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Tonic activation of $VPAC_1$ receptors by VIP modulates theta-burst induced LTP in the hippocampus: transduction pathways and GABAergic mechanisms.

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Abbreviations:

aCSF, artificial CSF solution; AP5, (2R)-amino-5-phosphonovaleric acid; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; GAT-1, GABA transporter 1; GF-109203X 3-[1-[3-(dimethylamino)propyl]-1H-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione; GRF, growth hormone-releasing factor; H-89, N-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide; KN-62, 4-[(2S)-2-[(5-isoquinolinylsulfonyl)methylamino]-3-oxo-3-(4-phenyl-1-piperazinyl)propyl] phenyl isoquinolinesulfonic acid ester; LTD, long-term depression; PACAP, Pituitary adenylate cyclase activating polypeptide; PG 97-269, [Acetyl-His^{1},D-Phe^{2},Lys^{15},Arg^{16},Leu^{17}]VIP(3-7)/GRF(8-27); PG 99-465, Myr-His^{1}[Lys^{12},$Lys^{27,28}$,$Gly^{29,30}$,Thr^{31}]VIP; SKF89976A, (1-(4,4-diphenyl-3-butenyl)-3-piperidinecarboxylic acid; TBS, theta-burst stimulation; VGCC, voltage-gated calcium channel; VIP, vasoactive intestinal peptide.

Abstract:

Background and purpose

Vasoactive intestinal peptide (VIP), acting on both $VPAC_1$ and $VPAC_2$ receptors, is a key modulator of hippocampal synaptic transmission, pyramidal cell excitability and synaptic plasticity phenomena, like long-term depression (LTD), partly through modulation GABAergic disinhibitory circuits. VIP effects on LTP and the involvement of disinhibition were scarcely investigated.

Experimental approach

The influence of endogenous VIP on CA1 LTP induced by TBS was evaluated in the CA1 area of hippocampal slices using field-excitatory electrophysiological recordings from young-adult Wistar rats using selective $VPAC_1$ and $VPAC_2$ antagonists. Phosphorylation of GluA1 AMPA receptor subunits and Kv4.2 potassium channels was evaluated in hippocampal membranes obtained from such slices by Western blot.

Key results

Here we show that VIP, acting on $VPAC_1$ (but not $VPAC_2$) receptors, is an endogenous inhibitor of hippocampal LTP induced by theta-burst stimulation (TBS) in the CA1 area of the hippocampus of young adult Wistar rats. This effect is dependent on GABAergic transmission and relies on the integrity of NMDA and CaMKII-dependent LTP expression mechanisms but not on PKA and PKC activity. Furthermore, it regulates the expression and Ser438phosphorylation of Kv4.2 potassium channels responsible for the A-current while inhibiting phosphorylation of Kv4.2 on Thr607.

Conclusions and implications

Altogether this suggests that endogenous VIP controls the expression of hippocampal CA1 LTP by regulating disinhibition through activation of $VPAC_1$ receptors in interneurons. This may impact the expression and phosphorylation of Kv4.2 K^+ channels at hippocampal pyramidal cell dendrites.

What is already known?

VIP modulates synaptic transmission in hippocampal CA1 synapses and endogenous VIP inhibits expression of hippocampal CA1 LTD through $VPAC_1$ receptor activation.

What this study adds

Endogenous VIP acting on $VPAC_1$ receptors inhibits hippocampal CA1 LTP through a mechanism dependent on GABAergic transmission and modulation of Kv4.2 K^+ channel function.

What is the clinical significance?

$VPAC_1$ receptors may constitute relevant pharmacological targets to treat altered GABAergic control of synaptic plasticity and cognitive disfunction in aging and epilepsy.

Keywords: VIP, LTP, $VPAC_1$ receptors, $VPAC_2$ receptors, interneurons, Kv4.2, hippocampus.

Introduction:

Vasoactive intestinal peptide (VIP) is an endogenous modulator of hippocampal synaptic transmission and excitability (Cunha-Reis and Caulino-Rocha, 2020). The actions of VIP in the hippocampus are mediated by $VPAC_1$ and $VPAC_2$ receptors belonging to the VIP/PACAP family of G protein-coupled receptors (Cunha-Reis et al., 2021). These receptors bind pituitary adenylate cyclase-activating polypeptide (PACAP) with similar affinity. A third receptor in this family, the PAC_1 receptor, binds preferentially PACAP over VIP. Exogenous PACAP is also able to modulate hippocampal synaptic transmission (Roberto et al., 2001).

Given that VIP is present in the hippocampus exclusively in interneurons (Acsády et al., 1996a) its actions are mainly dependent on GABAergic transmission (Wang et al., 1997; Cunha-Reis et al., 2004, 2005). VIP is expressed in three different populations of local interneurons with different target selectivity and is thus likely that it plays several distinct roles in the modulation of hippocampal synaptic transmission. Not surprisingly, endogenous VIP has opposing actions on GABA release when activating $VPAC_1$ or $VPAC_2$ receptors (Cunha-Reis et al., 2017). Furthermore, VIP enhancement of synaptic transmission to CA1 pyramidal cells involves inhibition of GABAergic interneurons that control pyramidal cell dendrites, leading to disinhibition (Cunha-Reis et al., 2004), an action mainly mediated by activation of $VPAC_1$ receptors located in the *strata oriens* and *radiatum* (Vertongen et al., 1997; Joo et al., 2004; Cunha-Reis et al., 2005). Yet, VIP also increases hippocampal pyramidal cell excitability in the absence of GABAergic transmission (Ciranna and Cavallaro, 2003; Cunha-Reis et al., 2004), through inhibition of the slow Ca^{2+}-activated K^+ current (IsAHP) (Haas and Gähwiler, 1992) and likely through activation of $VPAC_2$ receptors in the pyramidal cell layer of the Ammon's Horn (Cunha-Reis et al., 2006).

VIP is also crucial for hippocampal-dependent learning tasks (Takashima et al., 1993a, 1993b; Itoh et al., 1994; Ivanova et al., 2009) and VIP deficient mice fail to develop hippocampal-dependent learning skills like reversal learning (Stack et al., 2008). Synaptic plasticity phenomena are believed to underlie these hippocampal-dependent processes. Yang *et al.* have also shown that exogenously applied VIP enhances NMDA currents in CA1 pyramidal cells, an effect mimicked by $VPAC_2$ and to a lesser extent by $VPAC_1$ selective agonists (Yang et al., 2009). This suggests that either endogenous VIP or PACAP, the two endogenous agonists of this receptor, could contribute to NMDA-dependent hippocampal synaptic plasticity. Hippocampal long-term potentiation (LTP), long-term depression (LTD) and depotentiation are synaptic plasticity phenomena similarly dependent on NMDA receptor activity. We recently described that endogenous VIP, through $VPAC_1$ receptor activation, is an important endogenous modulator of NMDA-dependent hippocampal LTD and depotentiation in the CA1 area of the hippocampus. To date, no study has elucidated the role of endogenous VIP and PACAP and their relative importance in the modulation of hippocampal LTP.

LTP can be evoked *in vitro* by theta burst stimulation (TBS) (Rose and Dunwiddie, 1986; Larson and Lynch, 1988), a sequence of electrical stimuli that resemble CA1 pyramidal cell burst discharges observed during the hippocampal theta rhythm (4-10Hz), an EEG pattern that has been associated with hippocampal memory storage in rodents (Vertes, 2005; Larson and Munkácsy, 2015). Bursts repeated at the theta frequency induce maximal LTP due to the suppression of feedforward inhibition by the first burst or a priming single pulse, that allows for enough depolarization to activate NMDA receptors (Larson et al., 1986; Davies et al., 1990). This process is mediated by activation of $GABA_B$ autoreceptors, that strongly inhibit GABA release from feedforward interneurons (Davies and Collingridge, 1996). However, additional GABAergic mechanisms may contribute to TBS-induced LTP (Rodrigues et al., 2021).

In this paper we investigated the role of endogenous VIP in the modulation of hippocampal LTP induced *in vitro* by mild TBS and the receptors and transduction pathways operated by VIP in this modulation. A preliminary account of some of the results has been published as an abstract (Cunha-Reis et al., 2010).

Methods:

All protocols and procedures were performed according to BJP guidelines for experimental design, analysis, and their reporting. Animal housing and handing was performed in accordance with the Portuguese law (DL 113/2013) and European Community guidelines (86/609/EEC and 63/2010/CE). The experiments were performed on hippocampal slices taken from young-adult (6-7 weeks old) male outbred Wistar rats (Harlan Iberica, Barcelona, Spain) essentially as previously described (Aidil-Carvalho et al., 2017). Female rats were not used due to hormonal influences on LTP. The animals were anesthetized with halothane, decapitated, and the right hippocampus dissected free in ice-cold artificial cerebrospinal fluid (aCSF) of the following composition in mM: NaCl 124, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 26, $MgSO_4$ 1, $CaCl_2$ 2, glucose 10, and gassed with a 95% O_2 - 5% CO_2 mixture.

LTP experiments

Electrophysiological recordings were performed in hippocampal slices (400 µm thick) cut perpendicularly to the long axis of the hippocampus with a McIlwain tissue chopper. Slices were kept in a resting chamber in the same gassed aCSF at room temperature 22°C–25°C for at least 1 h to allow their energetic and functional recovery, then one slice at a time was transferred to a submerged recording chamber of 1 ml capacity, where it was continuously superfused at a rate of 3 ml/min with the same gassed solution at 30.5°C. Stimulation (rectangular pulses of 0.1 ms) was delivered through a bipolar concentric wire electrode placed on the Schaffer collateral/commissural fibres in the *stratum* radiatum. Two separate sets of the Schaffer pathway (S1 and S2) were stimulated (Fig. 1A). Responses were evoked every 10 s alternately on the two pathways, each pathway being stimulated every 20 s (0.05Hz). The initial intensity of the stimulus was that eliciting a field excitatory post-synaptic potential (fEPSP) of 600–1000 mV amplitude, while minimizing contamination by the population spike, and of similar magnitude in both pathways. Evoked fEPSPs were recorded extracellularly from CA1 *stratum* radiatum (Fig. 1A) using micropipettes filled with 4 M NaCl and of 2–4 MΩ resistance. The averages of six consecutive responses from each pathway were obtained, measured, graphically plotted and recorded for further analysis with a personal computer using the LTP software (Anderson and Collingridge, 2001). The fEPSPs were quantified as the slope of the initial phase of the potential.

The independence of the two pathways was tested at the end of the experiments by studying PPF across both pathways, less than 10% facilitation being usually observed. To elicit PPF, the two Schaffer pathways were stimulated with 50 ms interpulse interval. The synaptic facilitation was quantified as the ratio P2/P1 between the slopes of the fEPSP elicited by the second P2 and the first P1 stimuli.

