocols) had additional unpaired bursts of APs (evoked by a 550pA/250ms square current pulse every 4 seconds) to generate additional sAHP increment in membrane conductance besides that generated by STDP pairings (**Fig.6A**). In this way, we had the same number of pairings (60), but different levels of regenerative sAHP increments in membrane conductance (**Fig.6B**). Considering previous studies (in which 1.0Hz pairings were applied), a causal STDP protocol with lower frequency (0.25 Hz) will activate less sAHP, reducing the likelihood of EPSP shunting by regenerative sAHP activation during STDP, than one with higher frequency (1.0 Hz).

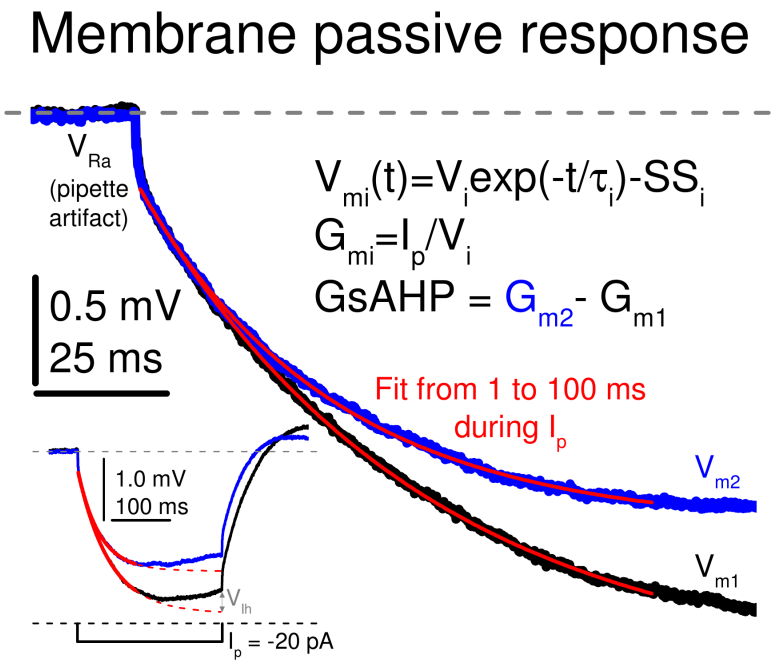

Figure 1. GsAHP estimation. Representative membrane responses to a hyperpolarizing current pulse (I_p). Red traces represent the fitting of an exponential model to the membrane response (V_{mi}). Black trace represents the basal response and blue trace the response during sAHP activation.

sAHP conductance (GsAHP)

To calculate sAHP conductance, a -20pA/250ms square current pulse (I_p) was employed (**Fig.1**). This voltage response was adjusted to an exponential curve between the initial 1-100 ms, to avoid I_h (V_{Ih}) dependent conductances, that activates after 100ms.

As depicted in **Fig.1**:"V_m" is the membrane voltage generated by I_p, "V_i" is the amplitude of the passive hyperpolarization, "τ" is the temporal constant and "SS_i" is the total amplitude of the passive hyperpolarization, including the uncompensated access resistance amplitude artifact (V_{Ra}). To calculate GsAHP (sAHP increment in membrane conductance in nS), we subtracted the G_{m1} (basal membrane conductance) from the G_{m2} (membrane conductance during sAHP activation). Each G_{mi} (nS) is calculated dividing I_p by V_i (mV).

PPi and coefficient of variation

PPi is calculated as the ratio of efficacy between successive responses ($PPi = R_2/R_1$, 80ms apart, **Fig.2E**), to determine variations in the Pr. Also, we employed the coefficient of variation (CV) method of eEPSC efficacy to asses the locus of plasticity (**Fig.4C**), whether pre or postsynaptic location (Bekkers and Stevens, 1990; Faber and Korn, 1991). The method consists in the assessment of CV before and after the plasticity protocol, where the ratio $r=CV^2_b/CV^2_a$ ($CV = \sigma/EPSC = [(1-p)/np]^{1/2}$; $EPSC = npq$; $\pi = EPSC_a/EPSC_b$) is 1 if only postsynaptic modifications are presents and $r\neq1$ if presynaptic changes occur (after="a", before="b", "standard deviation"=σ, "mean peak"=EPSC, "number of release sites"="n", "probability of release"="p", "quantal content"="q" and "modification factor"=π).

Data analysis

The analyses were made off-line in with Clampfit 10.4 and Origin 9. All parameters were

adjusted to normal distributions or linear regressions (Pearson's "r"). Their differences were assessed by parametric T-tests (single value, paired or two samples) or one-way ANOVA, with Bonferroni correction. The significance levels ("p") were represented by: * (0.05), ** (0.01) and *** (0.001). All values are presented as value±SEM. All eEPSC efficacy time courses were subjected to pair-sample T-test between baseline at 0-10 min and 20-30 min post-STDP to determine synaptic plasticity.

