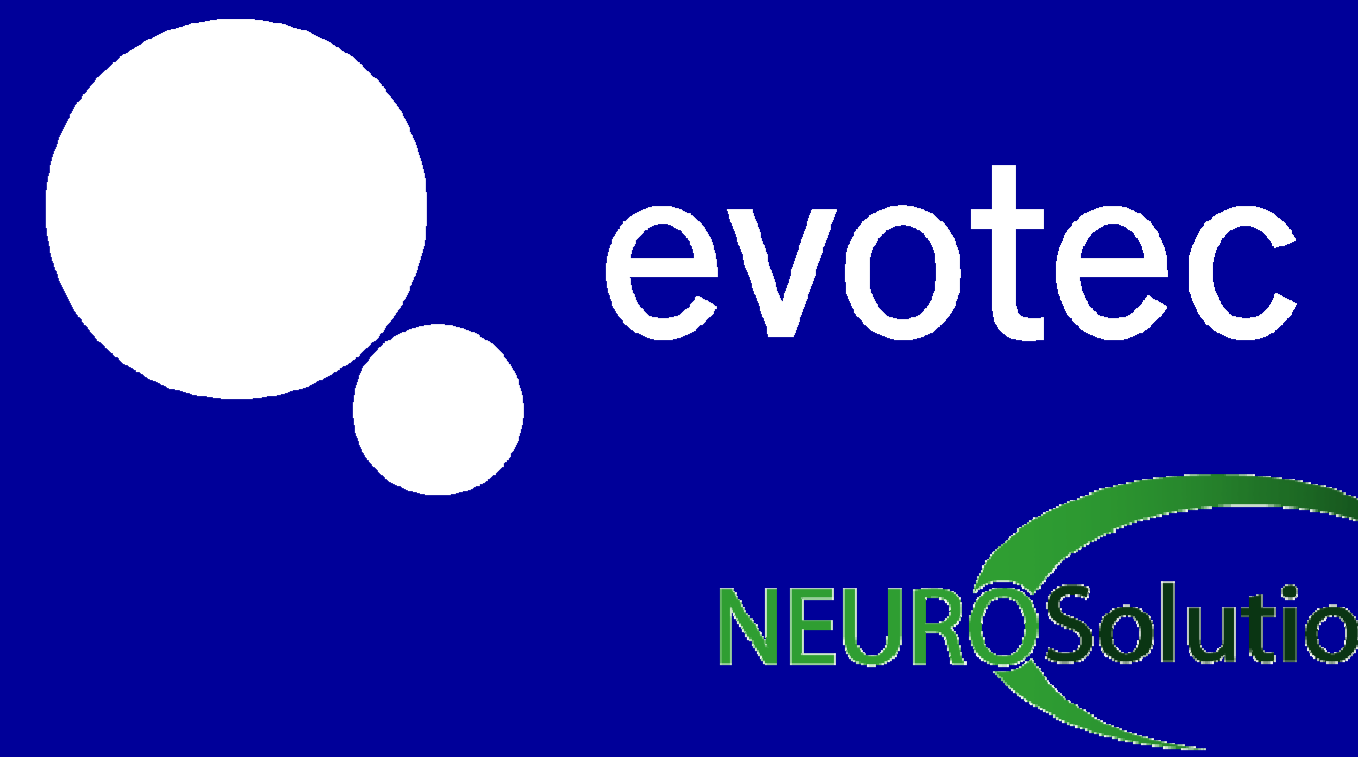


Selective blockade of NR2B subunit containing NMDA receptors enhances AMPA receptor-mediated EPSPs and sub-maximal LTP in hippocampal CA1 pyramidal neurons through disinhibitory effects