LTP was induced by a mild TBS pattern (five trains of 100 Hz, 4 stimuli, separated by 200 ms). The stimulation protocol used to induce LTP was applied after having a stable baseline for at least 20 min. The intensity of the stimulus was not changed during these stimulation protocols. LTP was quantified as the % change in the average slope of the potentials taken from 50 to 60 min after the induction protocol, in relation to the average slope of the fEPSP measured during the 10 min that preceded the induction protocol. Control and test conditions were tested in independent pathways in the same slice. In all experiments S1 always refers to the first pathway (left or right, randomly assigned) to which TBS was applied. Test drugs were added to the perfusion solution 20 min before TBS stimulation of the test pathway (S2) and were present until the end of the experiment except for the anti-VIP antibody that was present only until 20 min after TBS. When testing the effect of PG 97-269 in the presence of other drugs (bicuculine, SKF89976a, AP-5 or KN-62), these were added to the perfusion media at least 30 min before TBS stimulation of the control pathway (S1) and were present until the end of the experiment.

Western blot analysis of GluA1 and Kv4.2 phosphorylation

For western blot studies, hippocampal slices were prepared as described above and allowed for functional recovery. Slices were then placed in the electrophysiology chambers and superfused at a flow rate of 3 ml/min with gassed aCSF at 30.5 °C. Stimulation was delivered once every 15s in the form of rectangular pulses (0.1 ms duration) through a bipolar concentric wire electrode placed on the Schaffer collateral/commissural fibres in the stratum radiatum and lasted for 80 min (average duration of an electrophysiological experiment). In test chambers (but not in control chambers) TBS was applied 20 min after the beginning of basal stimulation to test but not to control slices either in the presence or the absence of the $VPAC_1$ antagonist PG 97-269 (100nM). Basal stimulation continued until the end of the experiment. Several slices and each of the four experimental conditions were tested for each animal. Hippocampal slices, minimum of four per condition, obtained from at least two animals were then collected in sucrose solution (320mM Sucrose, 1mg/ml BSA, 10mM HEPES e 1mM EDTA, pH 7,4) containing protease (complete, mini, EDTA-free Protease Inhibitor Cocktail, Sigma) and phosphatase (1 mM PMSF, 2 mM Na_3VO_4, and 10 mM NaF) inhibitors and homogenized with a Potter-Elvejham apparatus. The suspension was centrifuged at 1500g for 10 min and the supernatant collected and further centrifuged at 14000g for 12 min. The pellet was washed twice with modified aCSF (20mM HEPES, 1mM $MgCl_2$, 1.2mM NaH_2PO_4, 2.7mM NaCl; 3mM KCl, 1.2mM $CaCl_2$, 10mM glucose, pH 7.4) also containing protease and phosphatase inhibitors and hippocampal membranes were resuspended at a concentration of 1mg/ml protein concentration (Bradford assay) in modified aCSF. Aliquots of this suspension of hippocampal membranes were snap-frozen in liquid nitrogen and stored at -80°C until use.

Samples processed as above were incubated for 5 min at 95°C with Laemmli buffer (125mM Tris-BASE, 4% SDS, 50% glycerol, 0,02% Bromophenol Blue, 10% β-mercaptoethanol), run on standard 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membranes (Immobilon-P transfer membrane PVDF, pore size 0.45 µm, Immobilon). After blocking for 1 h with a 3% BSA solution, membranes were incubated overnight at 4°C with rabbit antiphospho-Ser845-GluA1 (1:2000, Chemicon, Cat# AB5849; RRID:AB_92079), rabbit antiphospho-Ser-831-GluA1 (1:3000, Chemicon, Cat# AB5847; RRID:AB_92077), rabbit anti-GluA1 (1:4000, Millipore, Cat# AB1504; RRID:AB_2113602), rabbit anti-Kv4.2 (1:1000, Millipore, Cat# 07-491; RRID:AB_310662), rabbit anti-phospho-Ser438-Kv4.2 (1:100, Santa Cruz Biotech, Cat# sc-135551; RRID:AB_10839526), rabbit anti-phospho-Thr607-Kv4.2 (1:100, Santa Cruz Biotech, Cat# sc-22254-R; RRID:AB_2131823), mouse monoclonal anti-phospho-Thr602-Kv4.2 (1:2000, Santa Cruz Biotech, Cat# sc-16983-R; RRID:AB_670816) or rabbit anti-beta-actin (1:10000, Proteintech, Cat# 60008-1; RRID:AB_2289225) primary antibodies. After washing the membranes were incubated for 1h with anti-rabbit or anti-mouse IgG secondary antibody both conjugated with horseradish peroxidase (HRP) (Proteintech) at room temperature. HRP activity was visualized by enhanced chemiluminescence with ECL Plus Western Blotting Detection System (GE Healthcare). Intensity of the bands was evaluated with the Image J software. Beta-actin density was used as a loading control. The % phosphorylation for each target on AMPA GluA1 subunits or Kv4.2 channels was determined by normalizing the band intensity of the phosphorylated form by the band intensity of the total GluA1 or Kv4.2. Some of the obtained results for phosphorylated Kv4.2 on Thr602 were not of the highest quality and although densitometric quantification has been performed the obtained results did not suggest significant changes. For this, and in agreement with the 3R policy, we did not consider further experiments to increase the sample load of the western blots. Additional experiments were not considered on the other two phosphorylation sites because the antibodies were discontinued by the supplier (Santa Cruz Biotech).

Materials:

VIP (Novabiochem), [Ac-Tyr^{1}, D-Phe^{2}] GRF (1-29), PACAP 6-38 (both Tocris Cookson, UK), PG 97-269, PG 99-465, Ro 25-1553 and [K^{15}, R^{16}, L^{27}] VIP (1-7) / GRF(8-27) (all Phoenix peptides, Europe) were made up in 0.1mM stock solution in CH_3COOH 1% (v v-1). H-89 (N- [2-((p-bromocinnamyl)amino) ethyl]-5-isoquinolinesulfonamide) (Calbiochem), KN-62 (4-[(2S)-2-[(5-isoquinolinylsulfonyl)methylamino]-3-oxo-3-(4-phenyl-1-piperazinyl)propyl] phenyl isoquinolinesulfonic acid ester, Tocris Cookson, UK) and GF-109203X (3-[1-[3-(Dimethylamino)propyl]-1H-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione) (Sigma/RBI) were made up in 5mM stock solutions in DMSO. The maximal DMSO and CH_3COOH concentrations used

were devoid of effects on tritium release. Bicuculline methochloride (Ascent Scientific, UK), AP-5 (D-(-)-2-Amino-5-phosphonopentanoic acid), and SKF89976A (1-(4,4-Diphenyl-3-butenyl)-3-piperidinecarboxylic acid) (both Tocris Cookson, UK), were prepared in aqueous solution. Mouse monoclonal anti-VIP IgG1 (Santa Cruz, Cat# sc-57499; RRID:AB_630434) was supplied as a suspension in PBS with <0.1% NaN3 and 0.1% gelatine. The maximal CH_3COOH and NaN_3 concentration added to the slices, 0.001% (v/v) and 0.0004% (p/v), were devoid of effects on fEPSP slope (n53). Aliquots of the stock solutions were kept frozen at 22°C until use. In each experiment, one aliquot was thawed and diluted in aCSF.

Statistics

Values are presented as the mean ± S.E.M of n experiments for electrophysiological studies and the mean ± S.E.M of n experiments performed in duplicate for western-blot studies. Statistical analysis was performed using GraphPad Prism version 6.01 for Windows. The significance of the differences between the means was calculated using the paired Student's t-test when comparing two experimental groups, or with one-way analysis of variance ANOVA when comparing more than two experimental groups. P values of 0.05 or less were considered to represent statistically significant differences.

Results:

Representative fEPSP obtained in hippocampal slices from young-adult rats (Fig. 1.A) under basal stimulation conditions (40-60% of the maximal response in each slice) had an average slope of 0.647±0.026 mV/ms (n=33). When mild TBS was applied to the control pathway (S1) an LTP was induced, (28.9±1.2% enhancement of fEPSP slope 50-60 min after TBS, n=33, Fig. 1.A). As previously described, this effect was absent (n=4) when slices were stimulated in the presence of the NMDA receptor antagonist AP-5 (100μM). A second TBS train delivered to the test pathway (S2) in the absence of drugs always resulted in a LTP of similar magnitude (% increase in fEPSP slope: 32.4±3.6%, n=4) to the one obtained in the control pathway (S1), showing that the LTP obtained is similar in S1 and S2. When the selective VPAC receptor antagonist, Ac-Tyr^{1}, D-Phe^{2} GRF (1-29) (300nM) was delivered to the slices 20 min before S2, TBS stimulation elicited a larger LTP, now increasing by 48.6±4.1% (n=5) the fEPSP slope (Fig 1.A-B), suggesting that VIP is an endogenous inhibitor of CA1 hippocampal LTP. This prompted us to test the involvement of each of the VIP-selective receptors in this action using the $VPAC_1$-selective antagonist PG 97-269 (Gourlet et al., 1997) and the $VPAC_2$-selective antagonist PG 99–465 (Moreno et al., 2000). The presence of PG 97-269 (100nM) 20 min before TBS in S2 elicited a larger LTP, increasing by 51.3±3.8% (n=7) fEPSP slope (Fig. 1.C). Conversely, the presence of PG 99-465 (100nM) from 20 min before TBS in S2 resulted in an LTP of similar magnitude to the one obtained in control conditions (% increase in fEPSP slope: 34.6±2.5%, n=6, Fig. 1.D). Ac-Tyr^{1}, D-Phe^{2} GRF (1-29) (300nM), PG 97-269 (100nM) and PG 99-465 (100nM), when added to the superfusion solution, did not significantly change fEPSP slope, suggesting that VIP does not have a tonic action on these receptors under basal stimulation conditions.