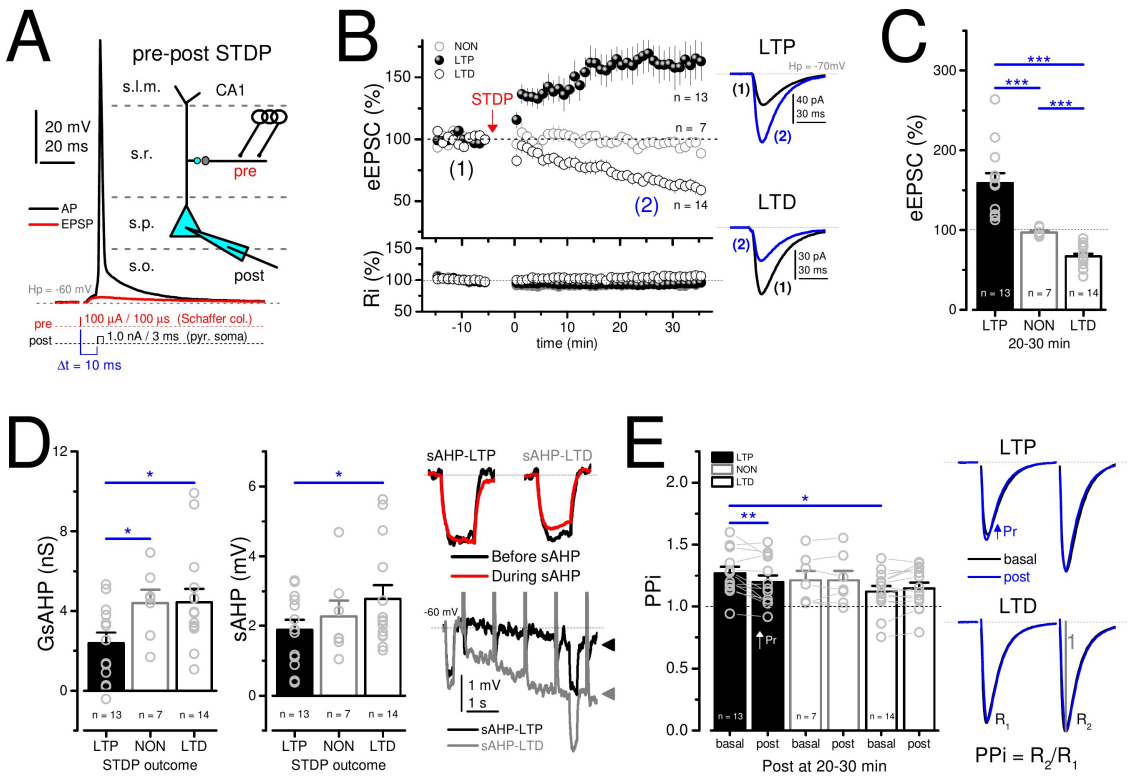

Figure 2. Regenerative GsAHP activation and plasticity outcome. A) Scheme of pre-post STDP applied on CA1 hippocampal glutamatergic synapses. **B)** Up, temporal course of eEPSC efficacy after STDP (All protocols included). Bottom, input resistance (R_i) temporal course. Right, representative eEPSCs for both LTP and LTD, black trace basal (1) and blue trace 20-30 min post STDP (2). **C)** Bars, mean eEPSC amplitude after STDP. **D)** Bars, Regenerative GsAHP and sAHP hyperpolarization during STDP, grouped by STDP outcome. Right-up, voltage responses to -20pA used to calculate GsAHP, before and during sAHP activation. Right-bottom, representative STDP sweeps fragments showing sAHP regenerative activation. Filled triangles indicate sAHP hyperpolarization level. **E)** Bars, PPi baseline and at 20-30 min after STDP,. Right, normalized (R2) representative recordings of paired pulse eEPSCs for LTP and LTD, with

paired pulse interval of 80 ms. Black traces are basal recordings and blue represent 20-30 min post STDP. (For all data Two-sample T-test was employed, except for "**E"** were paired-sample T-test was also used to compare basal and post PPi; "p" *0.05, **0.01 and ***0.001. % is percentage of baseline; 31 animals were employed)**.**

RESULTS

The sAHP conductance (GsAHP) regulates the direction of plasticity

It has been shown that the sAHP regulates the eEPSC plasticity, whether restricting the conditions necessary for their occurrence (Fuenzalida et al., 2007) or modifying its direction (Zaitsev and Anwyl, 2011). To determine if the sAHP activation level controlled the direction of synaptic plasticity, we then employed pre-post STDP protocols (T4sec, fT4sec, T1sec and fT1sec) in CA1 hippocampal glutamatergic synapses of pyramidal neurons, with variable sAHP activation (**Fig.2A and Fig.6A**) (Fernández de Sevilla et al., 2007; Fuenzalida et al., 2007).

We were able to generate plasticity with a single-AP STDP protocol in these conditions (**Fig.2B**). These pre-post STDP protocols generated three outcomes in eEPSC efficacy: LTP (159.1±12.3%), failure in plasticity (NON 97.1±2.0%) and LTD (66.7±3.8%) (**Fig.2B-C**, all STDP protocols included). The NON outcome group was expected because it was reported that the sAHP impairs LTP (NON outcome) by shunting of the eEPSP (**Fig.5D**) in the dendrite of rats below 30 postnatal days (Fuenzalida et al., 2007), however, the LTD outcome was unexpected. Due to the shunting effect we determined the GsAHP levels for all outcomes, being significantly greater for LTD and NON outcomes compared to LTP (**Fig.2D left**; LTP 2.39±0.53 nS; NON 4.40±0.66 nS; LTD 4.44±0.68 nS; Two-sample T-test: *$p<0.05$). On the other hand, the sAHP hyperpolarization was only significantly different between LTP and LTD, but neither LTP nor LTD were significantly different from NON (**Fig.2D right**, LTP 1.89±0.29 mV; NON 2.27±0.47 mV; LTD 2.77±0.40 mV; Two-sample T-test: *$p<0.05$). These data confirm that high GsAHP

activation (NON 4.40±0.66 nS) blocks the induction of LTP and also supports the observation that high GsAHP activation (LTD 4.44±0.68 nS) can also generate LTD in adult rats, in this way reverting the direction of plasticity in pre-post STDP protocols.

PPi levels even up after STDP

To asses if presynaptic modifications could be responsible for the result of the STDP protocols, we calculated the PPi (which is an indicator of Pr) for every experiment (**Fig.2E**). Basal PPi was significantly greater in LTP compared to LTD, but neither LTP nor LTD were significantly different from NON (**Fig.2E basal**, LTP 1.27±0.05; NON 1.21±0.08; LTD 1.12±0.04; Two-sample T-test: *p<0.05). PPi from LTP was significantly reduced after STDP, indicating a Pr increment for LTP (**Basal** 1.27±0.05, **Post** 1.20±0.05; Pair-sample T-test: **p<0.01). The PPi LTP modification accounted for 6% of relative eEPSC efficacy increment in R1 (with respect to R2). PPi presented no change for LTD and NON, suggesting that they had a postsynaptic expression mechanism. All PPi levels equalize after the STDP and were not significantly different between them (**Fig.2E post**, LTP 1.20±0.05; NON 1.21±0.08; LTD 1.15±0.05; Two-sample T-test: *p<0.05).

Together these results suggest that STDP at low Pr terminals (PPi=1.27±0.05) with low postsynaptic GsAHP (2.39±0.53 nS) results in LTP and fails to induce plasticity with high GsAHP (NON 4.40±0.66 nS). However, high Pr (PPi=1.12±0.04) with high GsAHP (4.44±0.68 nS) results in LTD. These results support the role of the postsynaptic sAHP as regulator of the direction of synaptic plasticity.

GsAHP reduction allows LTP only

Previous results suggests that the regenerative activation of GsAHP during STDP is responsible for blocking synaptic plasticity (NON) or producing LTD instead. Therefore, we expect that a

reduction of GsAHP results in LTP only. Consequently, we bath applied the sAHP blocker ISOP 50uM (NA agonist) to attenuate the sAHP (**Fig.3A;** basal 2.25±0.25 mV, ISOP 1.55±0.21 mV; Pair-sample T-test: **p<0.01) (Abel et al., 2004; Andrade et al., 2012; Pedarzani and Storm, 1993; Wu, 2004). We employed fT4sec and T1sec protocols only because they presented all 3 outcomes in similar proportions (LTP:NON:LTD proportions; **Fig.7**). Also, these protocols present the same GsAHP level and have intermediate sAHP activation between T4sec and fT1sec protocols (**Fig.6B;** T4sec 0.96±0.33 nS, fT4sec 3.35±0.42 nS, T1sec 3.60±0.24 nS, fT1sec 5.19±0.76 nS; Two-sample T-test: *p<0.05, **p<0.01 and ***p<0.001). T4sec was not employed because already activates little sAHP and results only in LTP. Also, ISOP significantly reduces the sAHP activation for fT4sec and T1sec protocols (**Fig.6C;** CN 3.53±0.20 nS, ISOP 2.14±0.44 nS; Two-sample T-test: **p<0.01 and ***p<0.001).

