THE CONSTITUTIVE ACTIVITY OF THE HISTAMINE H1 RECEPTOR INTERACTIONS WITH THE NMDA RECEPTOR : CONSEQUENCES IN EPILEPSY

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Summary

Objectives To explore the effects of H1 receptor signaling on NMDA and GABA responses.

Methods By making patch-clamp whole-cell recordings of neurons from primary rat hippocampal cultures, we observe the effects of several histaminergic agonists and antagonists on the rundown of NMDA and GABA currents..

Results Mepyramine, an H1 antagonist, at nanomolar concentrations increases significantly the NMDA responses by about 35 %, this effect is mimicked by another H1 antagonist, Triprolidine. Histamine alone has no effect on NMDA rundown but 100 μM Histamine can reduce partially the effect of Mepyramine. An H1 receptor agonist 2,3 bromophenyl histamine suppresses the effect of Mepyramine similar to Histamine. Our results suggest that the H1 receptor of Histamine is constitutively active, and its blockade by Mepyramine significantly increases the NMDA responses. Neither Mepyramine nor Histamine altered the rundown of GABA responses.

Significance Inhibition of H1 receptors with Mepyramine at nanomolar concentrations reveals an interaction between constitutive Histamine H1 receptor activity and the NMDAR responses. Curiously, Histamine alone does not alter NMDAR responses, but partially suppresses the effect of Mepyramine, suggesting Mepyramine acts as an inverse agonist to block the constitutive activity of the H1 receptor. The constitutive activity of the H1 receptor seems to be crucial in the regulation of NMDA receptor activity.

Keywords: Histamine H1 Receptor, Epilepsy, NMDA Receptor, Constitutive Activity

Keys Points

° The use of antihistaminic H1 drugs can facilitate the apparition of epileptic seizures,

°, Mepyramine, a highly potent H1 antagonist increased the NMDA responses during “run down” experiments, while Histamine as no effect.

° Mepyramine and Histamine show no effect on GABAR responses during “rundown” experiments.

° Constitutive activity of the Histamine H1 receptor seems to be crucial for the NMDA receptor activity.

INTRODUCTION

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As H1 antagonists are known to be proconvulsant and changes in both NMDA receptor (NMDAR) and $GABA_A$ receptor ($GABA_AR$) mediated currents are associated with epileptic neural activity, we sought to investigate possible effects of H1 antagonists on NMDAR and $GABA_AR$ receptor signalling.

Effects of Histamine are mediated by four Histamine receptor subtypes (H1, H2, H3, and H4), which are all G protein-coupled receptors. Blocking the H1 receptor (H1R) in the brain induces many “side effects“ such as a slowing of reaction times and somnolence (1). The first generation of H1R antagonists, including classical anti-allergy drugs, cause sedative and slowing effects on EEG (2) but have also been found to induce convulsions in healthy children and patients with epilepsy (3-5). H1R antagonists showed proconvulsive effects in mice and in rats (6,7). Moreover, H1R densities are increased in the brain in rats with genetically generalized epilepsies(8). suggesting that changes in H1R signalling may contribute to epileptogenesis.

The aetiology of epilepsy is complex. One putative mechanism triggering epileptic activity is the over-excitation of glutamatergic neurons via N-methyl-D-aspartate receptors (NMDARs) (9). The second mechanism implicates a reduction of GABAergic inhibition leading to the generation and spread of paroxysmal activities in neuronal networks(10).

Repetitive stimulation of either glutamatergic or GABAergic synapses in the rat hippocampus evokes, respectively, NMDAR and $GABA_AR$ receptor-mediated currents that progressively decrease in amplitude, a phenomenon referred to as “rundown”.

This electrophysiological approach is an interesting alternative to explore the mechanism of disease and the rundown has been found to be more pronounced in rodents with temporal lobe epilepsy (11) or in human partial epilepsy (12), suggesting it may provide important clues regarding the aetiology of this disease. The mechanism of the rundown was studied and is due to dephosphorylation of the $GABA_A$ receptors (13), in the case of NMDA current the blocking the

increase of intracellular calcium and increasing the intracellular ATP reduced rundown (14).

Finally, we reveal that the time course evolution of the current rundown correlates with the long-term action of anti-histaminergic drugs in the brain.

METHODS

Electrophysiological recording in neurons; Patch-clamp

Primary neuronal cultures from rat hippocampus and whole-cell patch-clamp recordings were performed as described (15). Ionic currents were recorded within large pyramidal neurons voltage clamped at -60 mV amplifier (AXOPATCH 1D). A rapid perfusion system was used for drug application. For the NMDAR experiments, the external solution for recording whole-cell currents contained 140 mM NaCl, 5 mM KCl, 1.2 mM CaCl2, 12 mM HEPES acid, 12 mM HEPES sodium, Glycine 5 µM, and 33 mM D-glucose. Tetrodotoxin (1 µM) was added to eliminate the voltage-gated sodium channel currents. The pH was 7.3 and the osmolarity was adjusted to 300 mOsm with D-glucose. The internal pipette solution contained 100 mM CsF, 40 mM CsCl, 1 mM CaCl2, 20 mM HEPES acid, and 10 mM EGTA. The pH was adjusted to 7.3 with CsOH. During the recording, NMDA (50 µM) was applied with a fast-step perfusion system for 2 seconds every 30 seconds for 30 minutes. For GABAR experiments, the external solution for recording whole-cell currents contained 135 mM NaCl, 3 mM KCl, 2 mM CaCl2, 10 mM HEPES acid, 1 mM MgCl2, 7 mM Triéthylamine chloride, and 10 mM D-glucose. Tetrodotoxin (1 µM) was added to eliminate the voltage-gated sodium channel currents. The pH was 7.3 and the osmolarity was adjusted to 300 mOsm with D-glucose. The internal pipette solution contained 130 mM CsF, 10 mM CsCl, 4 mM NaCl, 0,5 mM CaCl2, 10 mM HEPES acid, 5 mM EGTA and 7 mM Mg-ATP. The pH was adjusted to 7.3 with CsOH. The membrane potential was clamped at -80 mV so that GABA application evoked inward current. During the recording, GABA (100 µM) was applied for 1 second every 3 minutes for 30 minutes.