$VPAC_1$ and $VPAC_2$ receptors can be endogenously activated by both VIP and PACAP. To elucidate which of these peptides was responsible for $VPAC_1$ receptor activation leading to enhanced LTP we tested the influence of an anti-VIP antibody on LTP. Perfusion with mouse anti-VIP-IgG_1 (0.4μg/ml) resulted in an enhanced LTP magnitude of 48.7±2.9%, (n=5, Fig. 2.A), suggesting that VIP is the endogenous mediator of the tonic actions on $VPAC_1$ receptors that inhibit LTP. Since this did not exclude that endogenous PACAP, acting on other receptors could influence hippocampal LTP, we tested if PACAP 6-38, a selective PAC_1 receptor antagonist (Robberecht et al., 1992), could influence CA1 LTP. The presence of PACAP 6-38 (30nM) mildly enhanced the magnitude of LTP (% increase in fEPSP slope 43.8±4.0%, n=5, Fig. 2.B) suggesting an endogenous activation of PAC_1 receptors during TBS. PACAP 6-38 (30nM) or the anti-VIP IgG_1 (0.4μg/ml) did not significantly change fEPSP slope under basal stimulation conditions. To further understand the actions of VIP, we tested the influence of exogenously added VIP in the expression of LTP. When 1nM and 10nM VIP was added before S2 an enhancement of 20.4±4.3% (n=5) and 15.8±3.4% (n=5), respectively, in fEPSP slope was observed. TBS, applied in the presence of 1nM VIP, decreased LTP (% increase in fEPSP slope 14.6±1.1%, n=5,

Fig. 2.C), whereas the presence of 10nM VIP mildly enhanced LTP magnitude (% increase in fEPSP slope 43.7±4.0%, n=5, Fig. 2.D) compared to control conditions, suggesting that exogenously added VIP may be activating in a concentration dependent manner either different/additional receptors or distinct cellular and molecular pathways than endogenous VIP.

VIP-expressing interneurons promote either direct modulation of pyramidal cell excitability or disinhibition by two distinct indirect pathways regulating synaptic transmission to pyramidal cell dendrites (Cunha-Reis *et al.*, 2004). To elucidate the role of GABAergic transmission in $VPAC_1$ receptor-mediated modulation of CA1 LTP we tested the influence of PG 97-269 on LTP when fast GABAergic transmission was blocked using the selective $GABA_A$ receptor antagonist bicuculline. When added to the slices before S1 bicuculline (10μM) increased fEPSP slope by 41.5±4.9% (n=5). We thus reduced stimulation intensities to make fEPSP slopes of similar magnitude to the ones obtained in the absence of bicuculline (10μM). TBS stimulation of control pathway (S1) in the presence of bicuculine (10μM) caused an LTP of 25.1±4.7% (n=5, Fig. 3.A). When PG 97-269 (100nM) was added to hippocampal slices in the presence of bicuculline, TBS in S2 resulted in a similar LTP (% increase in fEPSP slope of 23.2±4.8%, n=5, Fig. 3.A), suggesting that $VPAC_1$ receptor mediated inhibition of LTP by endogenous VIP is dependent on GABAergic transmission.

$VPAC_1$ modulation of GABA release in the hippocampus may target GABA exocytosis or depolarization-induced reversal of the nerve terminal GABA transporter 1 (GAT-1) (Cunha-Reis et al., 2017). To test the involvement of this pathway on modulation of hippocampal LTP by endogenous $VPAC_1$ receptor activation we tested the effect of PG 97-269 on LTP upon inhibition of GAT-1 transporters with the selective antagonist SKF89976a. TBS delivered to S1 in the presence of SKF89976a (5μM) elicited an LTP, enhancing fEPSP slope by 33.8±2.6% (n=5, Fig. 3.B). When PG 97-269 (100nM) was applied to hippocampal slices in the presence of SKF89976a, TBS in S2 resulted in mildly larger LTP (% increase in fEPSP slope: 45.0±2.8%, n=5, Fig. 3.B), suggesting that $VPAC_1$ receptor mediated inhibition of LTP by endogenous VIP is only partially dependent on the presynaptic control of synaptic GABA by the GAT-1 transporter.

We further investigated if $VPAC_1$ mediated actions on LTP depended on the modulation of the TBS-induced NMDA-dependent component of LTP by testing the effect of PG 97-269 in the presence of the NMDA receptor antagonist (2R)-amino-5-phosphonopentanoate (AP-5). AP-5 (100μM) did not change fEPSP slope under basal stimulation. TBS stimulation of S1 in the presence of AP-5 (100μM) abolished LTP (% change in fEPSP slope of -0.7±3.2%, n=4, Fig. 4.A). In the presence of PG 97-269 (100nM) together with AP-5 (100μM), TBS in S2 also did not cause any long-lasting change in fEPSP slope (-0.4±1.9%, n=4, Fig. 4.A), suggesting that tonic $VPAC_1$ receptor-mediated inhibition of NMDA receptor activation.

LTP expression is described to rely on the Ca^{2+}-dependent activation and consequent auto-phosphorylation of Ca^{2+}/calmodulin dependent protein kinase II (CaMKII), that in turn promotes the recruitment of AMPA GluA1 subunits (Appleby et al., 2011). Yet this has also been reported as not essential for expression of NMDA-dependent LTP in the mouse (Cooke et al., 2006). The involvement of CaMKII in $VPAC_1$ receptor-mediated enhancement of TBS-induced LTP by endogenous VIP was investigated using the CaMKII selective inhibitor KN-62. TBS stimulation of S1 in the presence of KN-62 (50μM) suppressed LTP (% change in fEPSP slope: 1.0±0.7% (n=4, Fig. 4.B). TBS in S2 delivered in the presence of PG 97-269 (100nM) and KN-62 (50μM), elicited a very small LTP (% increase in fEPSP slope: 9.1±1.3%, n=4, Fig. 4.B). KN-62 (50μM) did not significantly change fEPSP slope under basal stimulation conditions. This suggests tonic $VPAC_1$ receptor-mediated inhibition of LTP is not fully dependent on CaMKII activity and involves additional transduction pathways.

To tackle this, we investigated the downstream targets of different intracellular kinases on AMPA GluA1 receptor subunits, previously reported to mediate expression of hippocampal LTP by promoting traffic or modifying opening probability of AMPA receptors (Lee et al., 2003). As we previously described (Rodrigues et al., 2021), 50 min after TBS we observed an enhancement of 50.2±9.8% (n=5, Fig. 5.C-D) in GluA1 phosphorylation in residue Ser831, targeted by both CaMKII and protein kinase C (PKC). No significant changes were observed in Ser845 phosphorylation, targeted by protein kinase A, (PKA) or in the total expression of GluA1 subunits (n=5, Fig 5A-B, E-F). Presence of the selective $VPAC_1$ receptor antagonist PG 97-269 (100nM) alone, did not change total GluA1 or in GluA1 phosphorylation (n=5, Fig 5. A-F). When TBS was delivered in the presence of PG 97-269 (100nM) the enhancement in GluA1 phosphorylation in residue Ser831 was not different ($P>0.05$) than the one observed in the absence of the $VPAC_1$ receptor antagonist (47.6±14.3%, n=5, Fig. 5.C-D) and phosphorylation on Ser845 was not altered. Altogether this suggests that $VPAC_1$ receptor activation by VIP during TBS LTP induction does not modify GluA1 phosphorylation and that the mechanism operated by $VPAC_1$ receptors to regulate LTP induced by TBS lies elsewhere.

We further investigated the involvement of the G_s/adenylate cyclase/PKA transduction system in $VPAC_1$-mediated inhibition of CA1 LTP induced by TBS. In the presence of the selective PKA inhibitor (Chijiwa et al., 1990) H-89 (5μM), the effect of PG 97-269 (100nM) on LTP induced by TBS was not changed (% increase in fEPSP slope: 44.9±5.8%, n=7, Fig. 6.A). In addition, we investigated the involvement of PKC on TBS-induced LTP. Upon selective inhibition of PKC with GF 109203X (1μM) (Toullec et al., 1991), the effect of PG 97-269 (100nM) on LTP induced by TBS was similarly not changed (% increase in fEPSP slope: 44.2±3.5%, n=7, Fig. 6.B). When present during S1, H-89 (5μM) and GF 109203X (1μM) did not affect LTP (Fig. 6.A-B).

Finally, additional pathways involved in $VPAC_1$ receptor modulation of hippocampal TBS-induced LTP, were investigated investigating the targets of different intracellular kinases on Kv4.2 K^+

channels, previously reported to contribute to LTP expression by suppressing the A-current and facilitating action potential backpropagation (Frick et al., 2004; Rosenkranz et al., 2009). When Kv4.2 phosphorylation was inspected 50 min after *mild TBS (5x4)*, an enhancement (167.8±67.2%, n=4, Fig. 7.A-B) was observed in Ser438 phosphorylation (a site targeted by CaMKII). The total expression of Kv4.2 subunits was also enhanced (n=5, Fig 7. E-F), yet no changes (n=3-4) were observed in Kv4.2 phosphorylation in two ERK phosphorylation sites Thr607 (Fig. 7.C-D) and Thr602 (not shown in Fig. 7). Basal phosphorylation (control conditions) at these two sites was low. When adding the selective $VPAC_1$ receptor antagonist PG 97-269 (100nM) in the absence of TBS no significant changes in total Kv4.2 levels or in Kv4.2 phosphorylation were observed (n=4, P>0.05, Fig 7. A-F). When TBS was delivered in the presence of PG 97-269 (100nM) the enhancement in Kv4.2 phosphorylation in residue Ser438 was abolished (n=4) and an enhancement of 61.1±18.0%, in Thr607 phosphorylation (n=3) was now observed. Altogether this suggests that $VPAC_1$ receptor activation by VIP during TBS LTP induction has a restraining effect on Thr607 phosphorylation, while facilitating Ser438 phosphorylation thus preventing a large LTP to occur.

Discussion:

In the present work we describe for the first time that: 1) VIP, acting on $VPAC_1$ (but not $VPAC_2$) receptors, is an endogenous inhibitor of hippocampal LTP induced by mild TBS in young adult Wistar rats, an effect dependent on GABAergic transmission; 2) endogenous PACAP, acting on PAC_1 receptors, mildly inhibits LTP; 3) Tonic $VPAC_1$-mediated inhibition of hippocampal LTP depends on of NMDA and CaMKII-dependent LTP expression mechanisms and is independent on PKA and PKC activity and 4) Inhibition of LTP by tonic $VPAC_1$ activation enhances expression and Ser438 phosphorylation of Kv4.2 potassium channels and inhibits their Thr607 phosphorylation but does not depended on AMPA GluA1 phosphorylation.

The physiology of VIP-expressing interneurons in the hippocampus has been target of active investigation in recent years (Tyan et al., 2014; Turi et al., 2019; Luo et al., 2020) yet the physiological role of endogenous VIP in the control of hippocampal circuits remains largely unexplored (Cunha-Reis and Caulino-Rocha, 2020). Endogenous VIP, modulates GABA release from hippocampal nerve terminals (Cunha-Reis et al., 2017), hippocampal CA1 LTD (Cunha-Reis et al., 2014), and is required for spatial discrimination in water maze learning (Glowa et al., 1992). The progeny of VIP-deficient female mice shows marked cognitive impairment in hippocampal-dependent tasks (Hill et al., 2007; Stack et al., 2008). VIP neuropeptide, stored in large dense-core granules, is released by long-lasting depolarization or high-frequency neuronal firing (Leenders et al., 1999), as likely occurs during TBS to allow its physiological control of CA1 LTP. Since our preliminary data suggested VIP was an endogenous modulator of hippocampal CA1 TBS-induced LTP (Cunha-Reis et al., 2010), we investigated the cellular mechanisms and hippocampal receptors mediating these effects, that may constitute important pharmacological targets to modulate hippocampal-dependent memory processes in pathological conditions like epilepsy, aging or neurodevelopmental disorders (Cunha-Reis et al., 2021).

