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Re: Dr MTA thesis

Date: 22 Jan, 2020

Dear reviewers, editor and evaluation panel members,

I thank the reviewers for their generous comments on my Dr MTA thesis. Please find below my point-to-point reply to questions raised by the reviewers.

Sincerely Yours,

Karri Lamsa

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Reviewer Gulyas Attila

Reviewer: Several homeostatic mechanisms have been demonstrated that fine tune cellular and network parameters, so that the network activity converges to a stable level. Therefore, parameters in a silent slice drift from normal values. The ACSF in the rodent experiments contained 2.5K 2.5Ca 1.3Mg, that results in a relatively low background activity level. Also, a cut was made between the CA3 and CA1 further decreasing network activity. The consensus nowadays is that a more excitable ACSF ~3.5K 1Ca 1Mg better approaches in vivo composition and will result in higher (more in vivo like) firing rates and spike distributons.

Reply: *The experimental conditions were deliberately used here to improve signal-to-noise resolution of experiments. Altogether, these allowed much improved detection of smallest changes in evoked EPSP amplitude and slope. First, in these conditions spontaneous neuronal firing and spontaneous occurrence of synaptic events are suppressed through lowered extracellular K^+ ($[K^+]_o$) hyperpolarising membrane potential (Vm) in neurons through K^+ electrochemical gradient. In addition, the increased extracellular total divalent cation concentration (3.8 mM rather than 2 mM) reduces activation of voltage-gated Na^+ and Ca^{2+} channels at resting membrane potential through the membrane surface screening effect. Indeed, lowering $[K^+]_o$ from 3.5 mM to 2.5 mM equals to ~8 mV negative shift in Nerstian K^+ reversal potential. Because average healthy neuron has roughly 0.05 permeability for Na^+ ions and 0.45 to Cl^- ions (relative to K^+ ions) the lowered $[K^+]_o$ here is expected to cause a 2-to-3 mV negative shift in resting membrane potential of hippocampal neurons*. In other words, abovementioned experimental modifications in the extracellular cation concentrations are not dramatic, and their effect on network excitability is modulatory *(Thompson SM & Gähwiler BH, J Neurophysiol 61:512-23, 1989). Second, a lesion between CA3 and CA1 areas prevents antidromic activation by the CA1 stimulation of excitatory recurrent connections in the CA3 area pyramidal cells. The lesioning of recurrent excitatory connections is highly useful because otherwise the elicited polysynaptic activity would challenge the analysis of Schaffer-collateral stimulation-evoked monosynaptic EPSC/Ps (Maccaferri G & McBain CJ, J Neurosci 16:5334-43, 1996). Third, increased extracellular Ca^{2+} levels promote synaptic release stabilising EPSP amplitude and slope in control conditions. Consequently, a reduced EPSP amplitude/slope variation increases statistical power of data recorded in control conditions.*

Reviewer: To what extent can the fact that the measurements were made in silent slices can influence the observed mechanisms?

Reply: *I assume that through 'hyperpolarising effect on Vm' both the lowered $[K^+]_o$ and the elevated $[Ca^{2+}]_o$ would facilitate 'anti-Hebbian' LTP which requires calcium-permeable (CP-) AMPARs (and is independent of glutamate NMDAR). CP-AMPARs are most conductive at hyperpolarized Vm. By depolarization, their ion permeability becomes gradually blocked by intracellular polyamine chains. Hence, membrane potential dependence of CP-AMPAR postsynaptic conductance (and calcium influx) is nearly a mirror image to that of NMDAR's. I have discussed these aspects in three review articles (Kullmann DM, Lamsa K. Nat Rev Neurosci 9:687-99, 2007; Kullmann DM, Lamsa K. J Physiol, 15:586:1481-6, 2008; Lamsa KP, Kullmann DM, Woodin MA. Front Synaptic Neurosci. 2:8, 2010). In addition, elevated $[Ca^{2+}]_o$ facilitates calcium influx through CP-AMPARs to postsynaptic CA1 interneurons during LTP induction protocol. This can promote induction of 'anti-Hebbian' LTP when neuron is at resting Vm (Camiré O, Topolnik L. J Neurosci. 34:3864-77, 2014).*
These modifications were principally made to facilitate detection and analysis of EPSP plasticity when testing existence of 'anti-Hebbian' LTP identified interneuron types.

Reviewer: Also, 100uM picrotoxin was used in the experiments. Why was it used? It switches off inhibitory feedback and influences network dynamics (activity level). Since the demonstrated plastic processes are sensitive to the activity level and the correlations, this intervention influences network behavior. Could the effects be demonstrated without blocking inhibition?