STDP with ISOP resulted in LTP only (**Fig.3B and C,** EPSC 135.0±6.2%) and presented no significantly different GsAHP nor sAHP hyperpolarization (**Fig.3D**, LTP 2.39±0.53 nS and 1.89±0.29 mV; ISOP 2.27±0.78 nS and 1.35±0.35 mV; Two-sample T-test). We assessed the differences in PPi indexes (Pr) due to ISOP, but basal PPi was not significantly different from LTP (**Fig3E**, LTP 1.27±0.05; ISOP 1.30±0.05; Two-sample T-test). PPi from ISOP was significantly reduced after STDP, indicating a Pr increment (**Fig.3E**, ISOP basal 1.30±0.05 and post 1.25±0.05; Paired-sample T-test: *p<0.05 and **p<0.01). All PPi even up post STDP, reduced PPi (augmented Pr) for LTP and ISOP (Post: LTP 1.20±0.05; ISOP 1.25±0.05; Two-sample T-test). ISOP presented a pattern of GsAHP, sAHP and PPi values similar to LTP, except for EPSC% (LTP 159.1±12.3% and ISOP 135.0±6.2%), indicating that a reduced GsAHP is unable to block LTP nor revert synaptic plasticity to LTD.

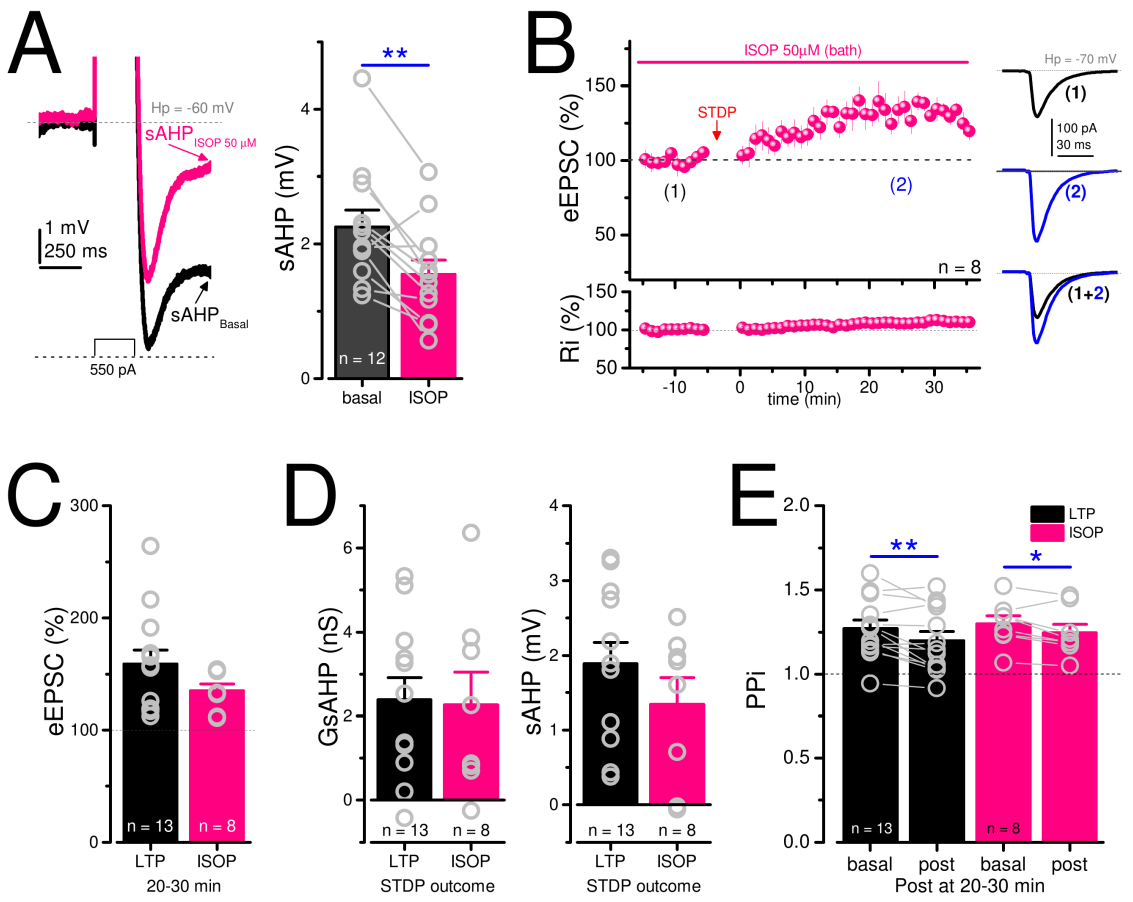

Figure 3. Effect of GsAHP reduction by ISOP. A) Representative current-clamp recording of sAHP showing the effect of ISOP application. Bars, sAHP amplitude 500 ms after the end of a depolarizing pulse. (Pair-sample T-test) **B)** Temporal course of eEPSC efficacy after STDP protocols in the presence of ISOP, as % of baseline. Bottom, input resistance (Ri) temporal course. Right, representative eEPSCs. **C)** Mean eEPSC amplitude as % of baseline. **D)** Regenerative sAHP conductance (GsAHP) and sAHP amplitude during STDP. **E)** Paired pulse index (PPi), a presynaptic indicator of short-term plasticity. (For "C", "D" and "E": Two-sample T-test was employed. For "E" basal-post Paired-sample T-test was employed: p *<0.05 and **<0.01; 11 animals were employed)

Regenerative sAHP activation produced by the STDP regulates plasticity

Previously, we have confirmed that the postsynaptic GsAHP controls the direction of plasticity. However, we have not discarded the possibility that the basal GsAHP levels (recorded previously to STDP) determine the the direction of plasticity and not the GsAHP produced by the STDP protocol itself. In **Fig.4A** we have found a significant linear relation between the

eEPSC efficacy (% of baseline after the STDP) and the regenerative GsAHP/sAHP reached during the STDP protocols (GsAHP r=-0.44 and p=0.003; sAHP r=-0.43 and p=0.004). This results show us that protocols that resulted in potentiation (LTP and ISOP) have lower levels of GsAHP than those that resulted depression (LTD) or those that failed to generate plasticity (NON). Also, In **Fig.6** we show that the regenerative sAHP level is proportional to the spiking frequency during the STDP. In **Fig.4B** we have found no significant linear relation between the eEPSC% efficacy and the basal GsAHP/sAHP (GsAHP r=-0.11 and p=0.53; sAHP r=-0.30 and p=0.08). All cells were subjected to the same depolarization protocol (550pA/250ms) 5 minutes after whole cell was achieved and previously to the STDP protocols (**Fig.7C**). All together, this results indicate that pyramidal neurons can produce multiple plasticity outcomes depending on the regenerative GsAHP/sAHP activated during the STDP protocols and not by the basal GsAHP/sAHP levels.