VIP acts through activation of two high-affinity receptors, $VPAC_1$ and $VPAC_2$ (Cunha-Reis et al., 2005, 2006, 2017) and is expressed in CA1 area in at least three different interneuron populations, basket cells (BCs, Fig. 8) which synapse mainly on pyramidal cell bodies and proximal dendrites, and several interneuron-specific (IS) interneuron populations (Acsády et al., 1996a, 1996b). We show that endogenous modulation of TBS-induced LTP by VIP depends on $VPAC_1$ receptor activation and GABAergic transmission, suggesting a mechanism involving disinhibition.

VIP-expressing BCs in hippocampal CA1, about 8% of the VIP-expressing interneurons in the hippocampus, are the main source of VIP that activates $VPAC_2$ receptors, since $VPAC_2$ receptors are mainly expressed in the *stratum piramidale (SP)* (Vertongen et al., 1997; Joo et al., 2004), where most VIP-expressing BCs synapses reside (Acsády et al., 1996b), and do not influence synaptic transmission to pyramidal cell dendrites. These effects may also be endogenously mediated by

PACAP, present in *SP* glutamatergic synapses (Solés-Tarrés et al., 2020). Although $VPAC_2$ receptor activation can enhance NMDA currents in CA1 pyramidal cells (Yang et al., 2009), it was not involved in the endogenous modulation of TBS-induced LTP reported in this work, suggesting BCs are not the cellular mediators of this effect.

$VPAC_1$ receptors, mainly expressed in the *stratum oriens (SO)* and *stratum radiatum (SR)* of the Ammon's Horn (Vertongen et al., 1997; Joo et al., 2004), can co-localize with glial markers, and mediate VIP enhancement of synaptic transmission to pyramidal cell dendrites by promoting disinhibition through pre- and postsynaptic modulation of GABAergic transmission (Cunha-Reis et al., 2004, 2005). Although immunohistochemistry never demonstrated $VPAC_1$ receptors in hippocampal interneurons, suggesting $VPAC_1$-mediated actions could be due to glial regulation of synaptic GABA availability, $VPAC_1$ receptor activation inhibits GABA release from hippocampal synaptosomes (Cunha-Reis et al., 2017), indicating the presence of $VPAC_1$ receptors in GABAergic synapses.

$VPAC_1$ receptor activation also mildly enhances NMDA currents in CA1 pyramidal cells (Yang et al., 2009), suggesting endogenous VIP or PACAP can directly influence NMDA-dependent hippocampal LTP. Moreover, enhancement in VIPergic inputs to parvalbumin-expressing BCs in the CA3 is implicated in hippocampal-dependent spatial learning (Donato et al., 2013). Yet, $VPAC_1$ receptor-mediated modulation of CA1 NMDA receptor-dependent LTD (Cunha-Reis et al., 2014) involves control of disinhibition, uncovering the importance of VIP-expressing IS interneurons in synaptic plasticity. In fact, disinhibition is crucial in the control of hippocampal synaptic plasticity (Artinian and Lacaille, 2018) and synaptic inhibition at pyramidal cell dendrites is crucial for Ca^{2+}-dependent input selectivity and precision of LTP induction (Müllner et al., 2015). The now described modulation of CA1 LTP by endogenous VIP is dependent on GABAergic transmission, implying that VIPergic modulation of hippocampal CA1 LTP targets disinhibition.

Several populations of CA1 VIP-expressing IS interneurons (Table 1, Fig. 8) may initiate VIP regulation of disinhibition (Acsády et al., 1996b, 1996a): 1) Interneurons expressing VIP and calretinin, with cell bodies near the *SP* and projecting to the *SO-alveus* border (VIP^+ CR^+ O/A or IS3 interneurons); 2) VIP-expressing interneurons located at the SR-SP border (VIP^+ $CR^{+/-}$ SR) or 3) at the *SR-SLM* border (VIP^+ IS2 interneurons) and projecting to the SR (Acsády et al., 1996b, 1996a) and 3) VIP long-range projecting (LRP) interneurons (Francavilla et al., 2018). Their main synaptic inputs, neurochemical markers and target selectivity are summarized in Table 1 yet given their complex interconnections it is challenging to infer which generates the tonic actions on $VPAC_1$ receptors inhibiting TBS-induced LTP. While VIP^+ IS2 interneurons are mainly targeted by the temporoammonic (TA) pathway, and thus not likely recruited by Schaffer collateral (SC) electrical stimulation, the dendrites of IS3 and VIP^+ $CR^{+/-}$ SR interneurons span all layers and are likely activated by both SC and TA pathways yet IS3 interneurons receive a predominant inhibitory drive from both VIP^+ IS2 and CR^+

IS1 interneurons (Luo et al., 2020) and likely GABAergic projections from the medial septum, important in regulation of hippocampal theta activity (Papp et al., 1999; Borhegyi et al., 2004). Although VIP-expressing IS3 interneurons play a crucial role in goal-directed spatial learning tasks (Turi et al., 2019) and are mainly recruited during theta oscillations (Luo et al., 2020), their activation during TBS-induced LTP may have little impact under SC stimulation, since OLM interneurons control the somatic propagation of otherwise spatially confined TA inputs to pyramidal cells during theta (Ang et al., 2005).

VIP^+ $CR^{+/-}$ SR interneurons are considerably less studied than IS3 or IS2 cells, but the neurochemical profile of their synaptic targets suggests they innervate SR CB^+ and CCK^+ Schaffer collateral-associated (SCA) interneurons (Acsády et al., 1996b; Turi et al., 2019), involved in feedforward inhibition (FFI) to pyramidal cell dendrites. TBS-induced LTP relies on the suppression of phasic FFI by the first burst, that allows for enough depolarization and temporal summation to activate NMDA receptors (Larson et al., 1986; Pacelli et al., 1989; Davies et al., 1990), canonically attributed to inhibition of GABA release from feedforward interneurons by $GABA_B$ autoreceptors (Cobb et al., 1999) yet disinhibition may play an important role in this process. FFI to CA1 pyramidal cells is mediated by physiologically and morphologically distinct GABAergic interneurons: perisomatic-targeting BCs and dendritic-targeting bistratified cells and SCA cells (Elfant et al., 2008; Ferrante and Ascoli, 2015). Our results suggest that VIP^+ $CR^{+/-}$ SR interneurons provide maximal feedforward disinhibition (and VIP release) during TBS, unleashing LTP at proximal pyramidal cell dendrites, targeted by the SC pathway. VIP $VPAC_1$ receptor-mediated inhibition of GABA release (Cunha-Reis et al., 2017) at VIP^+ $CR^{+/-}$ SR to SCA interneuron synapses during theta activity would curtail disinhibition. Accordingly, upon $VPAC_1$ receptor blockade, disinhibition would increase, eliciting the observed enhancement of TBS-induced LTP. Nevertheless, a few IS3 and VIP^+ LRP interneurons target bistratified interneurons (Francavilla et al., 2018; Luo et al., 2020) and can thus elicit this same effect. In agreement, we showed that increasing synaptic GABA availability through partial inhibition of GAT-1 reduced the effect of $VPAC_1$ receptor activation on TBS-induced LTP, suggesting that $VPAC_1$ receptors prevent GABA spill over that could activate tonic instead of phasic inhibition, that shunts neuronal excitability, controls neuronal input-output gain and involves distinct extra-synaptic $GABA_A$ receptors (Farrant and Nusser, 2005; Brickley and Mody, 2012). By controlling synaptic GABA levels (Cunha-Reis et al., 2017), VIP acting on presynaptic $VPAC_1$ receptors may provide timing precision to theta oscillation control by phasic inhibition.

In this work tonic $VPAC_1$ receptor activation during TBS-induced LTP did not significantly influence AMPA receptor GluA1 phosphorylation, either at Ser845 or Ser831, in agreement with previous observations showing that activation of hippocampal $VPAC_2$ (but not $VPAC_1$) receptors

promote GluA1 phosphorylation at Ser845 (Toda and Huganir, 2015), a PKA target site that is implicated in LTP maintenance and late-LTP (Benke and Traynelis, 2019).

Finally, we describe that tonic $VPAC_1$ receptor activation during TBS-induced LTP facilitates Ser438 phosphorylation and Kv4.2 expression yet restrains Kv4.2 Thr607 phosphorylation thus restraining LTP. Up-regulation of dendritic Kv4.2 mRNA by NMDA receptor activation (Jo and Kim, 2011) and TBS-induced enhancement of Kv4.2 phosphorylation in Ser438 following LTP in the hippocampus (Rodrigues et al., 2021) have previously been reported but the effect of $VPAC_1$ receptor activation is utterly new.

In conclusion, tonic $VPAC_1$ receptor activation during TBS-induced LTP in the CA1 area of the hippocampus restrains LTP induction and expression by regulating inhibition of feedforward inhibitory cells. This brings into question the hypothesis VIP $VPAC_1$ receptor ligands, by fine-tuning disinhibition, could constitute efficient and safer drugs to treat cognitive decline in aging or epilepsy.

References:

Acsády, L., Arabadzisz, D., and Freund, T.F. (1996a). Correlated morphological and neurochemical features identify different subsets of vasoactive intestinal polypeptide-immunoreactive interneurons in rat hippocampus. Neuroscience *73*: 299–315.

Acsády, L., Görcs, T.J., and Freund, T.F. (1996b). Different populations of vasoactive intestinal polypeptide-immunoreactive interneurons are specialized to control pyramidal cells or interneurons in the hippocampus. Neuroscience *73*: 317–334.

Aidil-Carvalho, M.F., Carmo, A.J.S., Ribeiro, J.A., and Cunha-Reis, D. (2017). Mismatch novelty exploration training enhances hippocampal synaptic plasticity: A tool for cognitive stimulation? Neurobiol. Learn. Mem. *145*: 240–250.

Anderson, W.W., and Collingridge, G.L. (2001). The LTP Program: a data acquisition program for on-line analysis of long-term potentiation and other synaptic events. J Neurosci Methods *108*: 71–83.

Ang, C.W., Carlson, G.C., and Coulter, D.A. (2005). Hippocampal CA1 circuitry dynamically gates direct cortical inputs preferentially at theta frequencies. J. Neurosci. *25*: 9567–9580.

Appleby, V.J., Corrêa, S.A.L., Duckworth, J.K., Nash, J.E., Noël, J., Fitzjohn, S.M., et al. (2011). LTP in hippocampal neurons is associated with a CaMKII-mediated increase in GluA1 surface expression. J Neurochem *116*: 530–543.