J. A. KEMP¹, R. JEGGO², A. WHYMENT², D. SPANSWICK² - ¹Evotec AG, Hamburg, Germany; ²Neurosolutions Ltd., Coventry, UK

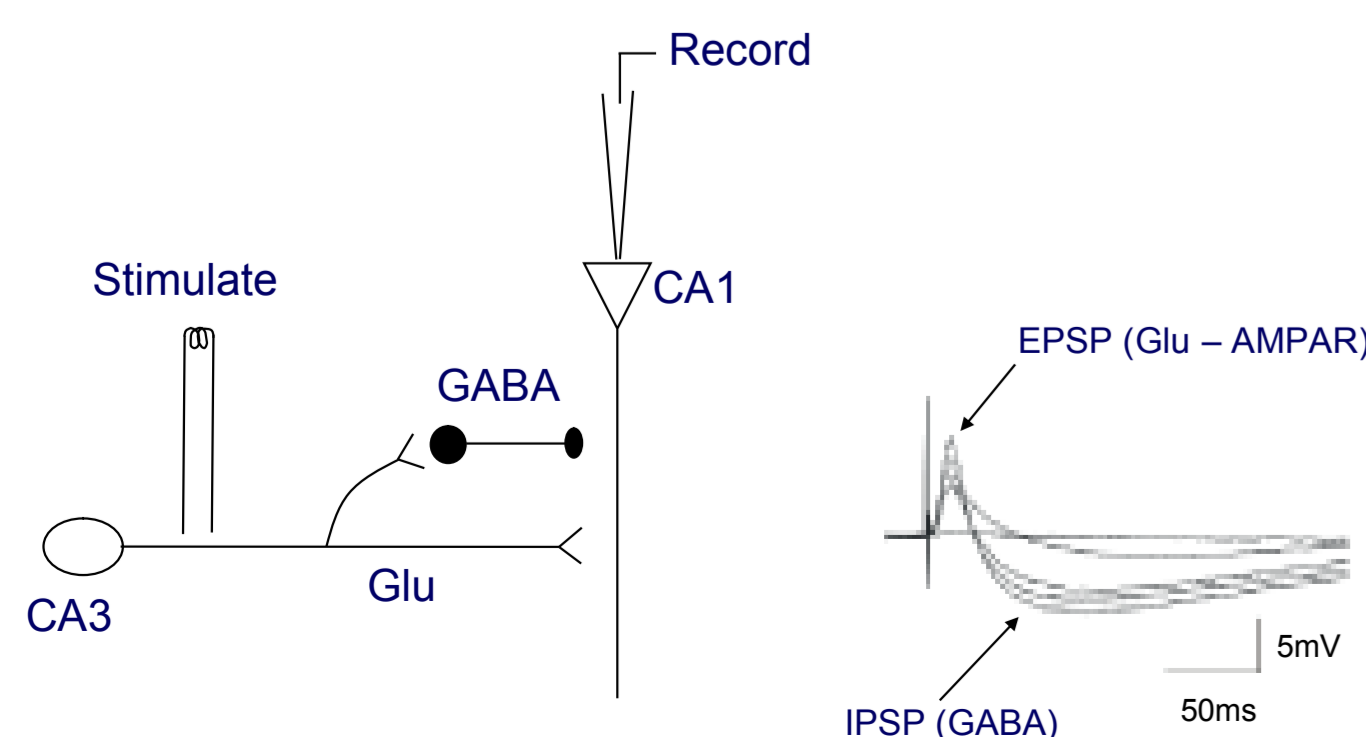
Introduction

NR2B subtype selective NMDA receptor antagonists, unlike non-selective NMDA receptor antagonists, do not produce marked stimulant or stereotypical behaviours in rodents, which are considered to reflect psychotomimetic properties in humans. In man, this has translated to high exposures being achieved without marked CNS or psychotic side-effects (Preskorn *et al.*, 2008; poster 641.22 this session). Furthermore, NR2B subtype selective antagonists have been shown to enhance performance in rats in certain cognitive tests of attention and working memory (Higgins *et al.*, 2005). We sought to examine the potential synaptic mechanisms that may underlie these behavioural findings by studying the effects of EVT 101 (poster 641.22 this session), and the related compound, EVT 105, novel NR2B subtype selective NMDA antagonists currently in clinical development, on synaptic transmission and long term potentiation in hippocampal slices from adult rats.