Reply: *A reason to use PiTX in many studies was technical and related to analysis of EPSP. Blocking $GABA_AR$ improves detection of EPSP/C plasticity in postsynaptic cell. Disynaptic feed-forward or feed-back IPSCs in CA1 neurons often overlap with monosynaptic EPSP rise slope and peak amplitude by following EPSC onset with short 2-5 ms delay (Pouille F, Scanziani M. Science. 293,5532:1159-63, 2001; Lamsa K, Heeroma JH, Kullmann DM. Nat Neurosci. 7:916-24, 2005). In addition, feed-back IPSCs can occur with additional few millisecond delay (Maccaferri G & McBain CJ, J Neurosci 16:5334-43, 1996). Overlap of EPSP and IPSP is problematic for the EPSP amplitude and slope analysis, because disynaptic IPSP characteristically show large cycle-by-cycle variation. This complicates EPSP analysis in plasticity studies. Therefore $GABA_ARs$ were often blocked in many (but not all) experiments.*

I would like to stress that we also have performed plasticity experiments with intact GABAergic transmission (in slices without PiTX). As an example, we demonstrated long-term potentiation of feed-forward IPSC probability in parallel with 'anti-Hebbian LTP' of EPSP taking place in local interneurons (Lamsa K, Heeroma JH, Kullmann DM. Nat Neurosci. 7:916-24, 2005; Nissen W, Szabo A, Somogyi J, Somogyi P, Lamsa KP. J Neurosci. 30:1337-47, 2010).
Thus, the studies demonstrate that interneuron LTP is generated in the presence of intact GABAergic transmission. In fact, we assume that intact GABAergic inhibitory transmission can facilitate 'anti-Hebbian' LTP, because it opposes postsynaptic depolarization.

Reviewer: Why was a different (3.5K, 1Ca, 3Mg) ACSF used in the human case? In some sense it is more excitable: higher K and lower Ca, though the high Mg blocks NMDA receptors and thus probably decreases excitability.
Reply: We use rodent recordings systematically in parallel with human ex vivo experiments (Szegedi V, Paizs M, Baka J, Barzó P, Molnár G, Tamas G, Lamsa K. Elife. 9,9. pii: e51691, 2020). In addition we studied plasticity parallel in a rat and in the human interneurons using this (3.5K, 1Ca, 3Mg) extracellular solution (Szegedi V, Paizs M, Csakvari E, Molnar G, Barzo P, Tamas G, Lamsa K. PLoS Biol. 14:e2000237, 2016).
Shortly, in the experiments we used similar solutions as earlier human slice studies have used; it allows us to make straight-forward comparison of various synaptic parameters between our data and features characterised by other laboratories in human neuron. This aspect is highly relevant since not many laboratories are yet working on intracellular parameters of human neurons and therefore 'human specific cellular data' are scarce. One major goal in the community at the moment is to compare each other's data.

Reviewer: The most difficult part for me to untangle was to understand the similarities and differences of the plastic processes in the different cases. First, it is difficult to compare the different cases, since different recording configuration were used, and the details given were not of similar depth. Second, it is important to distinguish the location of the plastic changes as well as the induction requirements. Plasticity can happen through changes of synaptic transmission or changes in the integrative properties of a neuron (change in resting potential, input resistance or firing threshold).
Reply: I apologize if the paradigm details were unequally explained for all experiments in the thesis. Yet, we have clarified them in individual original research articles.
I agree that the plasticity location (i.e. synaptic vs. non-synaptic) is a very valid point and that the thesis does not address it in depth. Indeed it was show shown (Ross ST, Soltesz I. Proc Natl Acad Sci U S A. 98:8874-9, 2001; Campanac E, Gasselin C, Baude A, Rama S, Ankri N, Debanne D. Neuron. 77,4:712-22, 2013) that in parallel with synaptic LTP, hippocampal interneurons can in addition generate intrinsic plasticity changes by high-frequency glutamatergic fiber activity. Such 'intrinsic LTP' in the CA1 area pv+ interneurons is caused by increased cellular excitability through downregulation of Kv1 channels and consequent increased firing responsiveness to a depolarizing pulse or EPSP (Campanac E, Gasselin C, Baude A, Rama S, Ankri N, Debanne D. Neuron 20;77:712-22, 2014). We cannot exclude such plasticity (among other possible changes altering intrinsic excitability) taking place in the CA1 interneurons during synaptic LTP or LTD in our experiments. However, we know for three reason that there is at least robust synaptic LTP or LTD in our experiments. First, our plasticity analysis primarily focus on EPSP amplitude and rise slope. Potentiation or depression of these two parameters at the same time indicates alteration in the synaptic strength. Second, the plasticity has presynaptic expression site indicated by altered synaptic probability (paired-pulse test) and EPSP peak amplitude co-efficient of variance (SD/mean -value). Third, we have tested homo- versus heterosynaptic nature of LTP and the LTD using two stimulated afferent pathways in parallel (see Lamsa et al. 2005a,b; Lamsa et al. 2007; Oren et al. 2009; Nissen et al. 2010; Szabo et al. 2012).