Artinian, J., and Lacaille, J.C. (2018). Disinhibition in learning and memory circuits: New vistas for somatostatin interneurons and long-term synaptic plasticity. Brain Res. Bull. *141*: 20–26.

Benke, T., and Traynelis, S.F. (2019). AMPA-Type Glutamate Receptor Conductance Changes and Plasticity: Still a Lot of Noise. Neurochem. Res. *44*: 539–548.

Borhegyi, Z., Varga, V., Szilágyi, N., Fabo, D., and Freund, T.F. (2004). Phase segregation of medial septal GABAergic neurons during hippocampal theta activity. J. Neurosci. *24*: 8470–8479.

Brickley, S.G., and Mody, I. (2012). Extrasynaptic GABA A Receptors: Their Function in the CNS and Implications for Disease. Neuron *73*: 23–34.

Chijiwa, T., Mishima, A., Hagiwara, M., Sano, M., Hayashi, K., Inoue, T., et al. (1990). Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma . J Biol Chem *265*: 5267–5272.

Ciranna, L., and Cavallaro, S. (2003). Opposing effects by pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal peptide on hippocampal synaptic transmission. Exp. Neurol. *184*: 778–784.

Cobb, S.R., Manuel, N.A., Morton, R.A., Gill, C.H., Collingridge, G.L., and Davies, C.H. (1999). Regulation of depolarizing GABA(A) receptor-mediated synaptic potentials by synaptic activation of GABA(B) autoreceptors in the rat hippocampus. Neuropharmacology *38*: 1723–1732.

Cooke, S.F., Wu, J., Plattner, F., Errington, M., Rowan, M., Peters, M., et al. (2006). Autophosphorylation of αCaMKII is not a general requirement for NMDA receptor-dependent LTP in the adult mouse. J. Physiol. *574*: 805–818.

Cunha-Reis, D., Aidil-Carvalho, F., and Ribeiro, J.A. (2014). Endogenous inhibition of hippocampal LTD and depotentiation by vasoactive intestinal peptide VPAC1 receptors. Hippocampus *24*: 1353–1363.

Cunha-Reis, D., and Caulino-Rocha, A. (2020). VIP Modulation of Hippocampal Synaptic Plasticity: A Role for VIP Receptors as Therapeutic Targets in Cognitive Decline and Mesial Temporal Lobe Epilepsy. Front. Cell. Neurosci. *14*: 153.

Cunha-Reis, D., Caulino-Rocha, A., and Correia-de-Sá, P. (2021). VIPergic neuroprotection in epileptogenesis: challenges and opportunities. Pharmacol. Res. *164*: 105356.

Cunha-Reis, D., Ribeiro, J.A., Almeida, R.F.M. de, and Sebastião, A.M. (2017). VPAC1 and VPAC2 receptor activation on GABA release from hippocampal nerve terminals involve several different signalling pathways. Br. J. Pharmacol. *174*: 4725–4737.

Cunha-Reis, D., Ribeiro, J.A., and Sebastião, A.M. (2005). VIP enhances synaptic transmission to hippocampal CA1 pyramidal cells through activation of both VPAC1 and VPAC2 receptors. Brain Res. *1049*: 52–60.

Cunha-Reis, D., Ribeiro, J.A., and Sebastião, A.M. (2006). VPAC2 receptor activation mediates VIP enhancement of population spikes in the CA1 area of the hippocampus. Ann. N. Y. Acad. Sci. *1070*: 210–214.

Cunha-Reis, D., Rodrigues, N.C., and Ribeiro, J.A. (2010). On the cellular and molecular pathways involved in the inhibition of LTP in the CA1 area of the hippocampus. J. Mol. Neurosci. *42*: 278.

Cunha-Reis, D., Sebastião, A.M., Wirkner, K., Illes, P., and Ribeiro, J.A. (2004). VIP enhances both pre- and postsynaptic GABAergic transmission to hippocampal interneurones leading to increased excitatory synaptic transmission to CA1 pyramidal cells. Br. J. Pharmacol. *143*: 733–744.

Davies, C.H., and Collingridge, G.L. (1996). Regulation of EPSPs by the synaptic activation of GABA(B) autoreceptors in rat hippocampus. J. Physiol. *496*: 451–470.

Davies, C.H., Davies, S.N., and Collingridge, G.L. (1990). Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. J Physiol *424*: 513–531.

Donato, F., Rompani, S.B., and Caroni, P. (2013). Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. Nature *504*: 272–276.

Elfant, D., Pál, B.Z., Emptage, N., and Capogna, M. (2008). Specific inhibitory synapses shift the balance from feedforward to feedback inhibition of hippocampal CA1 pyramidal cells. Eur. J. Neurosci. *27*: 104–113.

Farrant, M., and Nusser, Z. (2005). Variations on an inhibitory theme: Phasic and tonic activation of GABA A receptors. Nat. Rev. Neurosci. *6*: 215–229.

Ferrante, M., and Ascoli, G.A. (2015). Distinct and synergistic feedforward inhibition of pyramidal cells by basket and bistratified interneurons. Front. Cell. Neurosci. *9*: 439.
Francavilla, R., Villette, V., Luo, X., Chamberland, S., Muñoz-Pino, E., Camiré, O., et al. (2018). Connectivity and network state-dependent recruitment of long-range VIP-GABAergic neurons in the mouse hippocampus. Nat. Commun. *9*:.
Frick, A., Magee, J., and Johnston, D. (2004). LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. Nat. Neurosci. *7*: 126–35.
Glowa, J.R., Panlilio, L. V, Brenneman, D.E., Gozes, I., Fridkin, M., and Hill, J.M. (1992). Learning impairment following intracerebral administration of the HIV envelope protein gp120 or a VIP antagonist. Brain Res *570*: 49–53.
Gourlet, P., Neef, P. de, Cnudde, J., Waelbroeck, M., and Robberecht, P. (1997). In vitro properties of a high affinity selective antagonist of the VIP1 receptor. Peptides *18*: 1555–1560.
Haas, H.L., and Gähwiler, B.H. (1992). Vasoactive intestinal polypeptide modulates neuronal excitability in hippocampal slices of the rat. Neuroscience *47*: 273–277.
Hill, J.M., Cuasay, K., and Abebe, D.T. (2007). Vasoactive intestinal peptide antagonist treatment during mouse embryogenesis impairs social behavior and cognitive function of adult male offspring. Exp. Neurol. *206*: 101–13.
Itoh, S., Takashima, A., and Morimoto, T. (1994). Impaired spatial learning by vasoactive intestinal peptide in Morris water maze task in the rat. Can. J. Physiol. Pharmacol. *72*: 25–29.
Ivanova, M., Ternianov, A., Tashev, R., Belcheva, S., and Belcheva, I. (2009). Lateralized learning and memory effects of vasoactive intestinal peptide infused into the rat hippocampal CA1 area. Regul. Pept. *156*: 42–46.
Jo, A., and Kim, H.K. (2011). Up-regulation of dendritic Kv4.2 mRNA by activation of the NMDA receptor. Neurosci. Lett. *496*: 129–134.
Joo, K.M., Chung, Y.H., Kim, M.K., Nam, R.H., Lee, B.L., Lee, K.H., et al. (2004). Distribution of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide receptors (VPAC1, VPAC2, and PAC1 receptor) in the rat brain. J. Comp. Neurol. *476*: 388–413.
Larson, J., and Lynch, G. (1988). Role of N-methyl-D-aspartate receptors in the induction of synaptic potentiation by burst stimulation patterned after the hippocampal theta-rhythm. Brain Res *441*: 111–118.
Larson, J., and Munkácsy, E. (2015). Theta-burst LTP. Brain Res. *1621*: 38–50.
Larson, J., Wong, D., and Lynch, G. (1986). Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. Brain Res *368*: 347–350.
Lee, H.K., Takamiya, K., Han, J.S., Man, H., Kim, C.H., Rumbaugh, G., et al. (2003). Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory.

Cell *112*: 631–643.
Leenders, A.G., Scholten, G., Wiegant, V.M., Silva, F.H. Da, and Ghijsen, W.E. (1999). Activity-dependent neurotransmitter release kinetics: correlation with changes in morphological distributions of small and large vesicles in central nerve terminals. Eur J Neurosci *11*: 4269–4277.
Luo, X., Guet-Mccreight, A., Villette, V., Francavilla, R., Marino, B., Chamberland, S., et al. (2020). Synaptic Mechanisms Underlying the Network State-Dependent Recruitment of VIP-Expressing Interneurons in the CA1 Hippocampus. Cereb. Cortex *30*: 3667–3685.
Moreno, D., Gourlet, P., Neef, P. De, Cnudde, J., Waelbroeck, M., and Robberecht, P. (2000). Development of selective agonists and antagonists for the human vasoactive intestinal polypeptide VPAC(2) receptor. Peptides *21*: 1543–1549.
Müllner, F.E., Wierenga, C.J., and Bonhoeffer, T. (2015). Precision of Inhibition: Dendritic Inhibition by Individual GABAergic Synapses on Hippocampal Pyramidal Cells Is Confined in Space and Time. Neuron *87*: 576–589.
Pacelli, G.J., Su, W., and Kelso, S.R. (1989). Activity-induced depression of synaptic inhibition during LTP-inducing patterned stimulation. Brain Res. *486*: 26–32.
Papp, E.C., Hajos, N., Acsády, L., and Freund, T.F. (1999). Medial septal and median raphe innervation of vasoactive intestinal polypeptide-containing interneurons in the hippocampus. Neuroscience *90*: 369–382.
Robberecht, P., Gourlet, P., Neef, P. De, Woussen-Colle, M.C., Vandermeers-Piret, M.C., Vandermeers, A., et al. (1992). Structural requirements for the occupancy of pituitary adenylate-cyclase-activating-peptide (PACAP) receptors and adenylate cyclase activation in human neuroblastoma NB-OK-1 cell membranes. Discovery of PACAP(6-38) as a potent antagonist. Eur J Biochem *207*: 239–246.
Roberto, M., Scuri, R., and Brunelli, M. (2001). Differential effects of PACAP-38 on synaptic responses in rat hippocampal CA1 region. Learn. Mem. *8*: 265–271.
Rodrigues, N.C., Silva-Cruz, A., Caulino-Rocha, A., Bento-Oliveira, A., Ribeiro, J.A., and Cunha-Reis, D. (2021). Cellular and molecular mechanisms involved in LTP induced by mild theta-burst stimulation in hippocampal slices from young male rats: from weaning to adulthood. Eur. J. Neurosci. *und review*:
Rose, G.M., and Dunwiddie, T. V (1986). Induction of hippocampal long-term potentiation using physiologically patterned stimulation. Neurosci Lett *69*: 244–248.
Rosenkranz, J.A., Frick, A., and Johnston, D. (2009). Kinase-dependent modification of dendritic excitability after long-term potentiation. J. Physiol. *587*: 115–125.
Solés-Tarrés, I., Cabezas-Llobet, N., Vaudry, D., and Xifró, X. (2020). Protective Effects of Pituitary Adenylate Cyclase-Activating Polypeptide and Vasoactive Intestinal Peptide Against Cognitive Decline in Neurodegenerative Diseases. Front. Cell. Neurosci. *14*: 221.