Methods

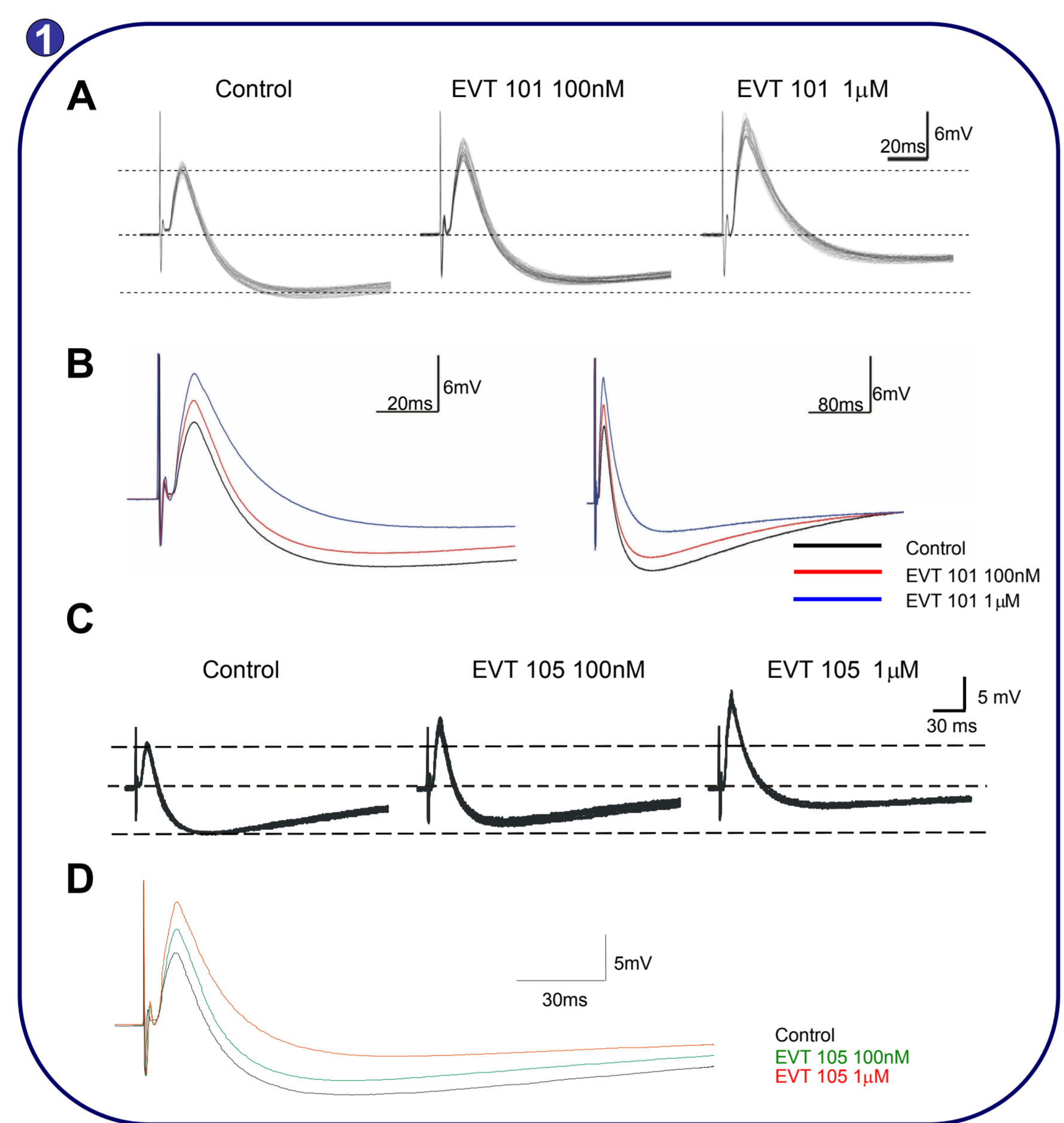
Sagittal hippocampal slices of 400µm thickness were prepared from male Wistar rats (5 - 8 weeks, 150-250g) in cooled artificial cerebrospinal fluid (aCSF; <4°C) using a microslicer (Leica VT1000S). Slices were subsequently maintained in oxygenated (95% O₂ - 5% CO₂) aCSF at room temperature for at least 1 hr prior to electrophysiological recording. Patch pipettes were pulled using a horizontal puller (Sutter Instrument Co, Novato, Ca, USA) from thin-walled borosilicate glass capillaries (Clarke Electromedical) and had resistances between 4-10 MΩ when filled with recording solution of the following composition (mM); Kgluconate 140, KCl 10, K₂EGTA 0.5, HEPES 10, Na₂ATP 2, pH 7.2. Recordings were obtained with a patch clamp amplifier (Axopatch-1D, Axon Instruments). The extracellular aCSF had the following composition (in mM): NaCl 127, KCl 1.9, KH₂PO₄ 1.2, MgCl₂ 1.3, CaCl₂ 2.4, NaHCO₃ 26 and D-glucose 10. Compounds were bath-applied from reservoirs connected to the aCSF flow line by manually operable three-way valves. Generally, antagonists were applied for at least 10 minutes to ensure equilibration in the recording chamber. Synaptic currents and potentials were evoked by electrical stimulation of the Schaffer collateral-commissural pathway with concentric bipolar stimulating electrodes (1-15V, 0.02ms, 0.03-0.05Hz).