Reviewer: The plasticity can also be homosynaptic, i.e. input specific, or heterosynaptic, affecting the function of the non-driven input too.
Reply: Please see my reply above. In various studies we have tested homo- versus heterosynaptic nature of LTP and the LTD using two stimulated afferent pathways in parallel showing that the plasticity is pathway specific.

Reviewer: It also can be Hebbian, that requires tight temporal correlation or (as shown by the candidate) anti-Hebbian. Therefore, it is crucial to define all details of the examined plasticity process with experiments addressing the conditions, time scale and mechanism of the plastic process in question. For example the classical NMDA mediated excitatory to excitatory plasticity that is present in the connections of most principal neurons (evidently with the exception of the mossy fiber input to granule cells) is: homosynaptic and therefore input and pathway dependent, it is highly sensitive to the temporal correlation of the activation of the pre and postsynaptic elements (STDP, Hebbian) , as well as it lasts for hours to days (some experimental approach allow this conclusion).

Reply: I agree and therefore Lamsa et al. 2007 (Science) specifically studied this aspect in the CA1 interneurons. It demonstrates that in the synapses where 'anti-Hebbian' CP-AMPAR-dependent LTP is present, the Hebbian NMDAR-dependent LTP is absent (and vice versa). This was also shown later by LeRoux et al (2013) (Le Roux N, Cabezas C, Böhm UL, Poncer JC. J Physiol. 591,7:1809-22, 2013).
In addition, our paper demonstrates temporal correlation of the pre- and postsynaptic firing in the Hebbian and in the anti-Hebbian LTP induction in interneurons. In that original research paper, we used various potential LTP induction protocols (presynaptic low-frequency pairing of postsynaptic depolarisation, high-frequency pairing, theta burst pairing) and tested these in Hebbian or anti-Hebbian manner. I have discussed spike-timing plasticity of interneuron LTP and LTD specifically in two reviews (Lamsa KP, Kullmann DM, Woodin MA. Front Synaptic Neurosci. 2:8, 2010; Kullmann DM, Lamsa KP. Neuropharmacology. 60:712-9, 2011).

-Reviewer: In the case of the presented papers it was not evident for all experiments whether the plasticity revealed was homo- or heterosynaptic and to what extent were precise temporal correlation needed. For example, in the 2007b paper the pathway specificity of the effect had been checked, while in other cases not.
-Reply: I am afraid this argument is not entirely true. Indeed, we demonstrate pathway specificity and temporal correlation of pre- and postsynaptic firing in Lamsa et al. 2007b (Science). In addition, we systematically tested and showed pathway specificity of the plasticity in other rodent ex vivo studies in this thesis (Lamsa et al. 2005; Nissen et al. 2010) as well as in our papers not included in thesis (Lamsa et al. 2007a; Oren et al. 2009; Szabo et al. 2012). Yet, I agree that pathway-specificity test (i.e. testing two afferent stimulation pathways simultaneously in postsynaptic interneurons) is missing in our study utilizing anaesthetized rat (in vivo) as well as in the study using human tissue material ex vivo. Reason for it is simply methodological; it is challenging to establish a recording configuration with two independent input pathways in vivo (when recording a postsynaptic response extracellularly by measuring the interneuron spike probability) or in ex vivo human tissue when measuring synaptically connected identified pre- and postsynaptic neurons. In other words, we have had to give up pathway specificity aspect in order to secure reasonable yield of data to test plasticity itself.