All the drugs; 2,3 Bromophenylhistamine dimaleate, Mepyramine, Triprolidine, and Histamine were added in all solutions bath and perfusion.

The rundown was measured by normalizing the currents evoked after the beginning of the recording. The data are expressed as a percentage of control +- standard deviations.

Immunohistochemistry ***Immunofluorescence of primary cultures.***

Primary cultures of hippocampal neurons were fixed with 2 % paraformaldehyde in phosphate buffer (0,1M pH 7,4) at room temperature for 10 min, rinsed for 48 h in 0.1M PBS phosphate buffer, primary cultures were double-immunolabeled for GABAAR/H1R ; NMDAR/H1R ; GFAP/H1R ; MBP/H1R ; HUC/D/H1R.

List of antibodies (dilution):

Immunofluorescent labeling

Anti-alpha 1 for GABAA receptor (1:1,000) (NeuroMab)

anti-NMDA (1:1000) Ionotropic glutamate receptor subunit GluN1 (Synaptic Systems - SYSY)

anti-H1 (1:100) ((Alomone Labs - AL)

anti-GFAP (1:750) (GFAP, Glial fibrillary acidic protein of astrocyte, (Chemicon, Temecula, CA)

anti-BMP, bone morphogenetic protein of oligodendrocyte, (1:250) (Chemicon, Temecula, CA)

anti-neuronal (protéine HUC/D.somatostatin (1:30) (Invitrogen)

Statistical analyses

After normalization to the first current recorded, the variation of the current amplitude was reported on the time curved and compared to control with a two-way ANOVA with Bonferroni adjustment. Significance was tested using a two-way ANOVA analysis for repeated measures followed by Dunnett's post hoc analysis (one-tailed distribution).

The area under the curve of the normalized current for the different conditions was compared to control conditions, thus a Dunnett's test was used as the samples were of the normal distribution.

RESULTS

Mepyramine modifies the NMDA rundown

In whole cells recordings from cultured hippocampal neurons, we observe a rundown of (fig 1 A) the amplitude of the NMDAR-mediated responses, as the amplitude decreased with the successive application of NMDA to a similar degree to that observed by Rosenmund et al,(14). The addition of Mepyramine 10 nM (fig 1 A) led to an increase in the current amplitude from 4 to 14 minutes. A second phase then followed in which responses decreased at the same rate observed during the rundown in the control condition (fig 1 B). The total NMDAR current during the 30 minutes of recordings is indicated by the area under the curve. Bath application of Mepyramine 10 nM led to a significant increase of 39% of the area under the curve compared to the control (NMDA alone) (fig1 C). 50 nM Mepyramine did not further alter rundown (increased by 37 % of the area). In contrast, 2 nM Mepyramine showed a moderate effect (increased by 14%) (fig1 C).

The effect of Mepyramine is specific to the rundown of NMDA currents.

Extending the inter-stimulus interval from 30 seconds to 3 minutes prevents NMDAR current rundown. We, therefore, tested if Mepyramine 10 nM altered NMDA currents directly. In this case, we first applied NMDA alone three times every 3 minutes and then repeated the experiment in the presence of Mepyramine. Mepyramine (10 nM) did not alter the NMDA current amplitude (n=10, data not shown), indicating the effect of Mepyramine on NMDA currents is specific to the rundown.

Triprolidine modifies the NMDA rundown

We next tested whether Triprolidine (fig 2), another H1 antagonist, affected the NMDAR rundown. Triprolidine 10 nM increased of the responses (20% increase of the area under the curve) but lower than Mepyramine.

Histamine decreases the effect of Mepyramine but alone has no effect on the NMDA rundown

Activation of H1 receptors with Histamine 100 µM did not alter the NMDAR rundown. We then asked if Histamine could suppress the effect of Mepyramine. Simultaneous bath application of Histamine 100 µM with Mepyramine 10 nM reduced the effect of the Mepyramine to about 50% compared to Mepyramine alone (area under the curve) (fig 3 A).

2,3 Bromophenyl Histamine decreases the effect of Mepyramine but alone has no effect on the

NMDA rundown

A high potent H1 agonist 2,3 Bromophenylhistamine dimaleate was tested (fig 3 B). With 10 µM, it can reduce significantly by about 50 % the effect of Mepyramine and Bromophenylhistamine was also tested alone has no effect on the NMDA rundown.

Mepyramine has no effect on GABA rundown

In whole cells recordings from cultured hippocampal neurons, we observe a rundown of the GABAA receptor (fig 4) the amplitude of the GABAA-mediated responses decreases with the successive application of NMDA to a similar degree to the one observed by Laschet et al.(12). When we add Mepyramine 10 nM, we do not observe any change in the rundown, the same observation with 50 nM (fig 4).

Histamine has no effect on GABA rundown

Histamine (n=6) alone has no effect on the GABA rundown.

H1 is not colocalized with NMDA or GABA receptor

We studied the localization of the H1R in the hippocampal cells culture, on neurons. The H1R immunoreactivity was observed (fig S1 A) but not on oligodendrocytes and astrocytes (fig S1 B, C). The co-expression of the NMDA receptor and the H1R was analyzed and there is no colocalization of the receptors in the neurons (fig S2 A).

The co-expression of the GABAA receptor and the H1R was also analyzed and there is no colocalization of the receptors neurons (fig S2 B).

DISCUSSION

Our results demonstrate that the Histamine receptor H1 is important in the functioning of the NMDA receptor, the blockage of this receptor induced a strong reduction of the rundown of the NMDA receptor holding the receptor in a more active statement. It can explain the proconvulsant

effect of the pharmacological class of the H1R antagonist.