Stack, C.M., Lim, M.A., Cuasay, K., Stone, M.M., Seibert, K.M., Spivak-Pohis, I., et al. (2008). Deficits in social behavior and reversal learning are more prevalent in male offspring of VIP deficient female mice. Exp. Neurol. *211*: 67–84.

Takashima, A., Maeda, Y., and Itoh, S. (1993a). Influence of chronic intracerebroventricular infusion of vasoactive intestinal peptide (VIP) on memory processes in Morris water pool test in the rat. Peptides *14*: 1073–1078.

Takashima, A., Maeda, Y., and Itoh, S. (1993b). Vasoactive intestinal peptide (VIP) causes memory impairment in passive avoidance responding of the rat. Peptides *14*: 1067–1071.

Toda, A.M.A., and Huganir, R.L. (2015). Regulation of AMPA receptor phosphorylation by the neuropeptide PACAP38. Proc. Natl. Acad. Sci.

Toullec, D., Pianetti, P., Coste, H., Bellevergue, P., Grand-Perret, T., Ajakane, M., et al. (1991). The bisindolylmaleimide GF 109203X is a potent and selective inhibitor of protein kinase C. J Biol Chem *266*: 15771–15781.

Turi, G.F., Li, W.K., Chavlis, S., Pandi, I., O'Hare, J., Priestley, J.B., et al. (2019). Vasoactive Intestinal Polypeptide-Expressing Interneurons in the Hippocampus Support Goal-Oriented Spatial Learning. Neuron *101*: 1150-1165.e8.

Tyan, L., Chamberland, S., Magnin, E., Camiré, O., Francavilla, R., Suzanne David, L., et al. (2014). Dendritic inhibition provided by interneuron-specific cells controls the firing rate and timing of the hippocampal feedback inhibitory circuitry. J. Neurosci. *34*: 4534–4547.

Vertes, R.P. (2005). Hippocampal theta rhythm: a tag for short-term memory. Hippocampus *15*: 923–935.

Vertongen, P., Schiffmann, S.N., Gourlet, P., and Robberecht, P. (1997). Autoradiographic Visualization of the Receptor Subclasses for Vasoactive Intestinal Polypeptide (VIP) in Rat Brain. Peptides *18*: 1547–1554.

Wang, H.L., Li, A., and Wu, T. (1997). Vasoactive intestinal polypeptide enhances the GABAergic synaptic transmission in cultured hippocampal neurons. Brain Res. *746*: 294–300.

Yang, K., Trepanier, C.H., Li, H., Beazely, M.A., Lerner, E.A., Jackson, M.F., et al. (2009). Vasoactive intestinal peptide acts via multiple signal pathways to regulate hippocampal NMDA receptors and synaptic transmission. Hippocampus *19*: 779–789.

	Dendritic tree	***Soma***	***Inputs***	***Projections***	***Target cells in CA1***
VIP$^+$ CCK$^+$ BCs	*Stratum Piramidale*	*Stratum Piramidale*	Schaffer collateral /commissural fibres	*Stratum Piramidale* and near *radiatum*	Pyramidal neurons
VIP$^+$ CR$^+$ IS2 INs	*Stratum lacunosum-moleculare*	*Stratum Radiatum / lacunosum-moleculare border*	Temporoammonic EC fibres	*Stratum radiatum*	VIP$^+$ CCK$^+$ BCs VIP$^+$ CR$^+$IS3 INs CB$^+$ SC-associated INs
VIP$^+$ CR$^+$ IS3 INs	All layers	*Stratum Piramidale/ radiatum border*	Schaffer collateral /commissural fibres Temporoammonic EC fibres IS1 and IS2 INs	*Stratum Oriens & Alveus*	Mostly OLM INs but also other *Oriens/Alveus* INs projecting to the *SLM*. BCs Bistratified cells
VIP$^+$ CR$^{+/-}$ SR/SP INs	All layers	*Stratum Piramidale/ radiatum border*	Schaffer collateral /commissural fibres	*Stratum radiatum and piramidale*	BCs Bistratified cells CB$^+$ SC-associated INs
VIP$^+$ LRP INs	*Stratum Oriens*	*Stratum Oriens*	Schaffer collateral /commissural fibres	*Stratum Oriens/ Piramidale* *Subiculum*	Mostly OLM INs BCs Bistratified cells Pyramidal cells and INs

Table 1 – Neuroanatomical features and connectivity of local-bound VIP-expressing interneurons in the CA1 area of the hippocampus. BCs – basket cells; CB – calbindin 28K; CCK - cholecystokinin; CR - calretinin; EC – Entorhinal cortex; INs – interneurons; IS – interneuron-specific; LRP – long-range projection; OLM – *Oriens-Lacunosum moleculare* (interneurons); SC – Schaffer-collateral; SP – *Stratum piramidale*; SR- *stratum radiatum*; VIP – vasoactive intestinal peptide(X$^+$); - expressing; (X$^-$) – non-expressing (Acsády et al., 1996a, 1996b; Tyan et al., 2014; Turi et al., 2019; Luo et al., 2020).

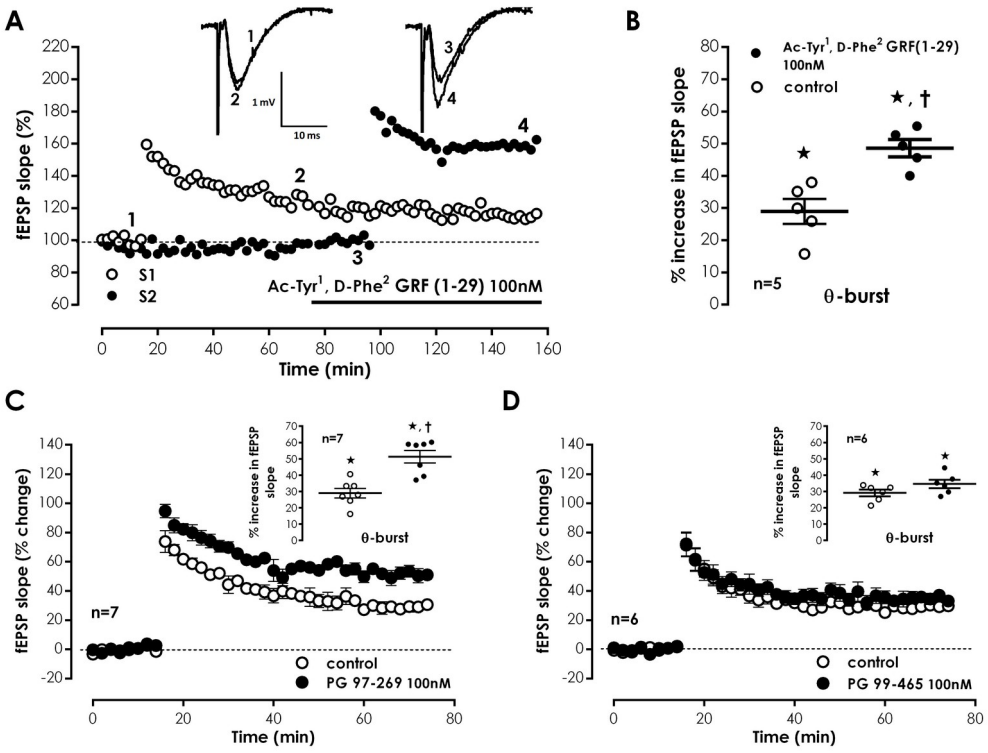

Figure 1 – Endogenous VIP impairs hippocampal CA1 long-term potentiation of synaptic transmission through $VPAC_1$ receptor activation. A. Time-course of changes in fEPSP slope caused by theta-burst stimulation (5 bursts at 5 Hz, each composed of four pulses at 100 Hz) in a typical experiment for which a control pathway (-○-, S1) was stimulated in the absence of added drugs and a test pathway (-●-, S2) was stimulated in the presence of the broad range VIP receptor antagonist Ac-Tyr[1], D-Phe[2] GRF(1-29) (100nM) in the same slice. ***Inset:*** Traces of fEPSPs obtained in the same experiment before (time points: **1** for S1 and **3** for S2) and 50-60 min after (time points: **2** for S1 and **4** for S2) theta burst stimulation. Traces are the average of eight consecutive responses and are composed of the stimulus artifact, the presynaptic volley and the fEPSP. **B.** LTP magnitude estimated from the averaged enhancement of fEPSP slope observed 50-60 min after theta-burst stimulation in the absence of added drugs (○) or in the presence (●) of Ac-Tyr[1], D-Phe[2] GRF(1-29) (100nM). **C.** and **D.** Averaged time-course of changes in fEPSP slope caused by theta-burst stimulation in the absence of added drugs (-○-) and in the presence of either the selective $VPAC_1$ antagonist PG 97-269 (100nM, -●-, **C.**) or the selective $VPAC_2$ antagonist PG 99-465 (100nM, -●-, **D.**). ***Inset:*** Magnitude of LTP estimated from the averaged enhancement of fEPSP slope observed 50-60 min after theta-burst stimulation in the absence of added drugs (○) or in the presence (●) of either PG 97-269 (100nM, **C.**) or PG 99-465 (100nM, **D.**). Individual values and the mean ± S.E.M are depicted (**B.** - **D.**). *$p < 0.05$ (Student's t test) as compared to the fEPSP slope before LTP induction; †$p < 0.05$ (paired Student's t-test) as compared with absence of drugs in the same slices (○, in the left).