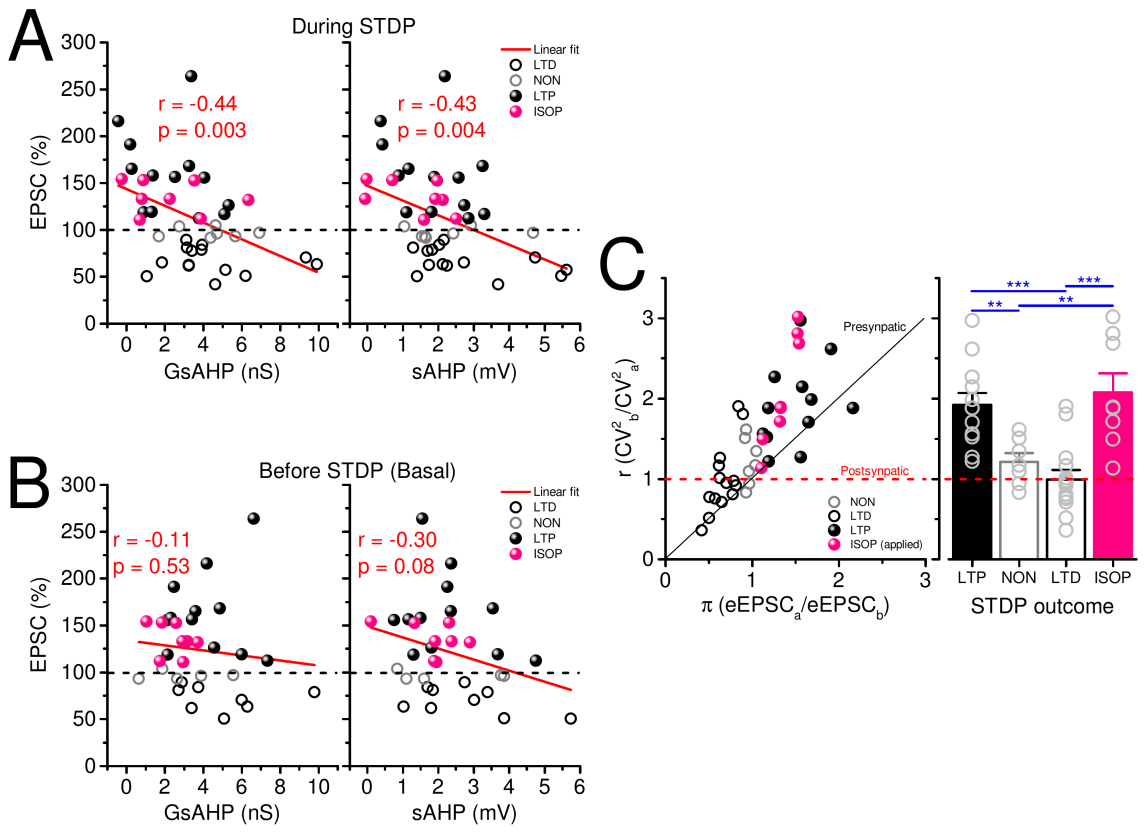

Figure 4. GsAHP regulation of eEPSC and site of expression. A) Diagram of eEPSC efficacy after the STDP against the regenerative GsAHP/sAHP during the STDP (all protocols included). Dashed line marks the 100% (basal efficacy). Red line, linear fit for all dots (Person's r and p of significance for the slope). **B)** Diagram of eEPSCs efficacy after the STDP against the basal GsAHP/sAHP evoked by a constant square current pulse (550pA/250ms, for all cases). Red line, linear fit for all dots (Person's r and p of significance for the slope). **C)** The plot of r (ratio of CVs squared) against π (ratio of eEPSCs) before and after the STDP. Red dashed line marks postsynaptic plasticity only. Black diagonal marks presynaptic changes in plasticity. Bars, quantification of "r", same scale than plot. (Two-sample T-test: *p<0.05, **p<0.01 and ***p<0.001; 36 animals were employed).

GsAHP dependent LTD has a postsynaptic site of expression

LTD and NON resulting STDPs presented no variation on the PPi after the STDP nor between their basal PPi levels (**Fig.2E**). These observations indicate that there was no change in the presynaptic Pr, however, a change in the number of release sites ("n") can be possible. To assess changes in "n" and "p" we employed the coefficient of variation (CV) method (**Fig.4C**) (Bekkers and Stevens, 1990; Faber and Korn, 1991). Changes in "p" and "n" could change the CV (increased "p" or "n" reduces CV), however, "q" is independent, consequently the ratio "r" (**Fig.4C** and methods) is equal to 1 if no presynaptic change occurred and different to 1 if a presynaptic change happened either in "p" or "n". In the **Fig.4C** scatter plot, we can see that the LTD and NON points are close to the red dashed line that marks r=1 and that ISOP and LTP appear along side the diagonal (r>1). In the bars, we can see that the r-values of NON and LTD are not significantly different from 1 (One-sample T-test; NON p=0.10 and LTD p=0.96) and significantly different from LTP and ISOP (Two-sample T-test: *p<0.05, **p<0.01 and ***p<0.001). Therefore, the GsAHP activation during a pre-post STDP determines the direction of plasticity in a postsynaptic manner, blocking LTP (NON) or producing LTD in glutamatergic synapses.