H1 antagonist modifies the NMDA rundown

The H1 antagonist effect is dose-dependent and is maximum with 10 nM Mepyramine about 40 % of increase of the current compared to control conditions, Mepyramine is a highly potent antagonist (16) of the receptor and to confirm this observation we tested the effect of Triprolidine, another formerly designated H1 antagonist. When we used 10 nm of Triprolidine the effect on rundown was the same as with Mepyramine but weaker certainly due to a lower affinity on the receptor compared to Mepyramine (16). During the experiments we observed with 10 nM Mepyramine a small increase of the NMDA current after 4 minutes of application of NMDA, during about 10 minutes with a 20 % maximal effect, this increase of the current is difficult to explain with a simple patch-clamp analysis of the whole-cell current, we observed more current passing through the NMDAR but the aspect of the recording line do not give any other special indication. After this 10 minutes under Mepyramine we have a decrease of the NMDA current and the slope of this decrease among the times is mimicking the slope of rundown in control conditions (fig 1 B), it seems that we have the same effect but with a decay of 10 minutes. Mepyramine holds up the beginning of the rundown! It is very important to notice that the same concentration of Mepyramine in no rundown condition (spacing out the applications) has no effect on the NMDA current. Finally, something happens during the rundown conditions that increase the NMDA currents. In order to characterize pharmacologically the effect of Mepyramine we have tested the effect on the rundown of Histamine (100 µM), alone, Histamine has no effect on NMDA rundown and when combining Histamine and Mepyramine to counteract Mepyramine, Histamine can reduce partially the effect of Mepyramine 10 nM about 50 % (fig 3A). The traditional maximal dose use of 100 µM Histamine can just partially reduce the effect of the Mepyramine which has a very high affinity with the H1R (16). Then we tested a highly potent agonist of the H1R 2,3 Bromophenylhistamine dimaleate at 10 µM (17,18), it also had no effect alone and reduce in the same range as Histamine the effect of Mepyramine on NMDA rundown. These two experiments confirm and characterize a pure H1 effect of Mepyramine on the NMDA rundown.

H1 receptor constitutive activity regulates the NMDA receptor activity.

H1R exhibits a constitutive activity (19). It is a crucial point in our experiments because when we

add Histamine 100 µM, we have no effect on NMDA rundown and when we block the H1R we diminish the rundown. This effect is due to the particular pharmacological properties of both Mepyramine and Triprolidine which are inverse agonists of the H1R (16). These two drugs can oppose the constitutive activity of the receptor. Finally, the constitutive activity of the H1R seems to be important for the NMDAR. The blockade of the constitutive activity by the H1R antagonist induces an increase of the NMDA current leading to an increase in excitability which is a theoretically proepileptogenic effect.

Is the H1 receptor pathway able to interact with NMDA receptor trafficking?

We first observed the aspect of the NMDA responses during the rundown in normal conditions and under Mepyramine more particularly the slopes of the current's traces during the 30 min of experiments and they were not significantly changed.

In order to increase the NMDA responses, the recruitment of a larger quantity of NMDAR at the membrane is a hypothesis, it could be due to a change in the receptor trafficking slowing the disappearance of the receptor at the membrane for example.

The accumulation of NMDAR is one possibility to explain the increase of the response with mepyramine and we can note that this accumulation is still observed during status epilepticus with an increase of the active NMDAR (20)

H1R is coupled to Gαq and its activation increases phosphotidyl inositol turnover (21) and induced [Ca2+]i increase (22), to abrogate this calcium rise could be enough to modulate the NMDAR. H1R also interacts with actin filament (23). Histamine reduces actin filament in endothelial cells and in T cells (24) could explain a part of our effect but we must notice that this is not observed for the moment in neurons. The calcium and ATP dependence of the rundown is well described (14) and this influences NMDAR activity by altering the state of polymerization of the actin filament (25). The rundown is attenuated if actin filaments are stabilized with a high concentration of intracellular ATP and calcium-induced actin depolymerization reduces NMDAR activity (25). The high intracellular concentration of calcium disturbs the connection between NMDAR and actin filament, otherwise, proteins kinase A and C phosphorylate NMDA and increase its activity (26,27) In this case, the H1R does not interact with these pathways, H1 has just an activating Phospholipase C effect which seems to have no consequence with MNDA rundown (28).

Another possibility is a direct interaction between the receptor and it is documented for example that both Histamine H1 and H2 receptors have been shown to co-localized with dopamine D1 and D2 in neurons (29) but in our experiments, we cannot observe any significant colocalization of the receptor NMDA with the H1R, it means that the effect that we observed is not linked to possible

direct contact of the receptors.

The complex role of Histamine and H1 receptor pathway in the brain role in epilepsy

The results of our experiments on the interaction of Mepyramine or Histamine on GABA rundown give us no evidence of any effect of these drugs, and we conclude that the GABAergic system is surely not involved in the proconvulsive effect of the H1 antagonist. In the brain, Histamine also acts as a positive allosteric modulator of the N-methyl-D-aspartate receptor (NMDAR) (30). It potentiates NMDA currents in isolated (31) and cultured (32) hippocampal neurons, and this effect results from direct interaction with NMDARs containing NR1 but this direct effect never was related to epileptiform activities. The results of Kamei clearly suggest that the epileptogenic activity of first-generation H1-antagonists is dependent upon a centrally acting Histaminergic mechanism through the Histaminergic receptors (7). Histamine had anticonvulsant effects, H3 receptor antagonists, which enhance endogenous Histamine release in the brain, have been demonstrated to have a potent anticonvulsant action. (33). Histamine attenuated amygdaloid-kindled seizures in rats effect (34), Histamine as also an anticonvulsant effect on pentetrazole (PTZ)-induced and electroconvulsive seizure threshold in mice (35). Both authors conclude that this effect is mediated by the H1R because the H1 antagonist can counteract the Histamine effect but we know that H 1 antagonist has proconvulsant effects and we propose that the Histamine effect on epilepsy is probably not due to direct interaction with the H1R but through another pathway. In our experiments, Histamine as well as a pure H1 agonist have alone no effect on the NMDA rundown they can just reduce the effect of the antagonist H1 drugs by competition interacting on the binding site of the drugs on the H1R. We can hypothesize that they are two different mechanisms one for the anticonvulsant effect of Histamine and one for the proconvulsant effect of H1 antagonist this indicates the crucial role of H1R in the central nervous system. Midzyanovskaya et al(8) describe an increase of the densities in the H1R in the brain of rats with generalized epilepsies and Kukko-lukjanov et al.(36) note that H1R knock out mice the status epilepticus is more severe than in control mice, they also conclude that the Histamine receptors H1 play a role in the control of epilepsy.