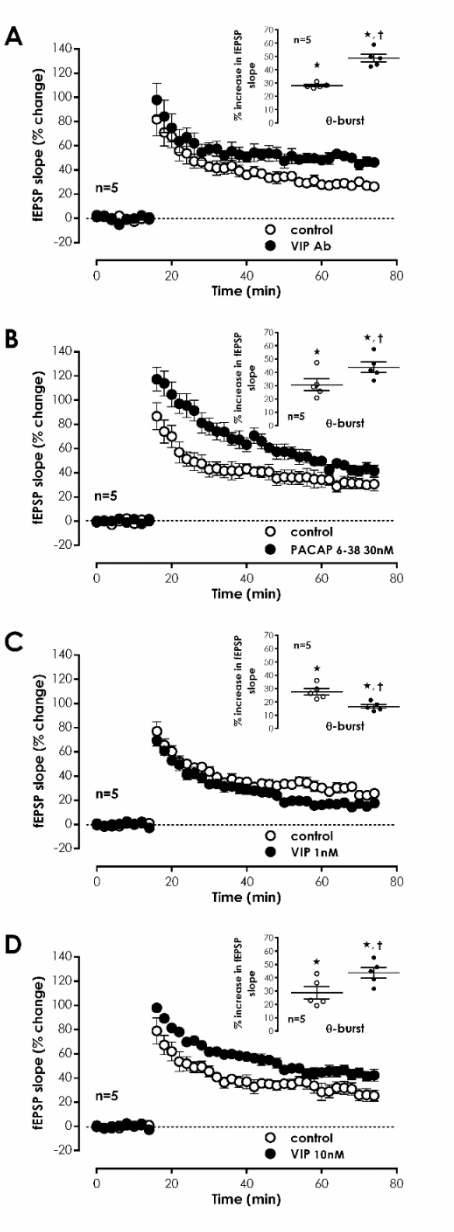
A
fEPSP slope (% change)
Time (min)
n=5
control
VIP Ab
% increase in fEPSP slope
θ-burst
B
PACAP 6-38 30nM
C
VIP 1nM
D
VIP 10nM

Figure 2 – Tonic actions of VIP and PACAP control hippocampal CA1 LTP. A. to **D.** Averaged time-course of changes in fEPSP slope caused by theta-burst stimulation in the absence of added drugs (-○-) and in the presence (-●-) of either a selective VIP antibody (0.4 µg/ml, **A.**), the selective PAC_1 antagonist PACAP 6-38 (30nM, **B.**), VIP (1nM, **C.**) or VIP (10nM, -**D.**). Control and test conditions (absence and presence of drugs) were tested in independent pathways in the same slice. ***Inset:*** Magnitude of LTP estimated from the averaged enhancement of fEPSP slope observed 50-60 min after theta-burst stimulation in the absence of added drugs (○) or in the presence (●) of either a VIP antibody (0.4 µg/ml, **A.**) the selective PAC_1 antagonist PACAP 6-38 (30nM, **B.**) or VIP in two different concentrations (1nM, **C.** and 10nM, **D.**). Individual values and the mean ± S.E.M are depicted. *$p < 0.05$ (Student's t test) as compared to the fEPSP slope before LTP induction; †$p < 0.05$ (paired Student's t-test) as compared with absence of drugs in the same slices (○, in the left).

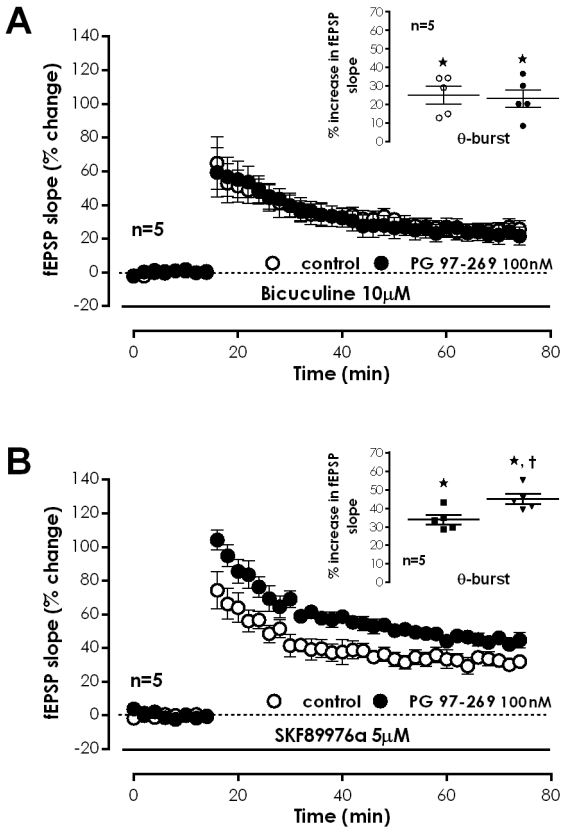

Figure 3 – VIP VPAC1 receptor mediated endogenous inhibition of hippocampal CA1 LTP is dependent on GABAergic transmission. A. and **B.** Averaged time-course of changes in fEPSP slope caused by theta-burst stimulation in the absence (-○-) and in the presence (-●-) of the selective $VPAC_1$ antagonist PG 97-269 (100nM) when either the selective $GABA_A$ receptor antagonist Bicuculline (10μM, **A.**) or the selective GAT_1 GABA transporter inhibitor SKF8997A (5μM, **B.**) were present throughout the experiment. Control and test conditions (absence and presence of PG 97-269) were tested in independent pathways in the same slice. ***Inset:*** Magnitude of LTP estimated from the averaged enhancement of fEPSP slope observed 50-60 min after theta-burst stimulation in the absence (○) or in the presence (●) the selective $VPAC_1$ antagonist PG 97-269 (100nM) when either the selective $GABA_A$ receptor antagonist Bicuculline (10μM, **A.**) or the selective GAT_1 GABA transporter inhibitor SKF8997A (5μM, **B.**) were present throughout the experiment. Individual values and the mean ± S.E.M are depicted. *p < 0.05 (Student's t test) as compared to the fEPSP slope before LTP induction; †p < 0.05 (paired Student's t-test) as compared with control conditions (absence of PG 97-269) in the same slices (○, in the left).

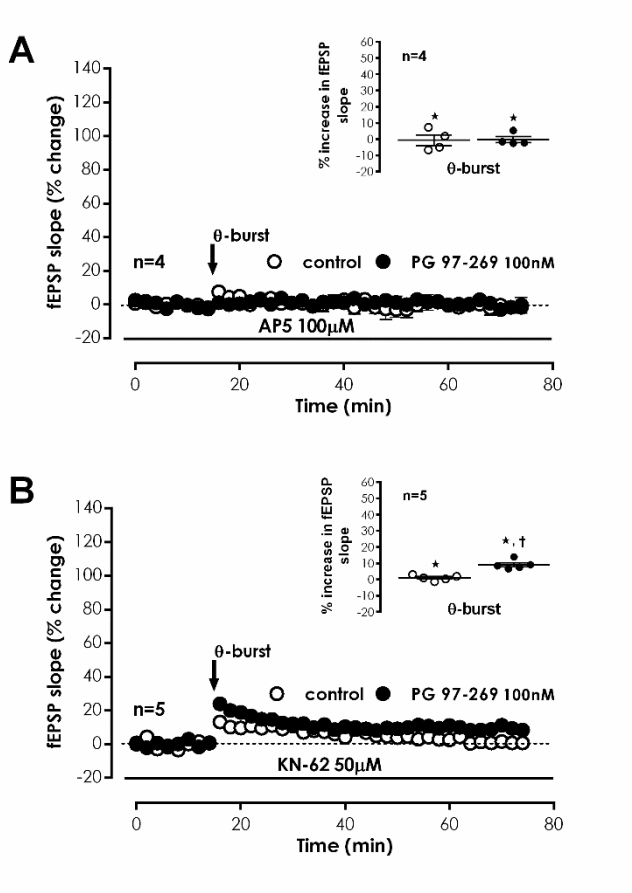

Figure 4 – VIP VPAC1 receptor mediated endogenous inhibition of hippocampal CA1 LTP is dependent on NMDA activation and CaMKII activity. A. and **B.** Averaged time-course of changes in fEPSP slope caused by theta-burst stimulation in the absence (-○-) and in the presence (-●-) of the selective $VPAC_1$ antagonist PG 97-269 (100nM) when either the selective NMDA receptor antagonist AP5 (100μM, **A.**) or the selective CaMKII inhibitor KN-62 (50μM, **B.**) were present throughout the experiment. Control and test conditions (absence and presence of PG 97-269) were tested in independent pathways in the same slice. ***Inset:*** Magnitude of LTP estimated from the averaged enhancement of fEPSP slope observed 50-60 min after theta-burst stimulation in the absence (open bar) or in the presence (black bar) the selective $VPAC_1$ antagonist PG 97-269 (100nM) when either the selective NMDA receptor antagonist AP5 (100μM, **A.**) or the selective CaMKII inhibitor KN-62 (50μM, **B.**) were present throughout the experiment. Individual values and the mean ± S.E.M are depicted. *$p < 0.05$ (Student's t test) as compared to the fEPSP slope before LTP induction; †$p < 0.05$ (paired Student's t-test) as compared with control conditions (absence of PG 97-269) in the same slices (○, in the left).

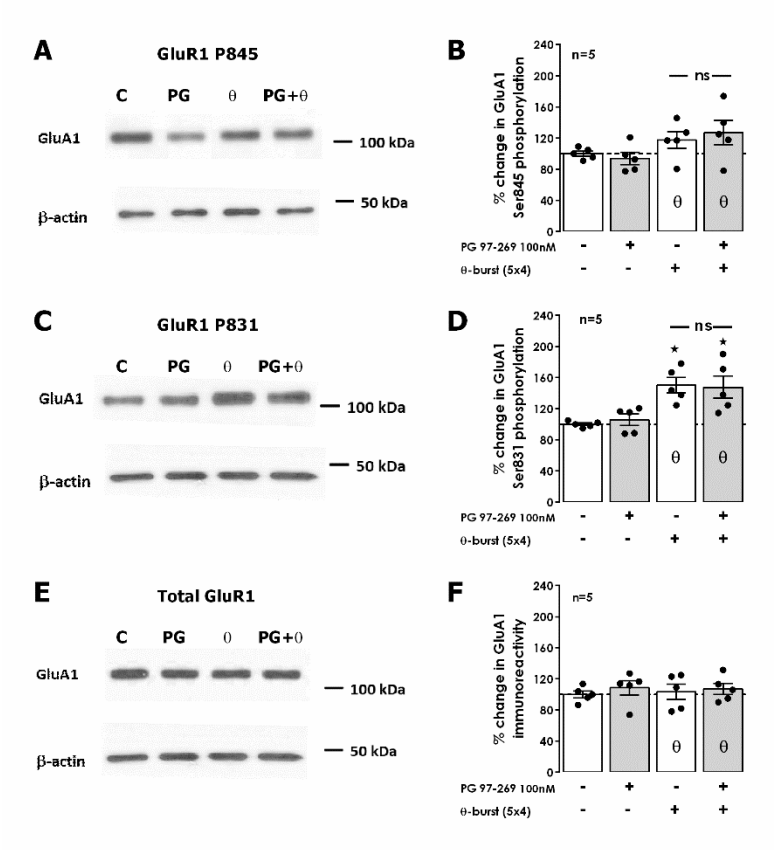

Figure 5 – Impact of VPAC1 receptor inhibition and of theta-burst stimulation on phosphorylation of hippocampal AMPA GluA1 subunits on Ser 845 and Ser 831. A., C., and **E.** Western-blot immunodetection of AMPA GluA1 phosphorylated forms in Ser845 and Ser831 and of total GluA1 subunits obtained in one individual experiment where hippocampal slices subjected to Schaffer collateral basal or theta-burst (θ) stimulation in the absence and in the presence (PG) of the selective $VPAC_1$ antagonist PG 97-269 (100nM). Slices were monitored for 50 min after theta-burst stimulation (or equivalent time for controls) before WB analysis. Total GluA1 immunoreactivity (**B.**) and **F.** % GluA1 phosphorylation in Ser845 (**D.**) or Ser831 (**F.**) residues are plotted. Individual values and the mean ± S.E.M of five independent experiments performed in duplicate are depicted. 100% - averaged GluA1 immunoreactivity or GluR1 phosphorylation obtained in control conditions (absence of theta-burst stimulation and PG 97-269). * P < 0.05 (*ANOVA*, Tukey's multiple comparison test) as compared to absence of PG 97-269 and theta-burst stimulation; ***ns*** represents non -significant differences P > 0.05 (*ANOVA*, Tukey's multiple comparison test) between respective bars.