-Reviewer: I miss a summary figure that collects and details the similarities and differences of the observed plasticity cases (question marks are valid elements). I also miss a synthesis in the conclusion chapter. An outlook to the literature (modeling can be useful here) would be useful in finding a function to these different forms of plasticity? How do they contribute to the stabilization of network dynamics and learning?
-Reply: I apologize these are missing in the thesis, although a simple summary is shown in figure 1 of the thesis on page 13. Regarding conclusions, I hope that citations in final paragraphs of the thesis to my review articles would help a reader. Naturally, referring to review articles does not fully compensate a missing comprehensive conclusions chapter. However, citations to my recent articles would help. I published a review article analysing all studies (to the best of my knowledge) in literature on the GABAergic interneuron postsynaptic long-term plasticity published by 2011 (Kullmann DM, Lamsa KP. Neuropharmacology. 60,):712-9, 2011). My earlier review articles (Lamsa and Kullmann 2007; Kullmann and Lamsa 2008; Lamsa, Kullmann and Woodin 2010) widely discuss synaptic long-term plasticity forms occurring in the hippocampal CA1 interneurons and speculates with reasin for their existence. My recent review (Lau and Lamsa 2019) recalls studies on hippocampal CA1 interneuron long-term plasticity in vivo.

Minor comment 1: "at both the PV+ basket cells as well as the ivy cells could generate either LTP or LTD in these conditions." They hypothesized brain state dependence, i.e. depending on the actual processing mode of the network, the same input or correlation might cause plasticity of different directions. This might argue somewhat against long-term plasticity. Brain states change on the scale of seconds, so this type of plasticity must be rather a short- or medium term one.
-Reply: As a matter of fact, we found in the study that a brain state did not (at least not alone) govern the direction of long-term plasticity generated in our experiments. This means that although brain state may affect the plasticity as a permissive mechanism and regulate the LTP or LTD strength and induction probability, direction of the plasticity (LTP or LTD) is probably governed by other factors such as the afferent fiber locus specificity or activity/plasticity history of fibers. The latter, known as metaplasticity, has been demonstrated to occur in interneuron of the hippocampal formation (Pelkey et al. 2006) where LTP is generated by after generation of LTD in the CA3 interneurons by similar consequent high-frequency stimuli. Reason for the switch of plasticity direction is activity-induced regulation of mGluR7 in glutamatergic synapses to interneurons. Specifically, presynaptic mGluR7which is responsible for LTD generation is internalized by high-frequency stimulation in naïve slices. The following high-frequency stimuli fail to cause mGluR7 activation and they elicit LTP instead (Pelkey KA, Lavezzari G, Racca C, Roche KW, McBain CJ. Neuron 7;46:89-102, 2005). Our aim was to test whether brain state (defined by underlying ongoing LFP oscillatory activity) might qualitatively regulate the long-term plasticity induced. But it did not.

Minor comment 2: p10 top: "Compound EPSCs in were confirmed by observing less than 100 pA increases in the evoked EPSC amplitude when gradually increasing stimulation intensity." It is difficult to understand the sentence.

-Reply: I agree this sentence sounds cryptic and I apologize this. The sentence means that when we studied large EPSCs elicited by extracellular glutamatergic fibre stimulation, we made sure that the final large amplitude EPSP composes of summation of various weak (less than 100 pA) EPSCs. When eliciting EPSC and increasing the stimulation intensity with small steps, the evoked postsynaptic EPSC also increased in graded manner by small amplitudes (less than 100 pA) correspondingly. This shows that the final EPSC used in the extracellular stimulation plasticity experiment, lacked the 'very large EPSC synapses'. The 'very large EPSC' connections between two neurons have postsynaptic amplitude of 500 pA or more. Therefore, less than 100 pA increments in postsynaptic EPSC (by increasing the stimulation intensity) indicate that the extracellularly-evoked postsynaptic 500 pA EPSC was a sum of 'small amplitude synapses' and did not include 'a very large EPSC' in it. The discrimination of 'very large EPSCs' is important here because we wanted to demonstrate in the experiment the 'weak' glutamatergic synapses can also experience long-term plasticity when many of them are activated at the same time.

-Minor comment 3: p19, 2nd para: "we found that the fast-spiking CA1 interneurons can *generate* either LTP or LTD following high-frequency glutamatergic fiber activity." Consider replacing the verb (*demonstrate, undergo)*!

-Reply: I agree there are various synonyms that can be used in this context. Yet, I think using 'generate' is not wrong, obscure or misleading either.

-Minor comment 4: Fig6: a schematic drawing of the experimental arrangement would be helpful

-Reply: I apologize that schematic showing experimental design is missing in the figure. Yet, we provide such in some original publication reporting these results.