We are now at the point where we have a lot of questions; the intracellular microenvironment with calcium (25) regulates the signaling of NMDAR and the role of actin filament seems to be crucial for the presence of new NMDARs at the membrane. but we will need another approach than whole-

cell recording to explore the intracellular movement of the NMDAR such as for example those from Ferreira et al, (37). Other questions, what happening in KO H1 mice? In human epileptic tissue, how is the NMDA rundown and how are distribute the H1R?

REFERENCES

1 Goldstein L, Murphree HB, Pfeiffer CC.
Comparative study of EEG effects of antihistamines in normal volunteers.
J Clin Pharmacol J New Drugs. 1968 Jan-Feb;8(1):42-53.

2 Kaneko Y, Shimada K, Saitou K, et al.
The mechanism responsible for the drowsiness caused by first-generation H1 antagonists on the EEG pattern. Methods Find Exp Clin Pharmacol. 2000 Apr;22(3):163-8.

3 Churchill JA, Gammon GD. The effect of antihistaminic drugs on convulsive seizures. J. Am. Med. Assoc., 141, 18–21 (1949).

4 Yokoyama H, Iinuma K. Histamine and Seizures: Implications for the Treatment of Epilepsy. CNS Drugs. 1996 May;5(5):321-30.

5 Yasuhara A, Ochi A, Harada Y, Kobayashi Y. Infantile spasms associated with a histamine H1 antagonist. Neuropediatrics, 29, 320–321 (1998).

6 Yokoyama H, Onodera K, Iinuma K, et al. Proconvulsive effects of histamine H1-antagonists on electrically-induced seizure in developing mice. Psychopharmacology (Berl). 1993;112(2-3):199-203.

7 Kamei C, Ohuchi M, Sugimoto Y, Okuma C. Mechanism responsible for epileptogenic activity by first-generation H1-antagonists in rats Brain Research 887 (2000) 183–186

8 Midzyanovskaya IS, Birioukova LM, Shatskova AB, van Luijtelaar G, Tuomisto LM.H1 histamine receptor densities are increased in brain regions of rats with genetically generalized

epilepsies. Epilepsy Res. 2016 Nov;127:135-140.

9 Meldrum B. The role of glutamate in epilepsy and other CNS disorders. (1994) Neurology 44(11, Suppl 8): S14-23.

10 Trevelyan AJ, Schevon CA. How inhibition influences seizure propagation. Neuropharmacology. 2013 69:45-54.

11 Palma E, Roseti C, Maiolino F, et al. GABA(A)-current rundown of temporal lobe epilepsy is associated with repetitive activation of GABA(A) "phasic" receptors. Proc Natl Acad Sci U S A. 2007 Dec 26;104(52):20944-8

12 Laschet J, Kurcewicz I, Minier F, Trottier S, Khallou-Laschet J, Louvel J, Gigout S, Turak B, Biraben A, Scarabin JM, Devaux B, Chauvel P, Pumain R. Dysfunction of $GABA_A$ receptor glycolysis-dependent modulation in human partial epilepsy. Proc Natl Acad Sci U S A. 2007 Feb 27;104(9):3472-7. Epub 2007 Feb 20.

13 Laschet J, Kurcewicz I, Minier F, et al . Dysfunction of $GABA_A$ receptor glycolysis-dependent modulation in human partial epilepsy. Proc Natl Acad Sci U S A. 2007 Feb 27;104(9):3472-7.

14 Rosenmund C, Westbrook GL. Rundown of N-methyl-D-aspartate channels during whole-cell recording in rat hippocampal neurons: role of Ca2+ and ATP. J Physiol. 1993 Oct;470:705-29

15 Burban A, Faucard R, Armand V, et al. Histamine potentiates N-Methyl-D-Aspartate receptors by interacting with an allosteric site distinct from the polyamine-binding site. *J Pharmacol Exp Ther* **332(3)**:912-21 (2010)

16 Fitzsimons CP, Monczor F, Fernández N, Shayo C, Davio C.
Mepyramine, a histamine H1 receptor inverse agonist, binds preferentially to a G protein coupled form of the receptor and sequesters G protein. J Biol Chem. 2004 Aug 13;279(33):34431-9. Epub 2004 Jun 10.

17 Leschke C1, Elz S, Garbarg M, Schunack W.

Synthesis and histamine H1 receptor agonist activity of a series of 2-phenylhistamines, 2-heteroarylhistamines, and analogues. J Med Chem. 1995 Apr 14;38(8):1287-94.

18 Leschke, C., Elz, S. and Schunack, W.
Synthesis and HI agonistic properties of 2-(3-bromophenyl)histamine and 2- thienyl analogues. XIIth Intern. Symp. Med. Chem.1992, Basel, P-062B

19 Bakker RA, Wieland K, Timmerman H et al.
Constitutive activity of the histamine H(1) receptor reveals inverse agonism of histamine H(1) receptor antagonists. Eur J Pharmacol. 2000 Jan 3;387(1):R5-7.