Recording set up

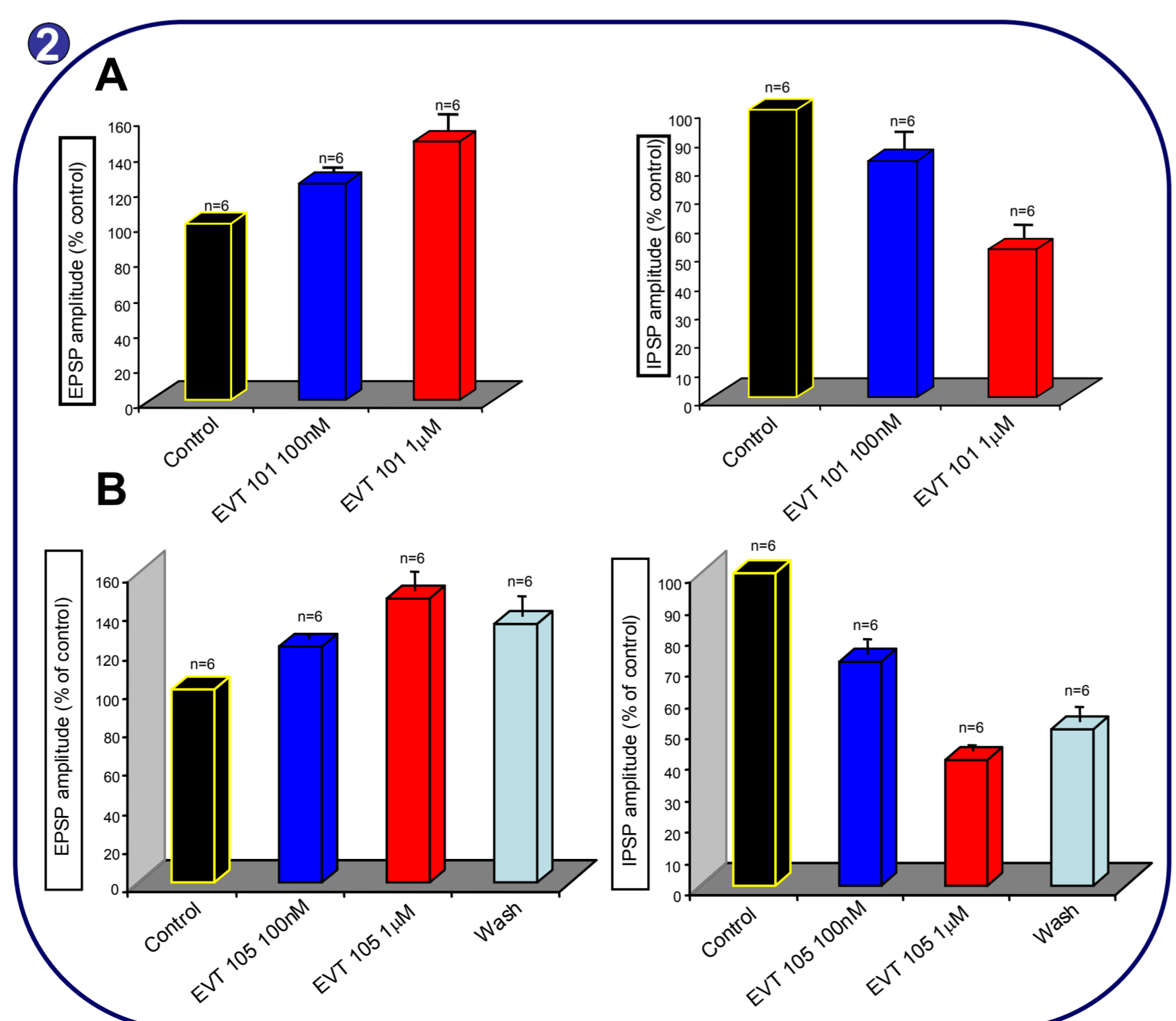


Results

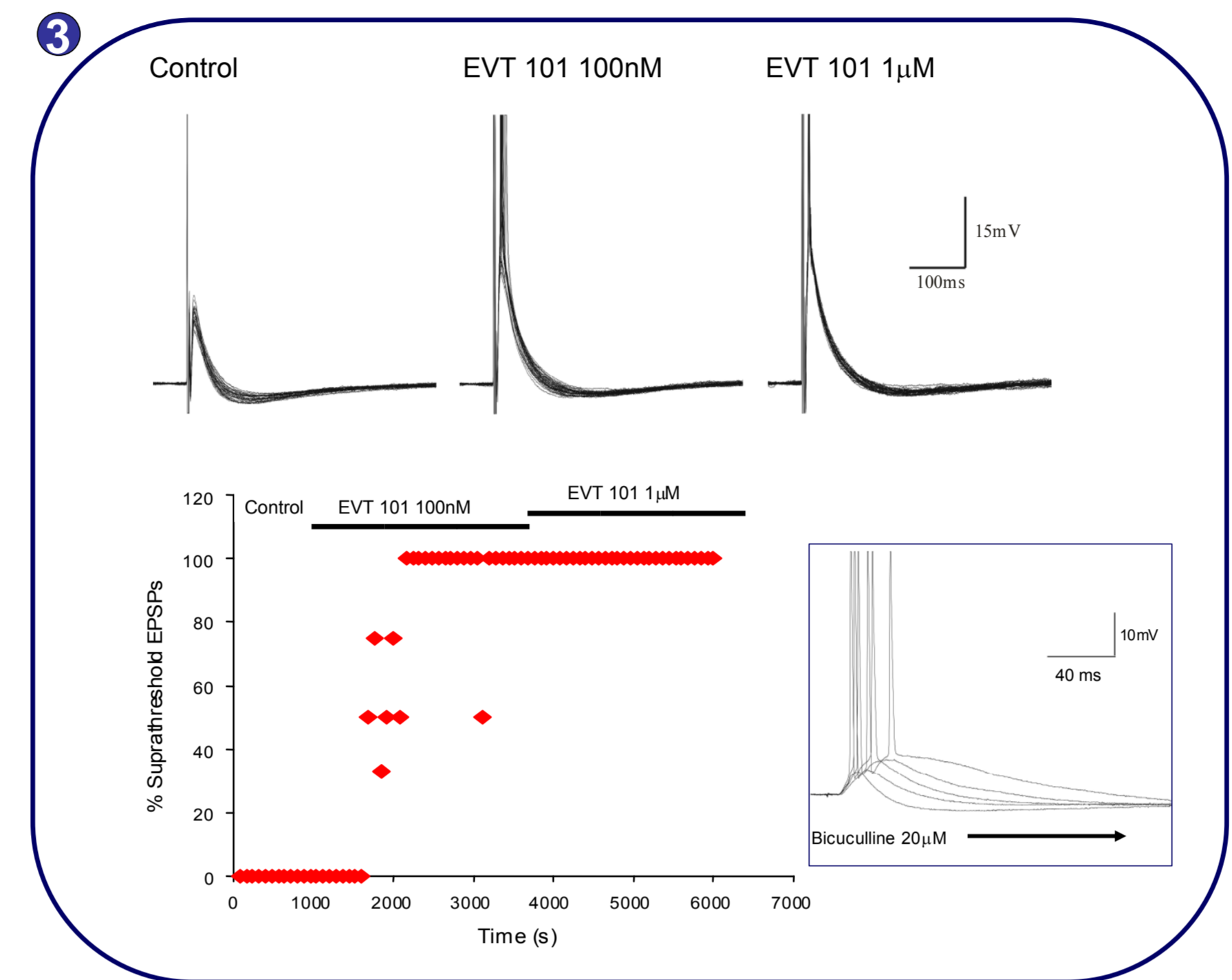
1 CA1 pyramidal neurones: EVT 101 and EVT 105 produced a concomitant increase in peak amplitude of EPSPs and a suppression of IPSPs evoked by stimulation of the Schaffer collateral pathway. Concentrations of 100 nM and 1 µM were used; approximately 10- and 100-fold above their IC₅₀s for blockade of NR2B receptors. **A & C** show at least 6 superimposed EPSP/IPSPs, **B & D** show the superimposed averages of at least 16 evoked responses.