Reviewer Kiss Tibor

-Reviewer: My first question is related to the applied perforated patch: The perforated patch feature is that electrical access to the cell interior is obtained through inclusion of pore-forming antibiotic molecules for example nystatin or amphotericin B in the patch area of membrane in contact with the patch pipette. Gramicidin used by the author shares the same basic principle with previously used methods of perforated patch recording, namely formation of channels selective to small ions and non-electrolytes, in addition; however, gramicidin channels lack of chloride permeability. It is well known however that upon activation, the GABAA receptor selectively conducts Cl− ions through its pore so my question is that gramicidin would not interfere with GABA activated channels someway?

-Reply: To the best of my knowledge, dimeric gramicidin pores or non-poor-forming monomers do not directly interact with GABA A or B receptors. In addition, transmembrane gramicidin pores pass through only small monovalent cations and low-weight non-charged molecules only. Therefore gramicidin-perforated patch is supposed to leave intracellular Cl- concentration as well as cytoplasmic biochemical signalling cascades (including G-protein mediated) untouched in a recorded cell and for this reason this recording method should enable intact GABA A or B receptor-mediated responses (assuming that physiological K^+ concentration is used in pipette filling solution).

We used gramicidin perforated-patch in plasticity studies, since it allows long-lasting membrane potential recording without dialysing cell cytoplasm or altering intracellular biochemical signalling (including intracellular Ca^{2+} activated pathways and Ca^{2+} -buffering mechanisms) critical for plasticity (see e.g. Isaac JT, Hjelmstad GO, Nicoll RA, Malenka RC. Proc Natl Acad Sci U S A 6;93:8710-5, 1996). Reason why we selected gramicidin (rather than amphotericin-B or nystatin) is because of its astonishing long-lasting stability in cellular recordings (Lamsa K, Heeroma JH, Kullmann DM. Nat Neurosci 8:916-24, 2005).

-Reviewer: So my question is in relation to the inclusion of QX-314 into the intracellular solution: why was it necessary to include this lidocaine derivate into the intracellular pipette solution both in perforated patch and whole cell experiments since it may interfere with the Ca-signals in the postsynaptic cell?

-Reply: I apologize that I haven't clarified this point well in the thesis, although the rationale is briefly mentioned in the original publications. There were two different reasons to use QX-314 in pipette filling solution in the experiments.

First, in the perforated patch recordings we included QX-314 (bromide salt) in pipette filling solutions because it serves as a good indicator of perforated-patch seal integrity since QX-314 cannot pass through gramicidin pores. As long as perforated-patch seal is intact and ionic conductance between cell cytoplasm and the pipette goes though the gramicidin pores and ion channels only, QX-314 doesn't block sodium action potentials. If the membrane seal is ruptured, QX-314 swiftly enters the cell and blocks firing. This rapidly tells us that perforated-patch recording has turned to a conventional whole-cell recording, and the recording should be terminated.
Second, in experiments with conventional whole-cell configuration we used QX-314 in filling solution to deliberately block action potential firing in the postsynaptic cell. Blocking spikes was necessary to get reliable current-voltage (I/V) measurement of EPSCs. EPSC I/V plot was critical for our studies because it shows rectification of (AMPAR-mediated) EPSCs. CP-AMPARs lack GluA2 subunit (as we also demonstrated in Szabo A, Somogyi J, Cauli B, Lambolez B, Somogyi P & Lamsa KP. J Neurosci 32:6511-6, 2012) and therefore their conductance shows strong inward rectification. Yet, the EPSC should be measured in a large membrane potential range from Em to +40 mV. For this purpose, spiking was blocked by QX-314. In PV+ interneurons Na^+ channel blockade is particularly important because these interneurons fire at very high frequency (300 Hz or more) and show weak Na^+ channel inactivation by depolarisation.