20 Naylor DE., Liu H, Niquet J, Wasterlain CG. Rapid surface accumulation of NMDAR receptors increases glutamatergic excitation during status epilepticus. Neurobiol Dis. 2013 Jun, 54 225-238

21 Claro E, Arbonés L, García A, Picatoste F.
Phosphoinositide hydrolysis mediated by histamine H1-receptors in rat brain cortex.Eur J Pharmacol.1986 Apr 16; 123(2):187-96.

22 Leurs R, Traiffort E, Arrang JM et al.
Guinea pig histamine H1 receptor. II. Stable expression in Chinese hamster ovary cells reveals the interaction with three major signal transduction pathways. Neurochem. 1994 Feb;62(2):519-27.

23 Rotrosen D, Gallin JI.
Histamine type I receptor occupancy increases endothelial cytosolic calcium, reduces F-actin, and promotes albumin diffusion across cultured endothelial monolayers. J Cell Biol. 1986 Dec;103(6 Pt 1):2379-87.

24 Ito T, Fujiyama T, Hashizume H et al.
Antihistaminic drug olopatadine downmodulates T cell chemotaxis toward CXCL10 by reducing CXCR3 expression, F-actin polymerization and calcium influx in patients with alopecia areata. J Dermatol Sci. 2013 Oct;72(1):68-71

25 Rosenmund C, Westbrook GL.
Calcium-induced actin depolymerization reduces NMDA channel activity. Neuron. 1993 May;10(5):805-14.

26 Chen L, Huang LY.
Sustained potentiation of NMDA receptor-mediated glutamate responses through activation of protein kinase C by a mu opioid. Neuron. 1991 Aug;7(2):319-26.

27 Raman IM, Tong G, Jahr CE.
Beta-adrenergic regulation of synaptic NMDA receptors by cAMP-dependent protein kinase. Neuron. 1996 Feb;16(2):415-21.

28 Nijmeijer S, Leurs R, Vischer HF.
Constitutive activity of the histamine H(1) receptor.
Methods Enzymol. 2010;484:127-47.

29 Zhuang QX, Xu HT, Lu XJ, Li B, Yung WH, Wang JJ, Zhu JN. Histamine Excites Striatal Dopamine D1 and D2 Receptor-Expressing Neurons via Postsynaptic H1 and H2 Receptors. Mol Neurobiol. 2018 Oct;55(10):8059-8070.

30 HaasHL, Sergeeva OA, Selbach O. Histamine in the nervous system. *Physiol Rev* **88**, 1183-241 (2008).

31 VorobjevVS, Sharonova IN, WalshI B, et al. Histamine potentiates N-methyl-D-aspartate responses in acutely isolated hippocampal neurons. *Neuron* **11**, 837-44 (1993).

32 Bekkers JM. Enhancement by histamine of NMDA-mediated synaptic transmission in the hippocampus. *Science* **261**, 104-6 (1993).

33 Bhowmik M, Khanam R, Vohora D Histamine H3 receptor antagonists in relation to epilepsy and neurodegeneration: a systemic consideration of recent progress and perspectives.Br J Pharmacol. 2012 Dec;167(7):1398-414

34 Kamei C, Ishizawa K, Kakinoki H, Fukunaga M. Histaminergic mechanisms in amygdaloid-kindled seizures in rats, Epilepsy Res.30 (1998) 187–194.

35 Hashem A., Frey HH. Histamine in brain its role in regulation of seizure susceptibility R Scherkl , Epilepsy Res 10(2-3) (1991);111-8

36 Kukko-LukjanovTK, Grönman M, Lintunen M, Laurén HB, Michelsen KA, Panula P, Holopainen IE. Histamine 1 receptor knock out mice show age-dependent susceptibility to status epilepticus and consequent neuronal damage Epilepsy Res 100(1-2):(2012) 80-92.

37 Ferreira JS, Papouin T, Ladépêche L, Yao A, Langlais VC, Bouchet D, Dulong J, Mothet JP, Sacchi S, Pollegioni L, Paoletti P, Oliet SHR, Groc L.
Co-agonists differentially tune GluN2B-NMDA receptor trafficking at hippocampal synapses.
(2017) *Elife*.6. pii: e25492. doi: 10.7554/eLife.25492.