2 CA1 pyramidal neurones: Mean data of the effects of (A) EVT 101 and (B) EVT 105 on evoked EPSPs and IPSPs in CA1 pyramidal neurones.

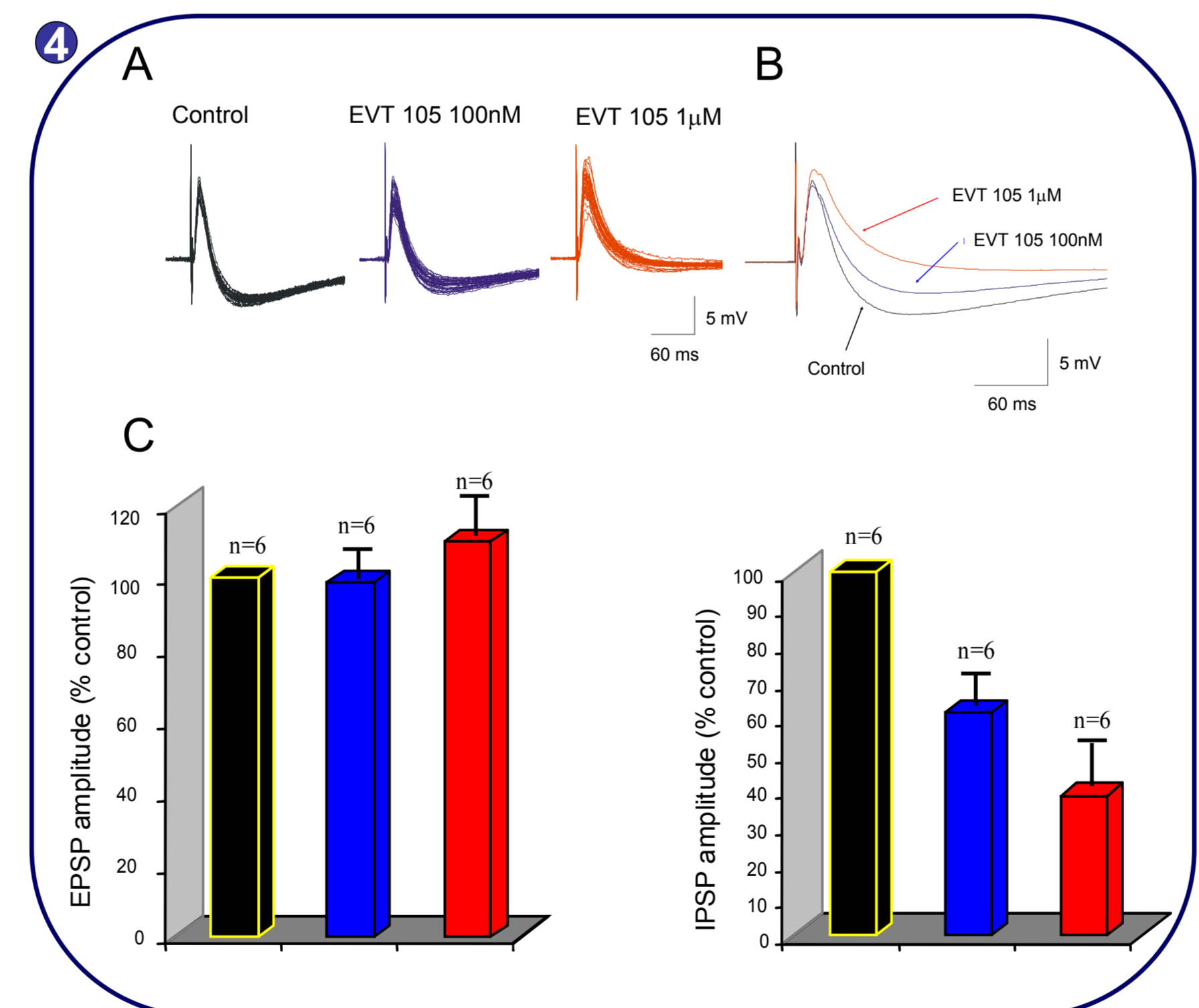


3 CA1 pyramidal neurones: EVT 101 and EVT 105 increased the probability of sub-threshold EPSPs reaching threshold for action potential firing but never induced burst firing or spontaneous burst firing, unlike the GABA_A receptor antagonist, bicuculline (inset)