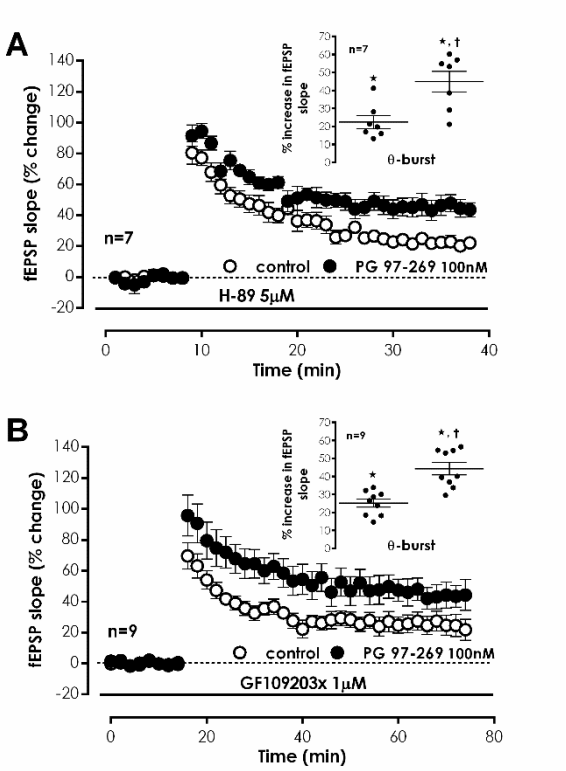

Figure 6 – VIP $VPAC_1$ receptor mediated endogenous inhibition of hippocampal CA1 LTP does not dependent on PKA and PKC activity. A. and **B.** Averaged time-course of changes in fEPSP slope caused by theta-burst stimulation in the absence (-○-) and in the presence (-●-) of the selective $VPAC_1$ antagonist PG 97-269 (100nM) when either the selective PKA inhibitor H-89 (5μM, **A.**) or the selective PKC inhibitor GF109203x (1μM, **B.**) were present throughout the experiment. Control and test conditions (absence and presence of PG 97-269) were tested in independent pathways in the same slice. ***Inset:*** Magnitude of LTP estimated from the averaged enhancement of fEPSP slope observed 50-60 min after theta-burst stimulation in the absence (○) or in the presence (●) the selective $VPAC_1$ antagonist PG 97-269 (100nM) when either the selective PKA inhibitor H-89 (5μM, **A.**) or the selective PKC inhibitor GF109203x (1μM, **B.**) were present throughout the experiment. Individual values and the mean ± S.E.M are depicted. *$p < 0.05$ (Student's t test) as compared to the fEPSP slope before LTP induction; †$p < 0.05$ (paired Student's t-test) as compared with control conditions (absence of PG 97-269) in the same slices (○, in the left).

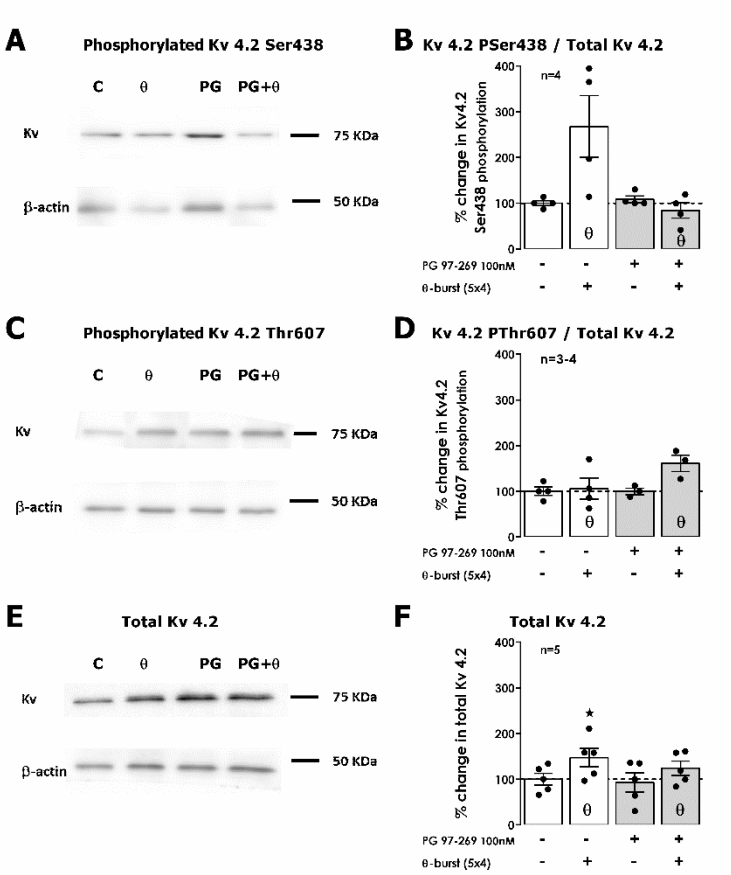

Figure 7 – Impact of $VPAC_1$ receptor inhibition and of theta-burst stimulation on phosphorylation of hippocampal Kv4.2 channels on Ser438, Thr802 and Thr807. Western-blot immunodetection of Kv4.2 phosphorylated forms in Ser438 (**A.**), Thr607 (**C.**) and total Kv4.2 (**F.**) obtained in one individual experiment where hippocampal slices were subjected to Schaffer collateral basal or theta-burst (**θ**) stimulation in the absence and in the presence (**PG**) of the selective $VPAC_1$ antagonist PG 97-269 (100nM). Slices were monitored for 50 min after theta-burst stimulation (or equivalent time for controls) before WB analysis. Total Kv4.2 (**F.**) immunoreactivity and phosphorylation in Ser438 (**B.**) or Thr607 (**D.**) residues normalized to the total variation in Kv4.2. Individual values and the mean ± S.E.M of 3-4 independent experiments performed in duplicate are depicted. 100% - averaged Kv4.2 immunoreactivity obtained in control conditions (absence of theta-burst stimulation for either no added drugs or the presence of the $VPAC_1$ antagonist PG 97-269 100nM). * represents $p < 0.05$ (*Student's t test*) as compared to absence of theta-burst stimulation.

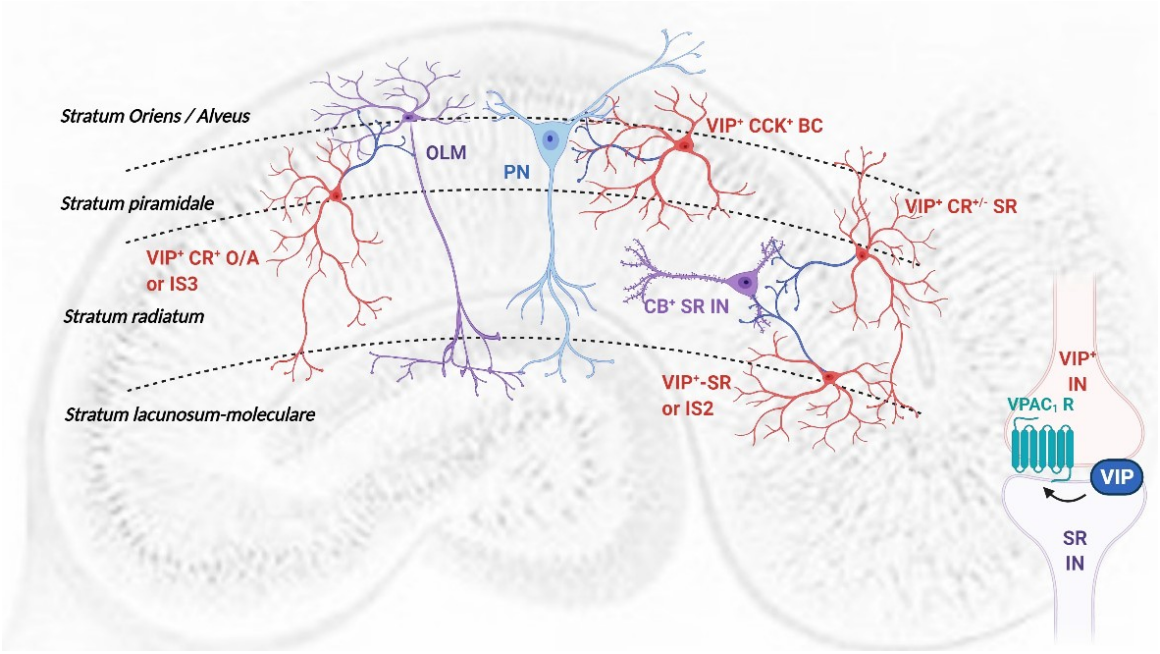

Figure 8 – Representation of local VIP-expressing interneurons in the CA1 area of the rat hippocampus: layer location and target selectivity. PN: pyramidal neuron (light blue); Interneurons (purple); VIP-containing interneurons (red) and their axonal projections (dark blue); OLM – *Stratum oriens* interneuron projecting to the *Stratum lacunosum-moleculare*; CB^+ SR IN – *Stratum radiatum* calbindin 28K-expressing local interneurons. *VIP^+-CCK^+ BCs*: VIP and CCK-expressing *basket cells*; *VIP^+ CR^+ O/A*: VIP and calretinin-containing interneuron-selective interneuron targeting the *stratum oriens / Alveus* or IS3 cells; *VIP^+ SR*: *stratum radiatum/lacunosum-moleculare* VIP-containing interneuron-selective interneuron targeting the *stratum radiatum* or IS2 cells and *VIP^+ $CR^{+/-}$ SR: stratum radiatum/ piramidale* VIP-containing interneuron-selective interneuron targeting the *stratum radiatum* (density of dendritic arborization is not represented; see Table 1 for details on main inputs).