-Reviewer: My question is: is it possible that the intrinsic properties of neurons building up neuronal microcircuits significantly contribute to the observed neuronal activity?
-Reply: This is a relevant question and because I haven't discussed the aspect much in the thesis I elaborate it here. Indeed, neuronal activity in hippocampal interneurons can cause long-term alterations in their excitability and EPSP/spike -coupling. Studies on fast-spiking (putative PV+ basket cells) in the hippocampal formation dentate hilar region as well as in the CA1 area have shown that high-frequency glutamatergic afferent pathway firing causes not only synaptic EPSC plasticity, but can induce permanent postsynaptic depolarisation of few millivolts through altered rate of electrogenic Na+/K+-ATPase pump function in the postsynaptic interneuron (Ross ST, Soltesz I. Proc Natl Acad Sci U S A 98:8874-9, 2001). Another reported non-synaptic long-term plasticity feature is downregulation of depolarisation-activated inhibitory potassium current (though downregulation of axonal Kv.1 channels and I_D current) reported in CA1 area PV+ interneurons (Campanac E, Gasselin C, Baude A, Rama S, Ankri N, Debanne D. Neuron 77:712-22, 2013).
In our studied we did not observe a depolarizing shift in postsynaptic interneuron resting membrane potential by high-frequency afferent stimulation, although this plasticity type is also induced by Ca^{2+} influx to postsynaptic cell via CP-AMPARs. Hence, we suggest that this phenomenon reported in unidentified dentate gyrus interneurons is probably specific to the hilar region and less likely in the hippocampal CA1 where our experiments took place.
Yet, it is possible (and even likely) that the PV+ interneurons we showed here to experience synaptic LTP or LTD also undergo Kv1 expression changes by high-frequency glutamatergic afferent fiber stimulation. However, our experimental protocol in brain slices did not allow us to detect such change, since we systematically studied the synaptic plasticity using subthreshold EPSPs and hence EPSP-spike coupling in these cells remained unstudied.
Yet, it is likely that for instance EPSP/spike potentiation or depression contributed to LTP and LTD we observed high-frequency afferent stimulation in vivo, since in those experiments the excitatory input strength was monitored though synaptically-elicited postsynaptic spike probability.

-Reviewer: Could you comment the possible contribution of intrinsic plasticity of individual neurons?
-Reply: In the study in vivo where we monitored excitatory pathway stimulation effect on synaptically-evoked interneuron spiking, axonal Kv1.1 or Kv1.2 channel down- or upregulation may contribute to the observed long-term plasticity of synaptically evoked spike probability and delay (Campanac E, Gasselin C, Baude A, Rama S, Ankri N, Debanne D. Neuron 77:712-22, 2013).
However, in the slice studies a subthreshold EPSP was systematically used to investigate glutamatergic pathway strength. Therefore it is unlikely that axonal Kv1 channels would contribute to the measured changes in EPSP in those plasticity experiments.
It is known in pyramidal cells that synaptic activity and consequent Ca^{2+} influx to cytoplasm can cause local changes in dendritic excitability. This means that a highly active glutamatergic excitatory pathway can elicit potentiation or depression of synaptic EPSP though plasticity of non-synaptic mechanisms which increase EPSP propagation along the dendrite towards soma. Such plasticity can show pathway specificity (assuming that the tested and control pathways are not terminating to same dendritic branches). However, because in our experiments the LTP or LTD was also associated with presynaptic changes it is unlikely that intrinsic plasticity in dendrites explain the long-term changes observed in EPSP slope and amplitude.

-Reviewer: Could you comment the possible contribution of non-synaptic release of neurotransmitters?

-Reply: I would argue that dendritic release of neurotransmitters or neuromodulators most likely have some effect on interneuron long-term plasticity, particularly facilitating or inhibiting its induction. However, we did not investigate it here and therefore we have no direct evidence for such action.
Yet, I would like to make a note that in few of studies I have recently investigated adenosine-mediated effect on basal synaptic transmission in identified hippocampal interneurons, including many CA1 area interneuron types. Although adenosine is not actually a neurotransmitter, is a non-synaptically released neuromodulatory substance it can affect synaptic and intrinsic plasticity since CA1 area interneurons show highly cell-type specific responses to adenosine receptors. Hence, it is likely that substances like adenosine, released non-synatically by neuronal activity affect both the synaptic- and nonsynaptic forms of long-term plasticity in these cells. However, this field is still very poorly known.

-Reviewer: It is also described by the author that NMDA receptor mediated EPSPs are recovered when whole-cell patch clamp recording is terminated. What was the time window for recovery? I guess that the recovery process in not unlimited it may depend on the duration of the whole-cell recording.
-Reply: Full recovery for NMDAR EPSCs was 10-15 min after the whole cell recording was terminated. We show this in a plot in Lamsa et al. 2005 figure 2, panel f. I agree with the Reviewer that duration of whole-cell recording is likely to affect the recovery; longer the cell is kept in whole-cell mode, longer and less complete is its recovery. To demonstrate reversible whole-cell effect on the NMDAR response strength we used relatively brief (5 minutes only) recording in whole-cell mode. In this set-up the NMDAR response was rapidly inhibited and fully recovered in 10-15 min time.