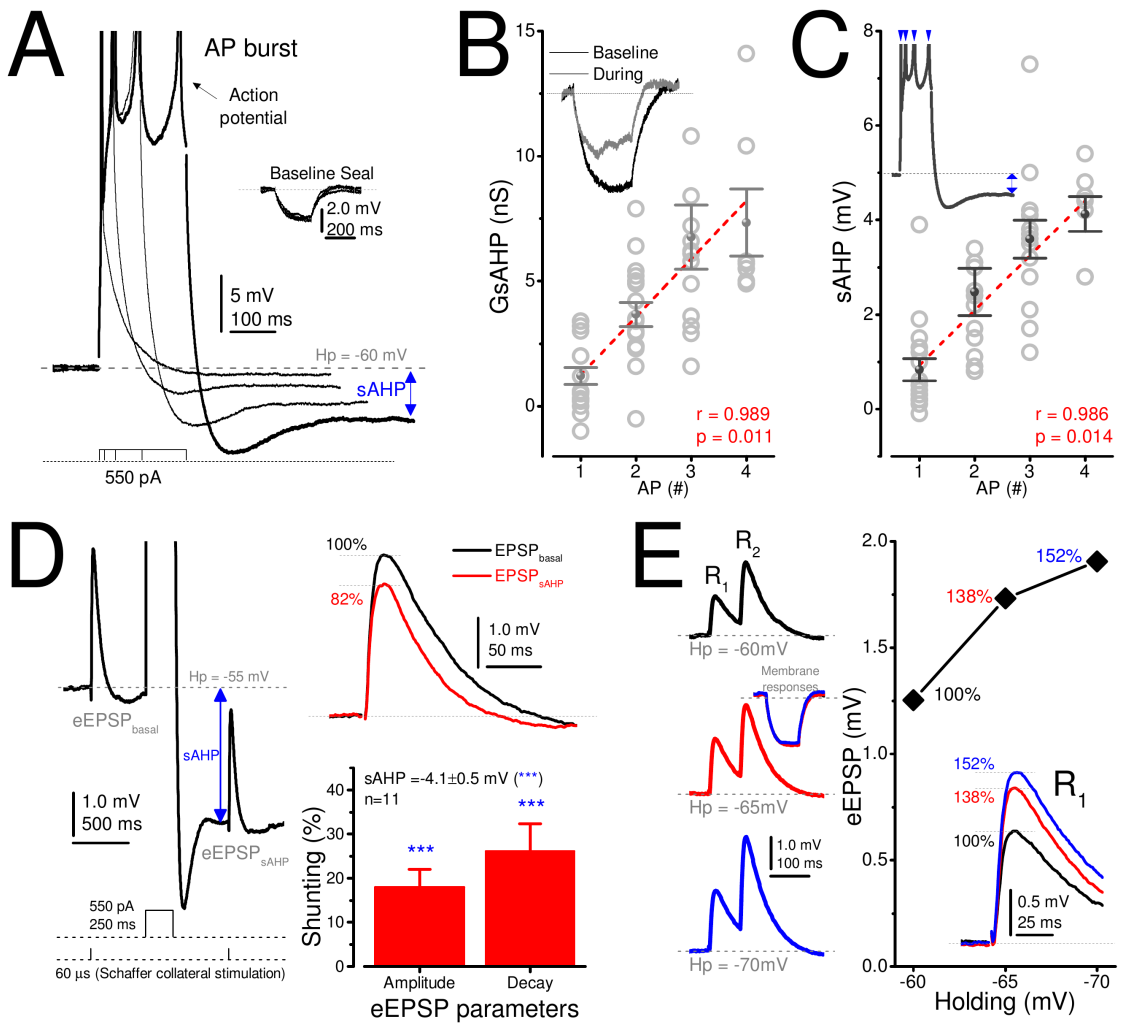

Figure 5. AP-number dependent GsAHP activation and shunting. A) Representative CC recordings of APs and sAHP amplitude. The protocol consisted in increasing duration constant intensity current pulses (550pA). Inset, sweeps of seal passive responses (-20pA) previous to depolarizing pulses. **B)** Graph showing AP-number dependence of GsAHP activation level, red dashed line represents linear fit. Overlaid seal inset, before and during the sAHP activation. **C)** Graph of sAHP-AP relation, with representative sweep of AP-evoked sAHP, red dashed line represents linear fit. sAHP hyperpolarization inset (Pearson's "r" and level of slope significance "p" included in both graphs). **D)** Representative trace of a shunted eEPSP during the sAHP. Right, superimposed representative shunted eEPSPs. Bars, percentage of attenuation (shunting) for EPSP amplitude, Tau of decay (Single-value T-tests, $p<0.001$***). **E)** Representative traces are showing the effect of hyperpolarization without membrane conductance change.(15 animals were employed)

Basal GsAHP activation is proportional to the number of APs

Previous studies have shown that the amplitude of the evoked sAHP is proportional to the

number of APs, however, a burst is needed to activate the sAHP significantly in younger rats (<p30) (Gustafsson and Wigström, 1981). Therefore, we decided to determine the contribution of every AP to sAHP in adult rats (~p60), employing the protocol depicted in **Fig.5A**. This protocol consisted in a constant amplitude depolarizing pulse, with increasing durations to evoke APs sequentially and in this manner assess the individual contribution of every AP. We determined a linear relation between the number of APs and the GsAHP activation level (**Fig.5B;** GsAHP r=0.989 and p=0.011) and hyperpolarization (**Fig.5C;** r=0.986 and p=0.014). Single AP can significantly activate GsAHP (1.21±0.34 nS; Single-value T-test: p=0.001) at Hp=-60mV, during less than 4 seconds, suggesting that spiking protocols with frequencies higher than 0.25Hz could regeneratively activate the GsAHP during STDP paradigms (**Fig.6**).

We verified that GsAHP significantly shunted the eEPSPs (**Fig.5D**). Also, the calculated GsAHP shunting was an underestimation of the effective eEPSP shunting, because hyperpolarization of the neuronal membrane generates larger eEPSPs due to the Na^+ driving force (**Fig.5E**). Therefore eEPSPs evoked during sAHP activation are shunted even if they have the same amplitude than the baseline eEPSPs.

GsAHP activation is proportional to the spiking frequency

To test if the STDP spiking frequency controls the amplitude of the regenerative sAHP, we developed two sets of spiking protocols: two at 0.25Hz (4 second period, T4sec and fT4sec) and at 1.0Hz (1 second period, T1sec and fT1sec) (**Fig.6A**). Control pulses (**Fig.6A** lower insets) were evoked at certain intervals (every 4-12 s) to assess the changes in the regenerative GsAHP (Fernández de Sevilla et al., 2007; Hausser, 2001; Rall, 1993; Staley and Mody, 1992). These protocols activated significantly different levels of regenerative GsAHP (**Fig.6B**; T4sec 0.96±0.33 nS, fT4sec 3.35±0.42 nS, T1sec 3.60±0.24 nS and fT1sec 5.19±0.76 nS; Two-sample T-test: *p<0.05, **p<0.01 and ***p<0.001), but 2 of them with different spiking frequencies

activated the same level of GsAHP/sAHP (fT4sec and T1sec).