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines

FIGURES

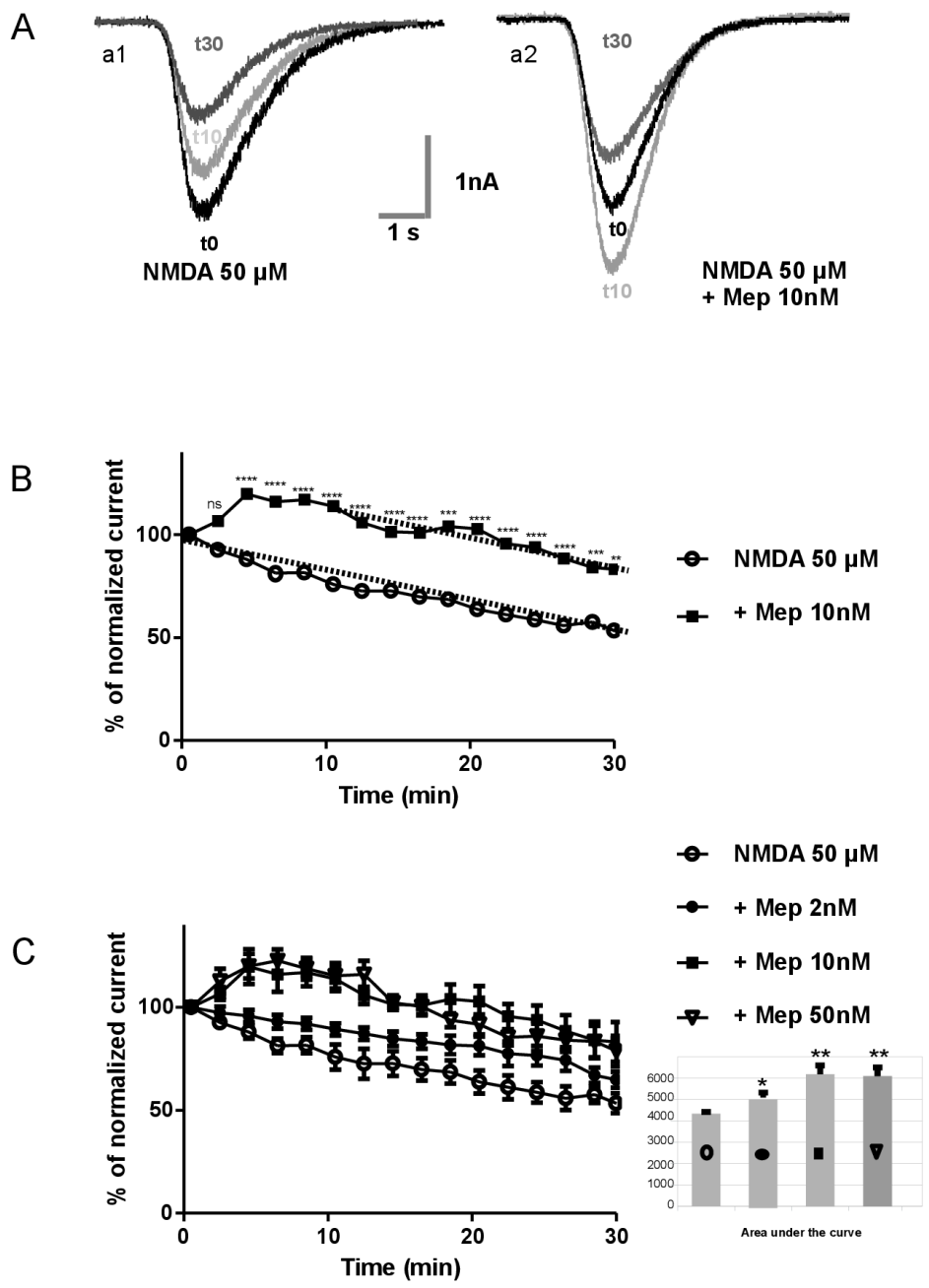

Fig 1

Figure 1.

Rundown of NMDA current; effect of Mepyramine **(Mep)**

Rundown of NMDA current during whole-cell recording, inward currents evoked by 2 s application of 50 µM NMDA at 30 s intervals in control and with Mepyramine.

A Currents evoked after the first application **t0**, 10 min **t10,** and 30 min **t30** of recording are superposed in NMDA 50 µM condition (a1) or in the presence of NMDA 50 µM + 10 nM of Mepyramine (a2).

B The graphs show the normalized currents in NMDA 50 µM condition (n= 10) and in the presence of NMDA 50 µM + Mepyramine 10nM (n=10) Currents were normalized to the first application. Note that the slope in both conditions is the same (dotted line). Statistical comparison of NMDA to NMDA +Mepyramine .*p<0,05, **p<0,01, ***p< 0,001, or ns.

C The graphs show the normalized currents in NMDA, 50 µM (n= 10) and in the presence of NMDA 50 µM plus Mepyramine 2, 10, and 50 nM (n=10 for each condition) Currents were normalized to the first application.

The area under the curve for all the conditions tested with statistical comparison *p<0,05, **p<0,01 or ns to NMDA 50 µM.

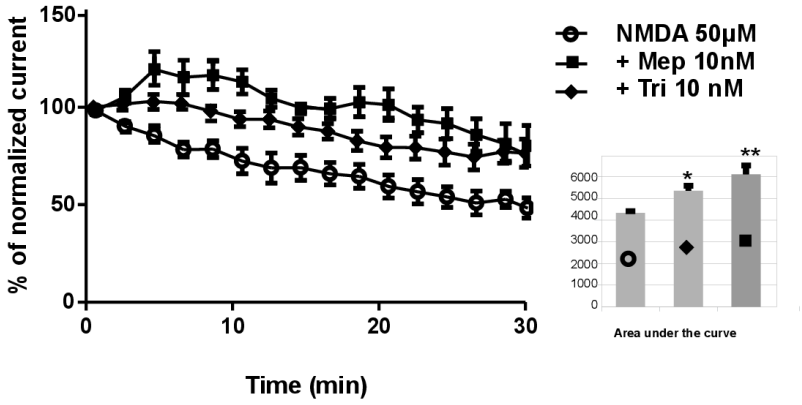

Fig 2

Figure 2. Rundown of NMDA current; effect of Triprolidine (**Tri)**. The graphs show the normalized currents in NMDA 50 µM red and in the presence of NMDA 50 µM plus Mepyramine 10 nM and NMDA 50 µM plus Triprolidine 10 nM (n=10) Currents were normalized to the first application.

The area under the curve for all the conditions tested with statistical comparison *p<0,05, **p<0,01 or ns to NMDA 50 µM.