-Reviewer: Neuropeptides are out of scope of this study but still may I ask your opinion or comment about the possible contribution of low molecular neuropeptides to the neuronal plasticity in investigated neuronal circuits?
-Reply: I would expect plasticity interneurons not to be much different from what has been found in principal cells regarding its regulation and dependence on nonsynaptically-released neuromodulators and neuropeptides. As I briefly mentioned above in an example, substances like adenosine (not a peptide though) are well-known modulators of LTP in principal cells and they will probably have some effect on long-term plasticity in interneurons too.
Similarly, although it still remains unknown, I would assume that neuropeptides such as BDNF (reviewed by Lu Y1, Christian K, Lu B. Neurobiol Learn Mem. 89:312-23, 2008), neuropeptide Y (Whittaker E1, Vereker E, Lynch MA. Brain Res. 827:229-33, 1999) or endogenous peptide ligands to μ-opioid receptors (reviewed in Sanderson TM, Georgiou J, Tidball P, Collingridge GL. Cell Rep 28:1117-1118, 2019.) modulating LTP or LTD in principal cells might have an effect on interneuron plasticity. In line with this, receptors for oxytocin, cholecystokinin and vasopressin or somatostatin are expressed all over the brain including interneurons. Some neuropeptides (such as neuropeptide S or somatostatin) have been shown to have a role as facilitating cognitive performance or ameliorating learning and cognitive in diseased brain. Many of these substances are released from neurons by neuronal activity akin to seen during LTP or LTD induction.
Therefore, although direct evidence for a role and modulation by various neuropeptides of the interneuron long-term plasticity is still missing, it is likely that many of these substances have important and specific roles in this regulation. Direct evidence for neuropeptides key role in improving cognitive performance and learning strongly suggest this.

Reviewer Kemenes Gyorgy

-Reviewer: The main criticism I have regarding the thesis and the applicant's published work in general is the apparent lack of discussion of the potentially evolutionarily conserved role of inhibitory interneurons in the regulation of neuronal network activity, including circuit plasticity – and not just between the rodent and human brain.
-Reply: I apologise that discussion for the evolutionary aspect of inhibitory interneurons and their plasticity is relatively thin in the thesis focusing on just human and rat (or mouse). However, it was my deliberate decision to keep this topic focused here for following reasons.
First, operation and plasticity of interneurons was studied in specific brain areas in mammals (hippocampus and neocortex) associated with higher level cognitive functions and the contextual learning processes behind it. Neocortex by definition is present only in mammals, and the hippocampal formation can be found in reptiles as well as in avians too but not in lower chordata or invertebrates. Higher level cognitive functions and specific brain areas where these take place are poorly studied and characterised in vertebrates other than mammals (perhaps excluding song-bird seasonal brain plasticity which however can be considered something not

discovered as such in the mammalian brain). Therefore, discussing the evolution of microcircuit processes of 'higher cognitive functions and memory processes behind them' was strictly restricted here to compare human and rat (or mouse) for which the brain organisation is largely similar - deviated only by 80 mya evolution- and in addition where the plasticity and learning takes place in microcircuits which are relatively well characterised (including consensus on many evolutionarily conserved neuron types in rodents and primates). Although behaviour and learning have been investigated in very different species of the animal kingdom, and various stunning studies of learning and behavioural adaptation can be found in bees and fruitflies, nematodes, octopussies and sea slugs, their entire nervous system large- scale organisation (and to some extent also small-scale anatomy e.g. lack of axonal myelin shield) differs from the mammalian brain so drastically that in the context of 'inhibitory microcircuits in higher brain functions' the comparison of evolutionary aspect would have simply required too much space here.
Second, data for species-specific cellular features comprise only a relatively small fraction of results in the thesis. four of five articles in this study were entirely based on studies utilising rodent brain tissue. Indeed, our only article here that compared species-specific features (other than rat vs. mouse) in brain circuits was the study published in PlosBiol (2016) utilising in parallel the human and rat neocortical tissue. I feel that elaborating the evolutionary aspect in the thesis would draw attention from main topics of the thesis (such as interneuron type specificity and the induction and expression patterns of plasticity).