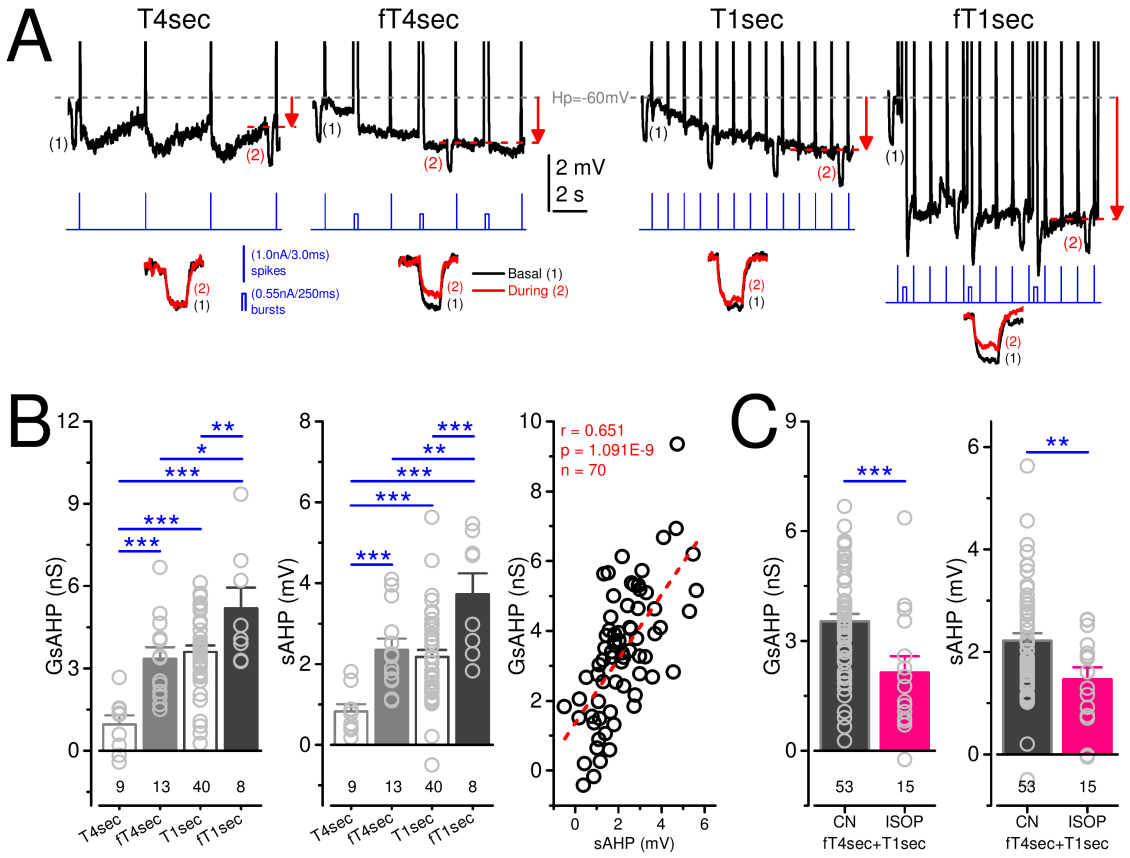

Figure 6. Spike frequency dependent GsAHP regenerative activation. A) Representative CC recordings of sAHP hyperpolarization due to repetitive AP spiking. Two protocol sets of 0.25Hz (T4sec and fT4sec) and 1.0Hz (T1sec and fT1sec). Red arrow, sAHP hyperpolarization. Blue lines, current injection protocols. Insets, hyperpolarizing passive responses to -20pA/250ms square current pulses, before (1) and during (2) sAHP regenerative activation. **B)** Bars, GsAHP and sAHP for every protocol (Two-sample T-test: *p<0.05, **p<0.01 and ***p<0.001). Scatter plot, sAHP linear relation between hyperpolarization and conductance. (Pearson's "r" and level of slope significance "p"). **C)** GsAHP regenerative activation for fT4sec and T1sec in normal conditions (CN) and under ISOP (Two-sample T-test: **p<0.01 and ***p<0.001).(62 animals were employed)

We determined that 4 seconds is the maximum interval interspike to maintain the sAHP regeneratively activated (**Fig.6A and B,** T4sec). We verified that different spike-frequencies activated fixed levels of regenerative sAHP and that forced protocols generated a significantly

higher sAHP level than their spiking only counterparts **(Fig.6B)**. In this way, we were able to dissociate the GsAHP activation level from the spike-frequencies for the STDP protocols. Also, the level sAHP hyperpolarization was linearly proportional to the increase in membrane conductance (GsAHP), as depicted in **Fig.6B** (scatter plot; Pearson's r=0.651 and p=1.091E-9). Also, we verified that the fT4sec and T1sec spiking protocols activated significantly lower level of regenerative sAHP when ISOP is present in the bath (**Fig.6C**; CN 3.53±0.20 nS and 2.22±0.15 mV; ISOP 2.14±0.44 nS and 1.47±0.23 mV; Two-sample T-test: **p<0.01 and ***p<0.001).

Therefore, single APs can activate the GsAHP for several seconds and the STDP spiking frequency determines the GsAHP regenerative level of activation.

The proportion of plasticity outcomes is dependent on the GsAHP level during the STDP

STDP protocols activate different GsAHP levels (**Fig.6B, and Fig.7B**), producing multiple outcomes by protocol (**Fig.7A**). These outcomes are shown as LTP:NON:LTD proportions that indicate the number of experiments that resulted in each outcome by every STDP protocol. In **Fig.7A**, from fT4sec to fT1sec the proportion of LTD outcomes rise (from 2/5 experiments in fT4sec to 4/6 experiments in fT1sec) and the proportion of LTP outcomes decay (from 4/4 experiments in T4sec to 1/6 experiments in fT1sec). Also, the ISOP fT4sec and T1sec protocols resulted only in LTP outcomes. In **Fig.7B,** the GsAHP and sAHP are plotted by STDP protocol and from T4sec to fT1sec the GsAHP/sAHP increases (T4sec 0.61±0.44 nS, fT4sec 3.05±0.63 nS, T1sec 3.46±0.36 nS and fT1sec 5.61±0.95 nS; Two-sample T-test: *p<0.05, **p<0.01 and ***p<0.001). These results together with the previous results (**Fig.6**) indicate that the proportion of outcomes varies with the amount of GsAHP activation during STDP.

In our experiments, the protocols with higher pairing frequencies are more likely to produce LTD (fT1sec at 1.00Hz resulted in 4/6 experiments and 5.61±0.95 nS) and lower frequencies

result in LTP (T4sec at 0.25Hz resulted in 4/4 experiments and 0.61±0.44 nS), which is contrary to previous results in which higher frequency of stimulation produces LTP and that do not considered sAHP activation (Bashir et al., 1991; Feldman, 2012; Kirkwood et al., 1993; Lisman and Spruston, 2005). fT4sec and T1sec protocols have equal GsAHP activation and consequently presented similar LTP:LTD proportions (2:2 and 6:8 respectively, **Fig.7A**), despite having different pairing frequencies.

As previously reported in **Fig.4B**, basal GsAHP values presented no relation with the plasticity outcomes (**Fig.7D;** Two-sample T-test). Basal sAHP activation protocol is depicted in **Fig.7C** (550pA/250ms).The GsAHP restricts the potentiation of eEPSCs to lower pairing frequencies (0.25 Hz) that activate lower levels of GsAHP (0.61±0.44 nS) and greater pairing frequencies (1.0 Hz) regeneratively activate higher levels of GsAHP (5.61±0.95 nS) resulting in LTD of eEPSCs.

Therefore, we concluded that the GsAHP activation level during STDP and not the pairing frequencies control the direction of plasticity.