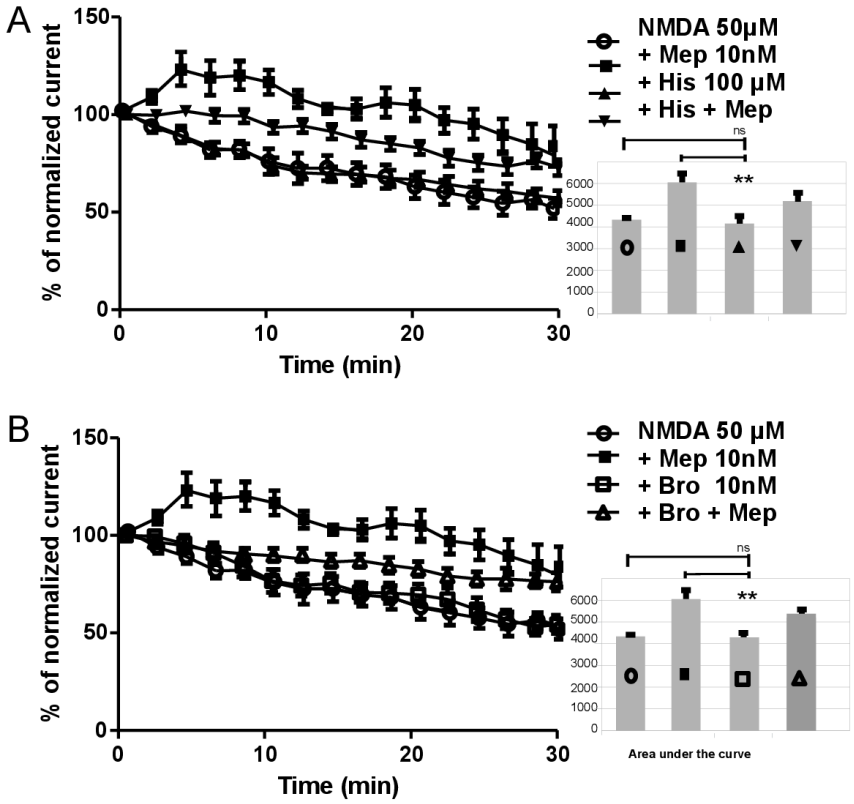

Fig 3

Figure 3. Rundown of NMDA current effect of Histamine (**HA)** and 2,3 Bromophenylhistamine dimaleate (**Bro)**,

A The graphs show the normalized currents in NMDA 50 µM red and in the presence of NMDA 50 µM plus Mepyramine 10 nM, with NMDA 50 µM plus Histamine 100 µM (n=10) and NMDA 50 µM +Histamine and Mepyramine 10 nM (n=10). Currents were normalized to the first application. The area under the curve for all the conditions tested with statistical comparison *p<0,05, **p<0,01 or ns to Mepyramine 10 nM.

B The graphs show the normalized currents in NMDA 50 µM red and in the presence of NMDA 50 µM plus Mepyramine 10 nM, with NMDA 50 µM plus 2,3 Bromophenylhistamine dimaleate 10 µM (n=10) and in NMDA 50 µM plus 2,3 Bromophenylhistamine dimaleate and Mepyramine 10 nM (n=10). Currents were normalized to the first application. The area under the curve for all the conditions tested with statistical comparison *p<0,05, **p<0,01 or ns to Mepyramine 10 nM.

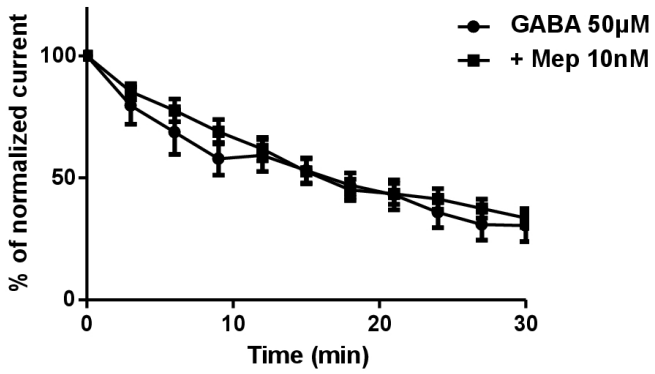

Fig 4

Figure 4. Rundown of GABA current; effect of Mepyramine **(Mep)**

Rundown of GABA current during whole-cell recording, inward currents evoked by 1 s application of 100 µM GABA at 3 min intervals in control (n=12) and Mepyramine 10 nM presence (n=10).