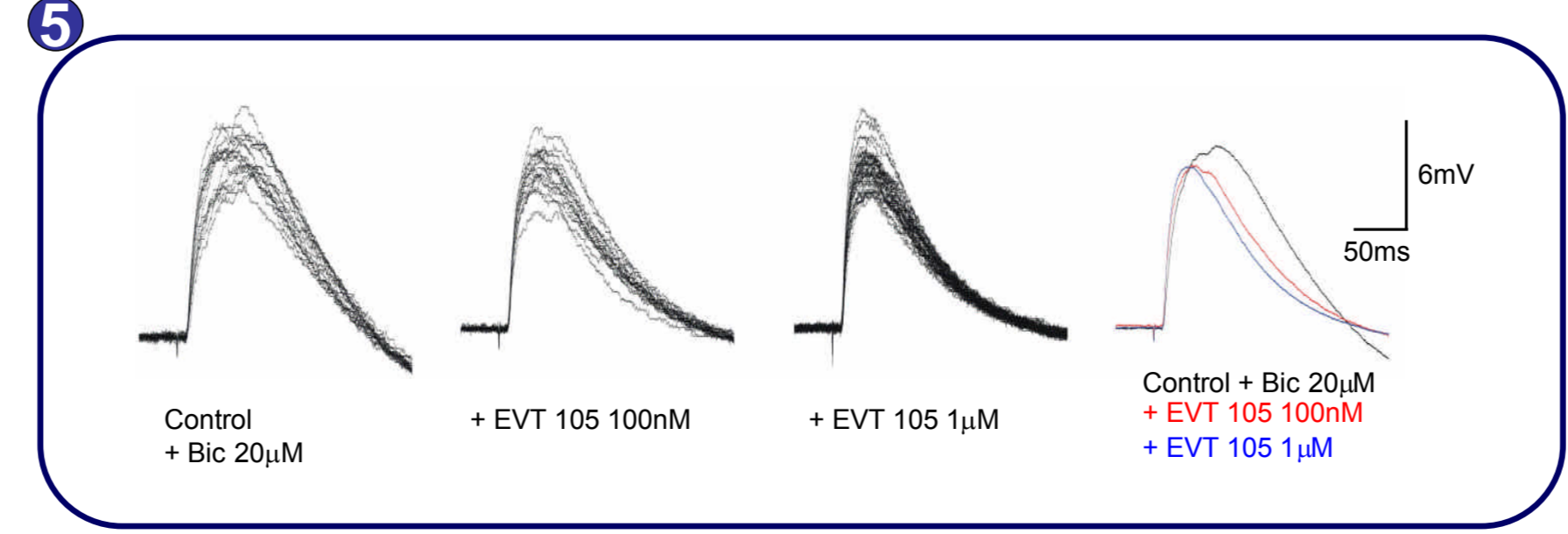


4 Stratum Radiatum interneurons: EVT 101 and EVT 105 did not produce the same increase in size of EPSPs as in CA1 cells although they produced a similar decrease in the size of the IPSP

A shows at least 8 superimposed EPSP/IPSPs, **B** shows the superimposed averages of at least 16 evoked responses and **C** shows the mean data from 6 interneurons



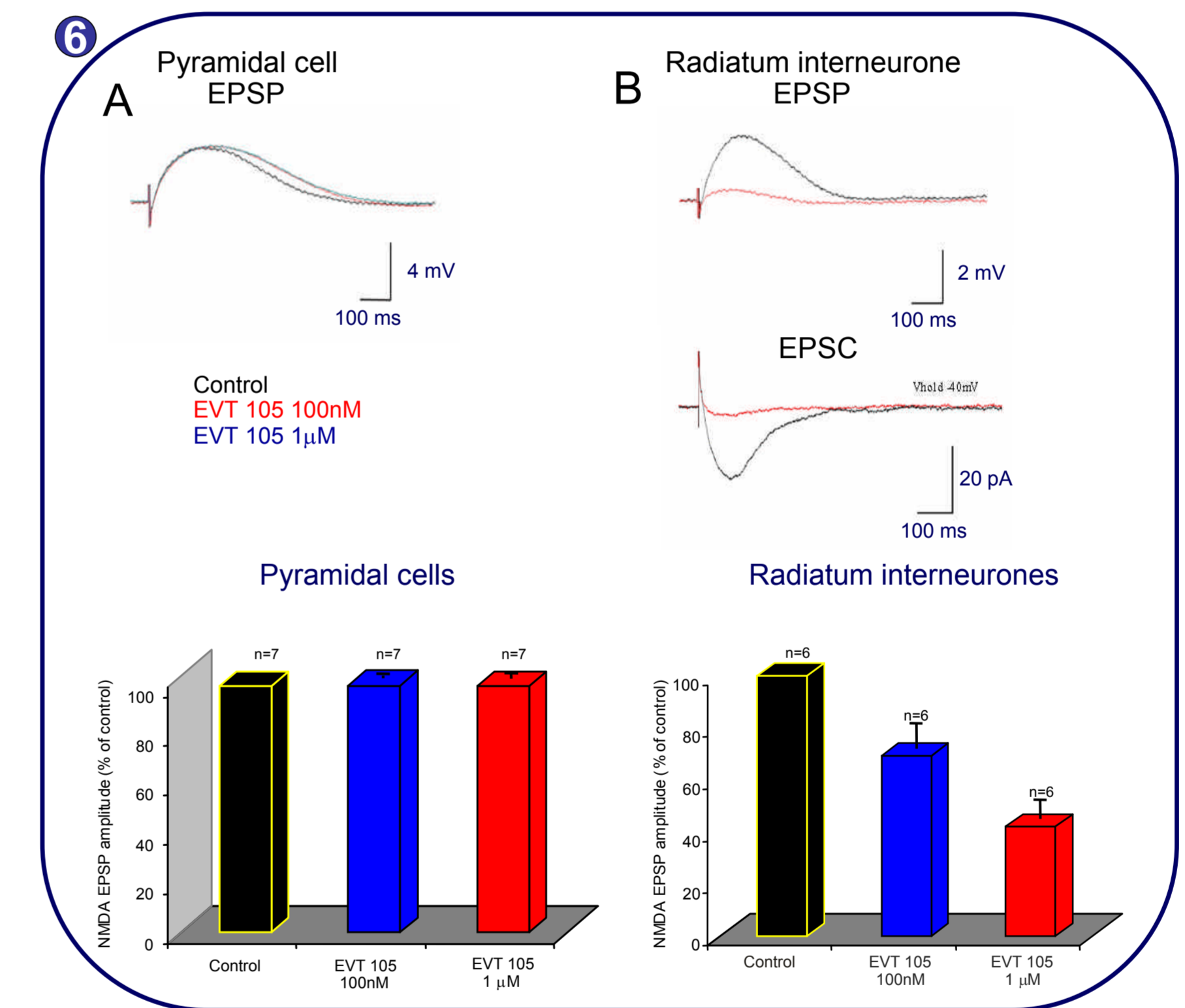
5 CA1 pyramidal neurones: Blockade of fast GABAergic inhibition with bicuculline (20 µM) occluded the potentiating effect of EVT 105 on EPSPs