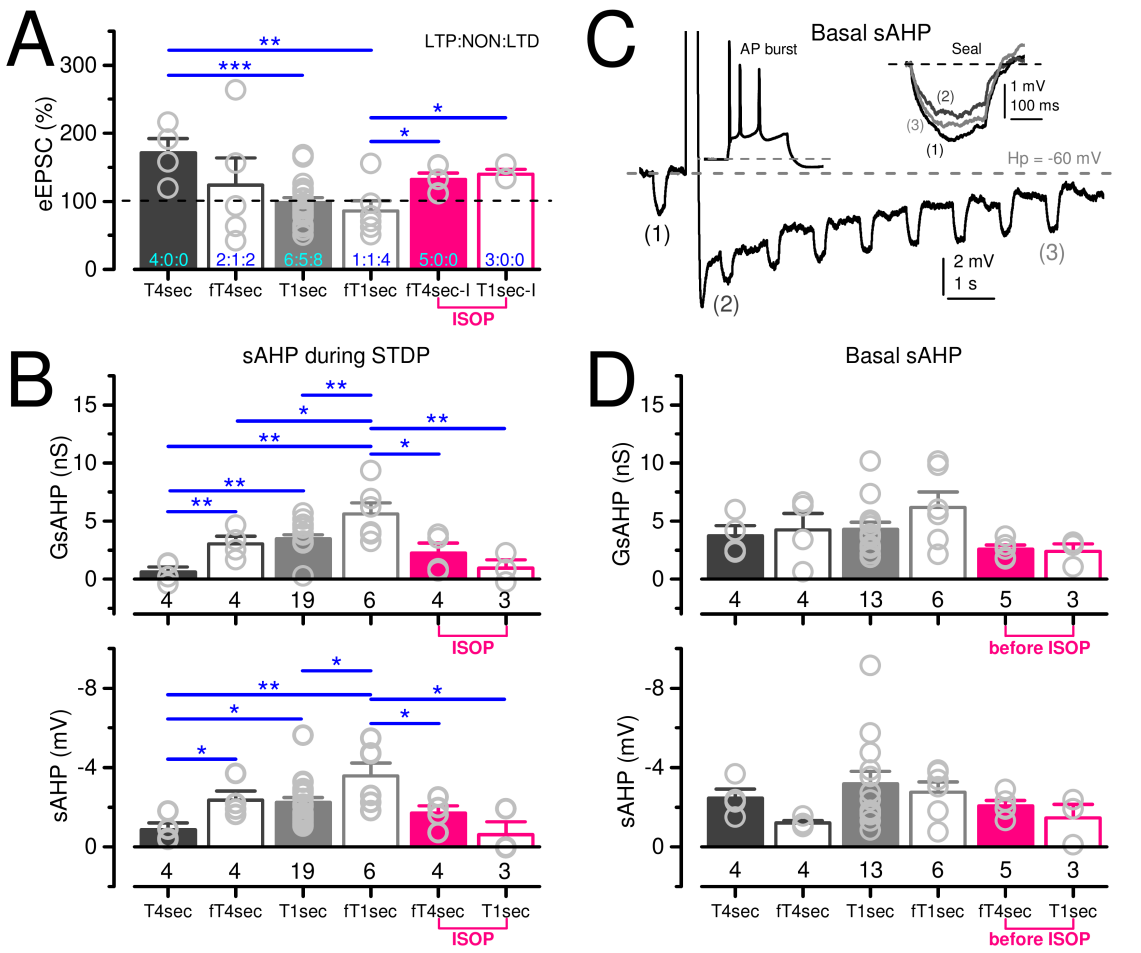

Figure 7. GsAHP activation by STDP protocol. A) eEPSCs efficacy 20-30 min after each STDP protocol. Inside every bar are the number of plasticity outcomes by STDP protocol, showed as proportions (LTP:NON:LTD). **B)** sAHP conductance and hyperpolarization by protocol. **C)** Representative recording of sAHP evoked by single 550pA/250ms square pulse. Each number is the response to a -20pA/250ms current pulse ("Seal" inset) during the sAHP time course. "AP-burst" is the spike train generated by the depolarizing pulse. **D)** GsAHP/sAHP evoked by "C" protocol for the same neurons in "B", before STDP protocols. For all figures only significant differences are shown (Two-sample T-test: *p<0.05, **p<0.01 and ***p<0.001).(36 animals were employed)

DISCUSSION

In this study, we have determined the effect of sAHP activation during different STDP protocols in CA1 hippocampal pyramidal neurons of adult rats. We have found evidence of a new form of postsynaptic pre-post LTD, generated by the GsAHP activation during the STDP that controls the direction of plasticity.

1. Higher GsAHP activation produces LTD in neurons of adult rats

We calculated the GsAHP by grouping the STDP outcomes (i.e., LTP, NON or LTD). In this way, it was possible to discern that those that resulted in LTD or NON had higher GsAHP activation (4.44±0.68 nS and 4.40±0.66 nS respectively) than those that resulted in LTP (2.39±0.53 nS) (**Fig.2D**). The LTD effect of GsAHP was an unexpected finding, because previous works in young animals (<p30) concluded that the GsAHP activation only blocks the mechanisms necessary to generate LTP (NON), by changing the temporal course of the EPSP rendering inefficient the nonlinear bAP depolarization and requiring a reduction in the window of plasticity to recover the LTP induction (Fernández de Sevilla et al., 2007; Fuenzalida et al., 2007). However, in our experiments in older rats (p60), we observed a significant depression of eEPSCs when the GsAHP was regeneratively activated in high Pr synapses (**Fig.2E;** PPi=1.12±0.04). Neither change in the PPi occurred after STDP in the LTD group (**Fig.2E**)**,** nor in the CV analysis (r=1) implying postsynaptic modifications only (**Fig.4C**). These observations suggest that the LTD generated by the GsAHP is not a presynaptic reduction of the Pr as is with

the work of Zaitsev and Anwyl in 2011, nor a reduction of the number of release sites but a postsynaptic mechanism. It has been reported that repetitive dendritic spine depolarization without NMDA currents results in synaptic depression (LTD), as is the case for some post-pre STDP protocols or uncorrelated activation of the synaptic spine (Feldman, 2012; He et al., 2015; Lisman and Spruston, 2005). This NMDAR-independent mechanism could be a way with which GsAHP produces LTD through shunting of the eEPSC. This plastic phenomena could also correspond to one of those reported by Banerjee et al., 2014: a postsynaptically expressed form of LTD with postsynaptic NMDAR dependence.

Also, there was a superposition in the GsAHP activation levels for certain STDP outcomes (**Fig.2D**), this was probably due to the differences in the distribution and density of Ca^{2+}-dependent K^+ channels throughout the neuronal dendrite which are more concentrated in the basal than the distal dendrite, where distal synapses are less shunted than proximal ones (Bekkers, 2000; Power et al., 2002; Sah and Bekkers, 1996; Storm, 1990; Trimmer, 2015) . Also, this superposition could be generated by an imprecise estimation of the GsAHP because our recordings were made in the neuronal soma. Therefore we can only estimate the effective shunting exerted by the GsAHP over distal glutamatergic synapses. Another reason could be the thermal noise present in the membrane during the estimation of the basal membrane conductance with the initial test pulse (right before the GsAHP activation) during STDP protocol, that was calculated from a single sweep (not from average recordings).