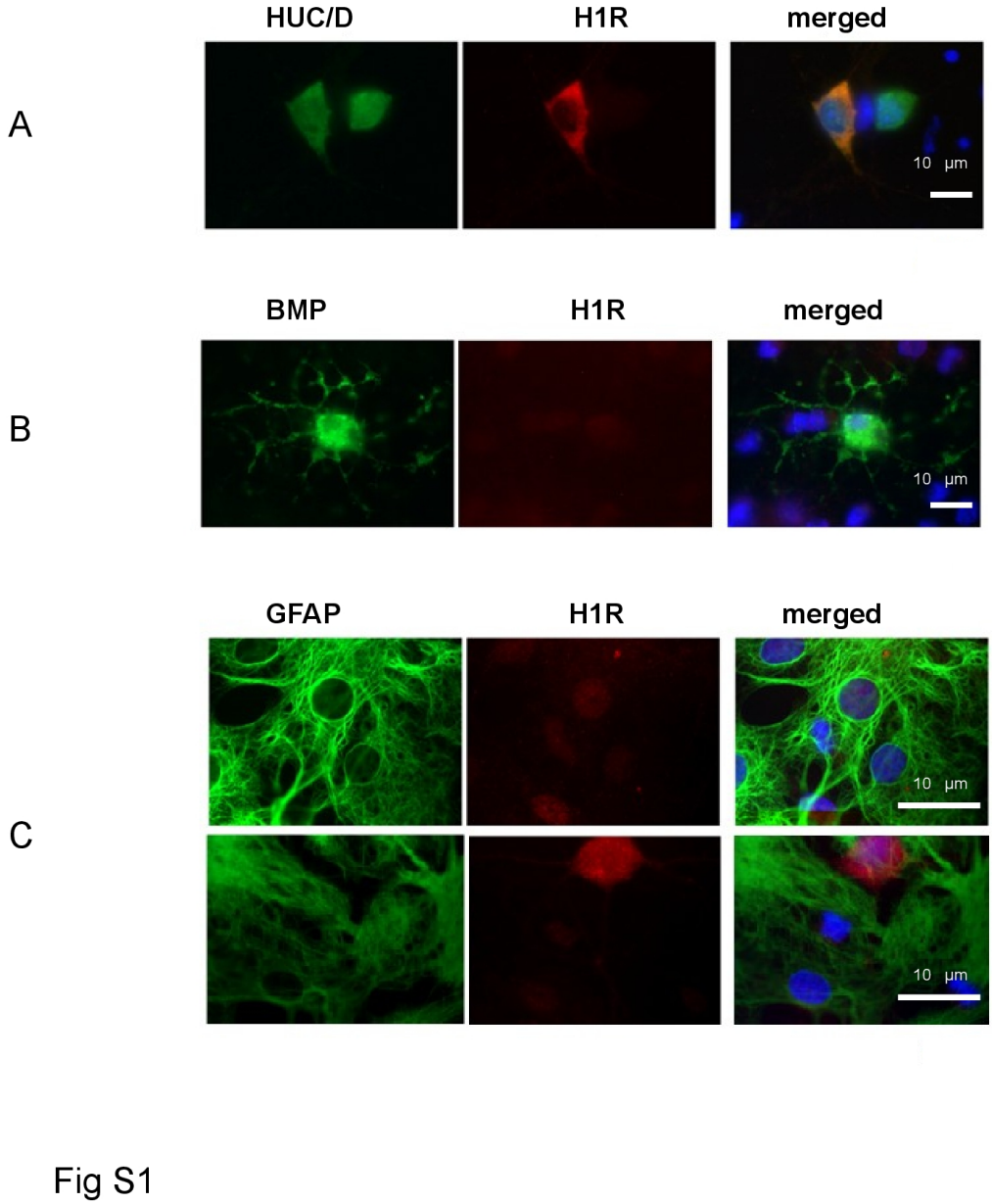

Fig S1

Figure S1. Immunostaining for HUC/ D (A), BMP (B) and GFAP (5C) co-expression with the H1 receptor in cultured hippocampal cells. Scale bar, 10 µm.

In the culture of the hippocampal cell, we found an expression of the H1 receptor in 79 % of the

neurons (HUC/ D positive cells) (n=395 on 500 observed cells), we also observe that none of the glial cells was H1 receptor positive.

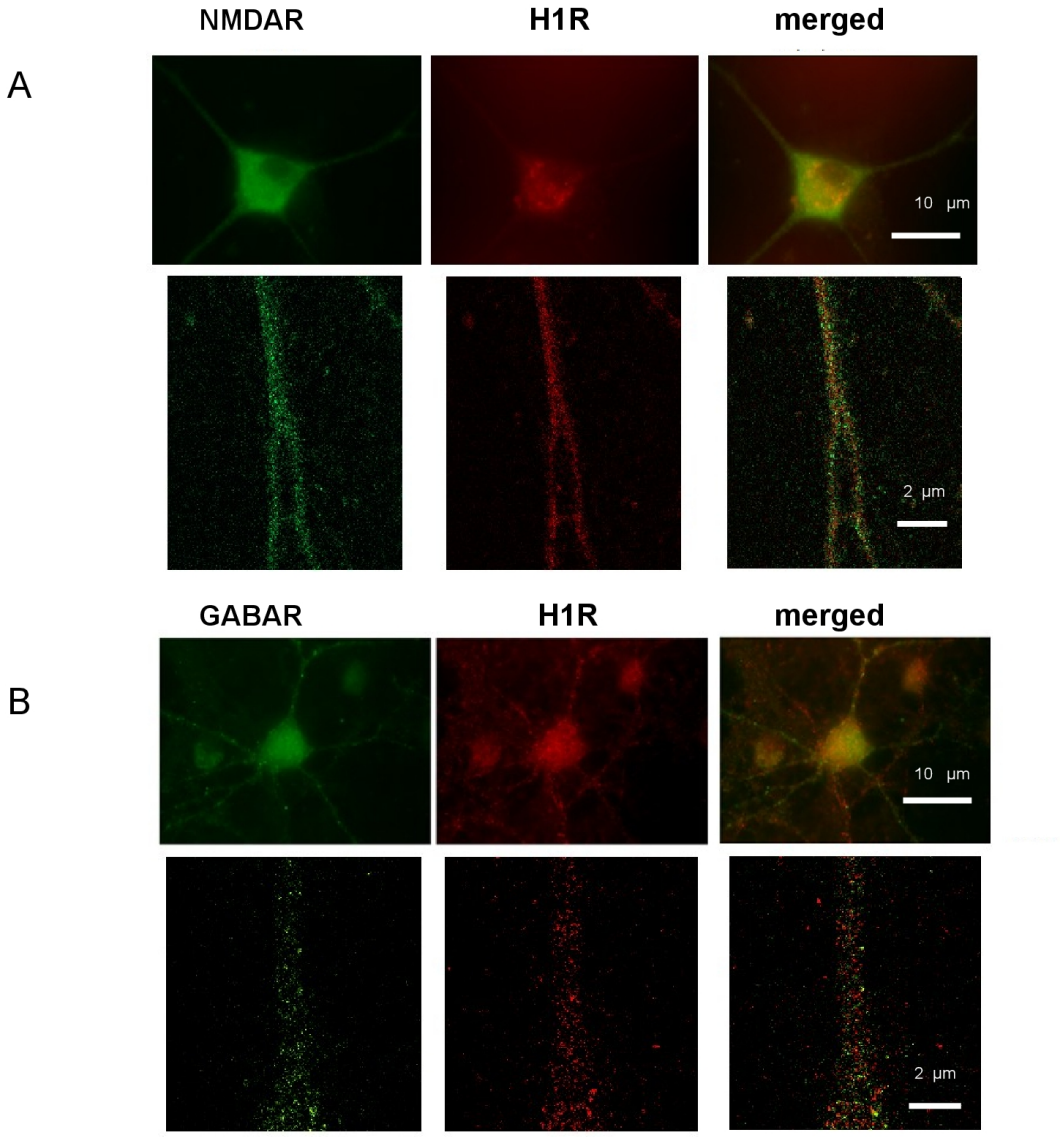

Fig S2

Figure S2. Immunostaining fo**r** NMDA receptor (A) and receptor $GABA_A$ receptor (B) co-expression with the H1 receptor in cultured hippocampal cells. Scale bar 10 µm and 2 µm as indicated