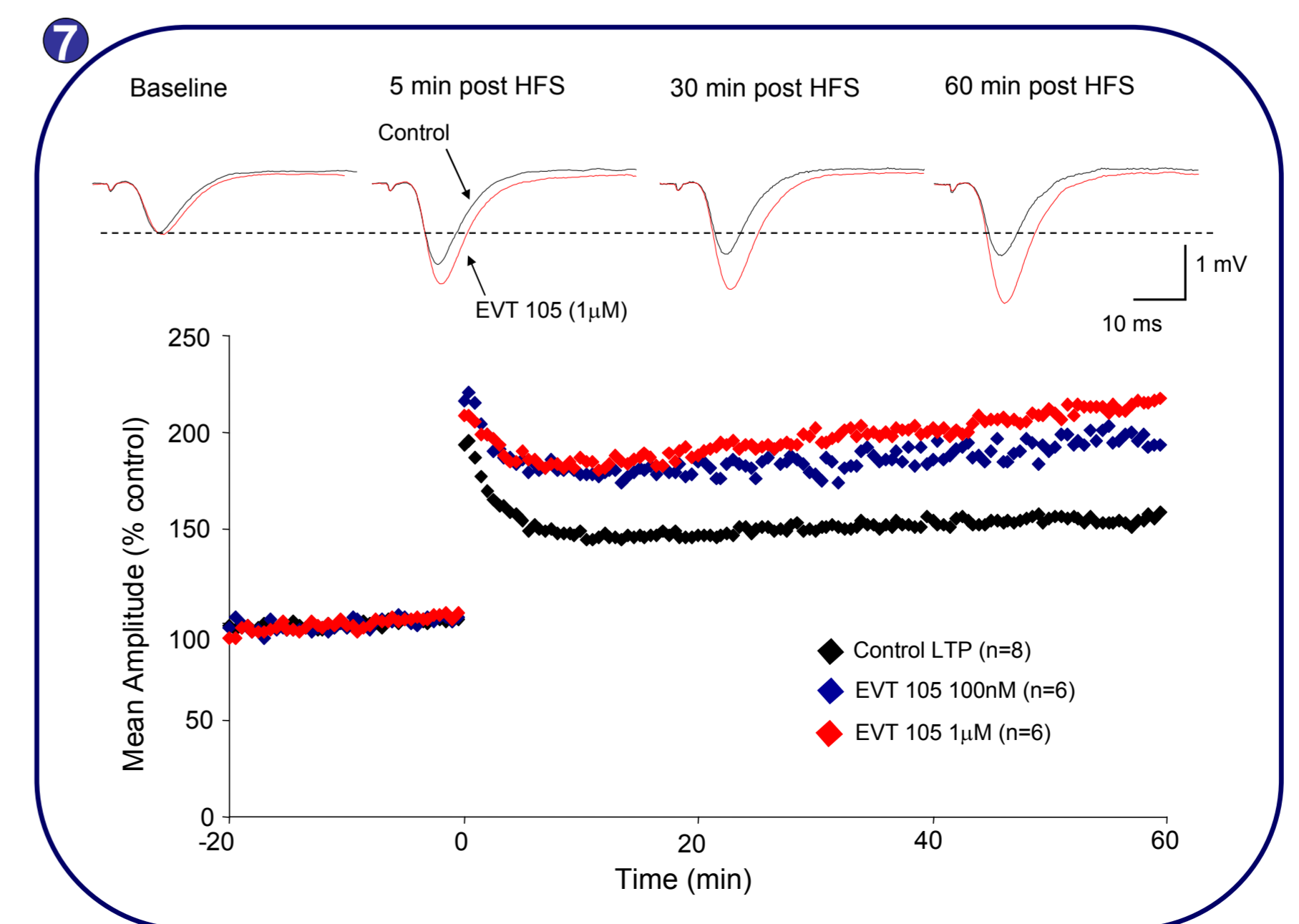
These results were consistent with EVT 101 and EVT 105 having disinhibitory effects on synaptic transmission in the CA1 region of the hippocampus, indicating that NR2B receptors may play a more predominant role in the synaptic excitation of inhibitory interneurons than of CA1 pyramidal neurones.