2. GsAHP reduction through NA activation with ISOP

Given that spiking protocols >0.25Hz can only increase the GsAHP activation during the STDP protocols, we employed the NA agonist ISOP to specifically reduce the GsAHP (**Fig.3A**) without significantly affecting the EPSCs (Li and Horn, 2008; Pedarzani and Storm, 1993; Zhang et al., 2013). In this way, we were able to reduce the GsAHP generated by fT4sec and

T1sec STDP protocols (**Fig.6C**) and therefore changing the outcomes of these protocols, passing from mixed (i.e., LTP:NON:LTD) results to LTP only (**Fig.3B and C, and Fig.7A and B**). In **Fig.7A** GsAHP reduction results in LTP only, however, its GsAHP/sAHP was not significantly different from fT4sec nor T1sec (**Fig.7B**). This is because fT4sec and T1sec presented mixed outcomes, where LTP/low-GsAHP and LTD/high-GsAHP present overlappings in their distributions with those of ISOP. On the other hand, the basal GsAHP measurements presented no significant difference between them, neither GsAHP nor sAHP, supporting the observation that the GsAHP activation during STDP is responsible for the direction of plasticity. Cholinergic modulation can reduce the sAHP through the M1 receptor that activates the secondary G_s pathway, however, the central pathway of this receptor is G_q that activates the PLC-IP3 pathway and also can have another secondary pathway Gi , therefore in our conditions GsAHP attenuation with cholinergic agonists is not specific and could significantly affect the presynaptic excitability as well as other K^+ conductances.

This results suggest that the attentional or emotional states where NA agonists are released could modulate the glutamatergic synaptic plasticity through the reduction of the GsAHP that is activated during STDP paradigms in the hippocampus of adult rats.

3. Adult GsAHP is regeneratively activated by STDP spiking

Single-APs can activate the GsAHP for several seconds (**Fig.5 and Fig.6**), allowing the construction of STDP protocols with different spiking frequencies and different GsAHP activation levels. This was demonstrated through the use of two types of protocols: simple spiking and forced GsAHP at two different pairing frequencies (0.25 and 1.0 Hz, **Fig.6A**). This allowed us to dissect the real contribution of the GsAHP over the frequency of the pairings (**Fig.6B**). If the frequency of the pairings was the determinant of the resultant plastic changes, different frequency protocols (fT4sec and T1sec, **Fig.6A**) but with the same GsAHP activation

(**Fig.6B and Fig.7B**) would have produced different outcomes, however, this was not the case (**Fig.7A**). In fact, these two protocols with different spiking frequencies but similar GsAHP activation produced similar LTP:LTD proportion (2:2 and 6:8, **Fig.7A**). In the **Fig.7A**, T4sec and fT1sec resulted in opposite LTP:LTD proportion (4:0 and 1:4, respectively) due to their level of sAHP activation and not by their respective pairing frequencies. In fact, if we compare spiking-only protocols (T4sec and T1sec) we can reach the conclusion that GsAHP is proportional to the frequency of spiking, therefore higher frequencies produce LTD and lower frequencies result in LTP, but this is contrary to the commonly accepted paradigm that higher frequencies are more likely to result in LTP (Bashir et al., 1991; Feldman, 2012; Kirkwood et al., 1993).

This restriction of the LTP plasticity to lower frequencies (0.25Hz) in adult animals could be a neuroprotective mechanism against intense bursting activity, like that observed in epilepsy that could produce uncontrolled LTP, that consequently could drive an increase in efficacy of synaptic transmission producing more spontaneous bursting and in this way augmenting the severity of seizures through the development of the pathology. This mechanism could counterbalance excessive neuronal firing activity, like those observed during epileptogenesis and in this way block unregulated plasticity (Fernández de Sevilla et al., 2006).

4. GsAHP through development

During development the progressive increment in the GsAHP changes the rules of plasticity: first, by making harder to produce LTP by a reduction of the window of coincidence in young rats of p30 (Fernández de Sevilla et al., 2007; Fuenzalida et al., 2007) then in adult rats of p60 (as demonstrated in our present work) GsAHP restricts the pairings that generate LTP to lower frequencies (0.25Hz) that minimally activate the GsAHP and not to higher pairing frequencies that result in LTD. Therefore in adult rats (p60), high frequency pairings (1.0Hz) normally depress the synaptic efficacy except if the GsAHP is reduced by the activation of NA agonist,

showing a diversification in the coding frequencies. This changes in the rules of synaptic plasticity through development suggests that in adulthood plasticity must be highly regulated to specific circumstances in which several conditions must be present, probably to preserve the fine tuning of the hippocampal circuits, or maybe to diversify the frequency codings.

On the other hand, the increment in the density of the channels that generate the GsAHP could be a mechanism by which the hippocampus can selectively block the LTP in some neurons and in this way separate groups of neurons to control their direction of plasticity independently (i.e., neurons with no GsAHP generate LTP only and neurons with high GsAHP could only generate LTD). This form of regulation can be useful for situations where several neurons could be activated or silenced simultaneously by different ambient conditions such as exploration or mating, or physiological states such as sleep and arousal, specifically through the alternate activation of adrenergic and cholinergic transmission. This results support previous findings in which the GsAHP reduction acts as a switch that allows synaptic plasticity related to memory and learning (Cohen-Matsliah et al., 2010; Matthews et al., 2009).

5. Future perspectives

In the present work, the exact biochemical mechanism by which the postsynaptic GsAHP generates pre-post LTD at the postsynaptic site remained unknown, leaving several questions with respect to what are the molecular changes occurring through development that end in this manifestation of plasticity that was not reported in young rats (<p30). Also, we only explored the causal activation of the STDP, leaving the post-pre STDP paradigm unexplored, which can contribute to elucidate the entire picture of what are changes in the regulation of plasticity in adult rats. However, the precise mechanisms of the GsAHP dependent LTD remain unknown, opening a new perspective to investigate new mechanisms of regulation emerging through development.

CONCLUSION

In this study we have determined the regulatory effect of GsAHP activation during STDP protocols in CA1 hippocampal pyramidal neurons of adult rats, acting as a switch that determines the direction of synaptic plasticity either by blocking the induction of LTP or by producing LTD.

Our main findings are:

1. Postsynaptic activation of GsAHP during STDP less than 2.39±0.53 nS resulted in LTP and greater than 4.44±0.68 nS resulted in LTD of eEPSC-efficacy. STDP protocols with GsAHP activation between these levels of conductance produced mixed outcomes (i.e., LTP, NON or LTD).
2. GsAHP attenuation by ISOP (NA activation) during the STDP protocol resulted in LTP only.
3. GsAHP activated during STDP spiking restricts LTP to 0.25 Hz pairings and either block LTP (NON) or produce LTD with 1.0 Hz frequency pairings .

We have shown a new depressive (LTD) effect of postsynaptic GsAHP activation over glutamatergic synapses of pyramidal neurons.

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