-Reviewer: There are numerous examples of studies from both invertebrate and lower vertebrate model systems pre-dating or contemporary to the applicant's work where the roles of inhibitory neurons have been elucidated in the regulation of network activity, such as central pattern generation or indeed network and behavioural plasticity, and yet there is no reference to these in the thesis, or as far as I can see, in the applicant's published work. I would have welcomed a brief discussion of the broader evolutionary context of the importance of inhibitory interneuronal activity/plasticity to be included in a Doctoral Thesis and also in the original work underpinning it and was somewhat disappointed not to find it there. I am a neuroscientist working with an invertebrate model system and when we publish our findings we always put them into the context of relevant findings in other invertebrate as well as vertebrate model systems; it would be nice if this were reciprocated by neuroscientists using vertebrate models.
-Reply: I fully agree that specifically in small circuitry level (such as canonical feed-forward, feed-back and lateral inhibition configurations) a comprehensive evolutionary review including analogous operations in invertabrates and the vertebrates would have brought various tremendously exciting and important aspects in the discussion. Yet, for the two reasons I have mentioned above, I have left it out of the current thesis.
I would like to add that I have great enthusiasm toward the evolutionary aspect and I am always amazed how conserved many physiological key features are in nerve cells in the animal kingdom. Operation of excitatory and inhibitory neurotransmitter mechanisms including their synthesis, release, signalling mechanisms and synaptic regulation are largely standard in the animal kingdom (excluding some evolutionary specializations such as the inhibitory function of glutamate in vertebrate retina). Similarly, axon potential mechanism characterised by Hodkin and Huxley in a giant squid axon similarly apply to the mammalian neurons. Again, canonical organisation of inhibitory circuits, utilising GABA or glycine as a transmitter, are responsible for repetitive rhythmic pattern generation in invertebrate ganglia as well and in vertebrate spinal cord.
Fundamental work done with invertebrate neurons and neural circuits show us how small scale system operations can be applied to processes in mammalian brain; an example for this is Kandel's work demonstrating cellular mechanisms of behavioural learning in sea slug nervous system to follow largely similar rules and mechanisms as we now know are operating in the mammalian brain.

-Reviewer: Another area that the applicant could have addressed in a bit more detail in the discussion section of the thesis is how long-term synaptic and non-synaptic (intrinsic) plasticity may work together to result in network and behavioural level plastic changes. It is now well documented in both vertebrates and invertebrates that non-synaptic as well as synaptic plasticity can be a substrate for long-term memory and in the thesis the applicant does refer to the fact that GABAergic neurons undergo a wide range of synaptic and non-synaptic activity-induced plasticity processes. It would have been helpful if the thesis had explained briefly what the main findings were of the studies where both types of plasticity were investigated in the same neurons.
-Reply: This question was partly raised by other reviewers too, and I summarise some points here. Although not much is known about interneurons' long-term plasticity yet, some previous studies have show that neuronal activity in local circuits can cause long-lasting changes in intrinsic properties of cortical interneurons.
Indeed, neuronal activity in hippocampal interneurons can cause long-term alterations in their excitability and EPSP/spike -coupling. Studies on fast-spiking (putative PV+ basket cells) in hippocampal formation dentate hilar region as well as in the CA1 area have shown that high-frequency glutamatergic afferent pathway firing causes not only synaptic EPSC plasticity, but can induce permanent postsynaptic depolarisation of few millivolts through altered rate of electrogenic Na+/K+-ATPase pump function in interneurons (Ross ST, Soltesz

I. Proc Natl Acad Sci U S A 98:8874-9, 2001). Another reported non-synaptic long-term plasticity feature is downregulation of depolarisation-activated inhibitory potassium current (though downregulation of axonal Kv.1 channels and I_D current) reported in CA1 area PV+ interneurons (Campanac E, Gasselin C, Baude A, Rama S, Ankri N, Debanne D. Neuron 77:712-22, 2013).

It is possible that for instance in our experiments in vivo, where plasticity was monitored through interneuron spiking probability, both synaptic and non-synaptic mechanisms contribute to the long-term changes observed in the glutamatergic pathway's excitatory efficacy in the interneurons. Similarly, some of our recordings ex vivo demonstrating interneuron plasticity through disynaptic IPSC probability could partially be explained by both synaptic EPSP potentiation and non-synaptic plasticity in interneurons. Yet, how these mechanisms contribute to plasticity of behaviour of animal is virtually unknown. However, some studies on freely moving rats measuring neuronal discharge in the hippocampus through multichannel electrodes suggest that spatial learning is associated with persistent changes in GABAergic interneuron firing rates (Dupret D, O'Neill J, Csicsvari J. Neuron. 10,78:166-80, 2013). Such results suggest that reconfiguration of GABAergic inhibitory interneuron activity, through synaptic or non-synaptic interneuron plasticity, occurs during long-term spatial memory encoding in a novel environment where new memory maps are required for the navigation between places.