Therefore, we investigated this directly by examining the effects of EVT 105 on isolated NMDA receptor-mediated synaptic responses in both CA1 pyramidal cells and stratum radiatum interneurons. NMDA receptor-mediated responses were isolated by perfusion with the GABA_A receptor antagonist, bicuculline (20µM), the GABA_B receptor antagonist, CGP55845 (200nM), the non-NMDA receptor antagonist, NBQX (10µM) and depolarisation of the membrane potential to -48 to -40mV.

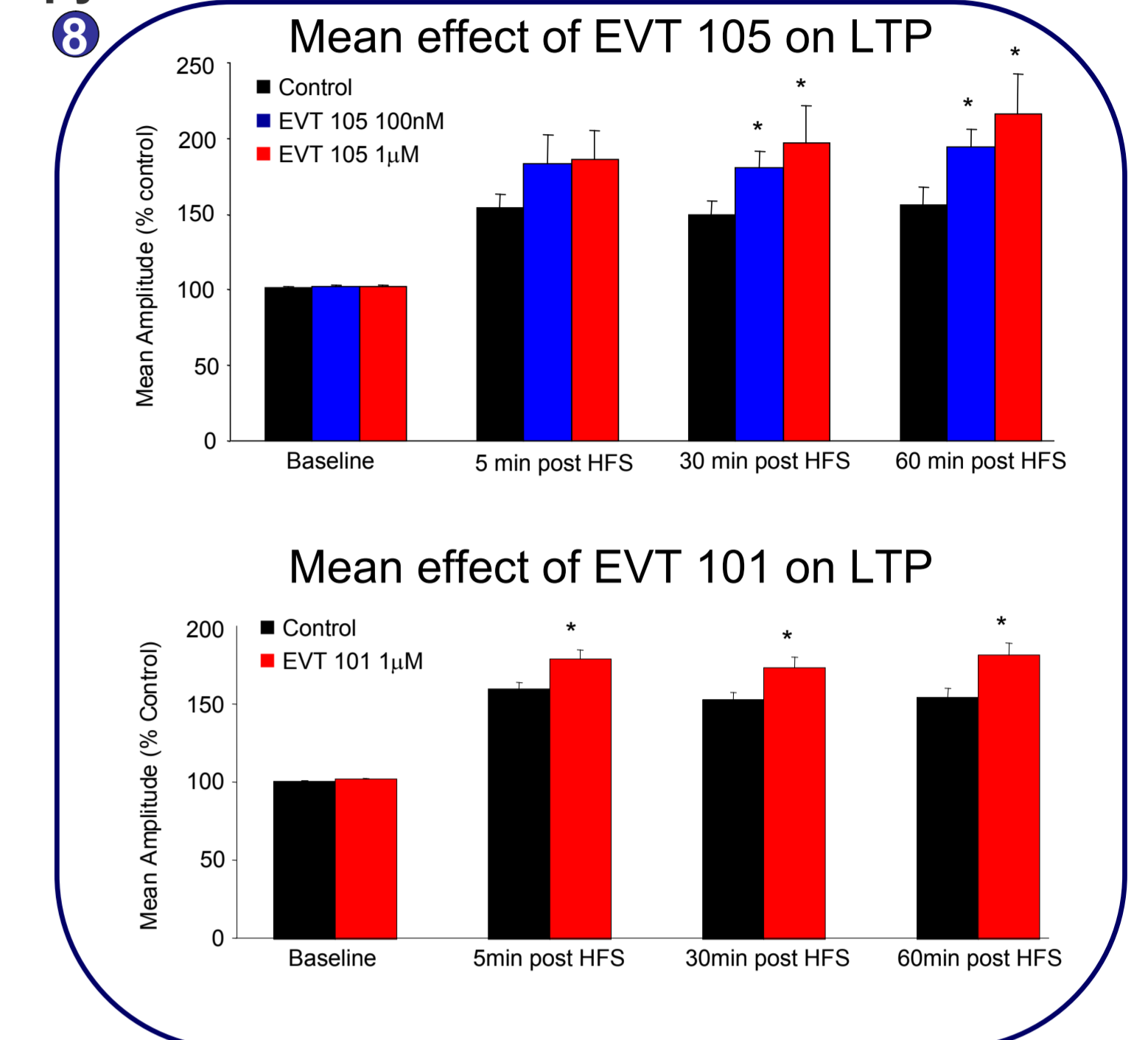
6 EVT 105 had no effect on the isolated NMDA receptor-mediated EPSP in A) CA1 pyramidal cells but produced a profound inhibition of the evoked EPSP and EPSC in B) stratum radiatum interneurons.



As these findings indicated that the NR2B subtype selective antagonists produced disinhibition without blocking NMDA receptor-mediated transmission on CA1 pyramidal cells, we examined the effect of EVT 101 and EVT 105 on the induction and maintenance of submaximal LTP following high frequency stimulation (HFS) of the Schaffer collateral pathway.



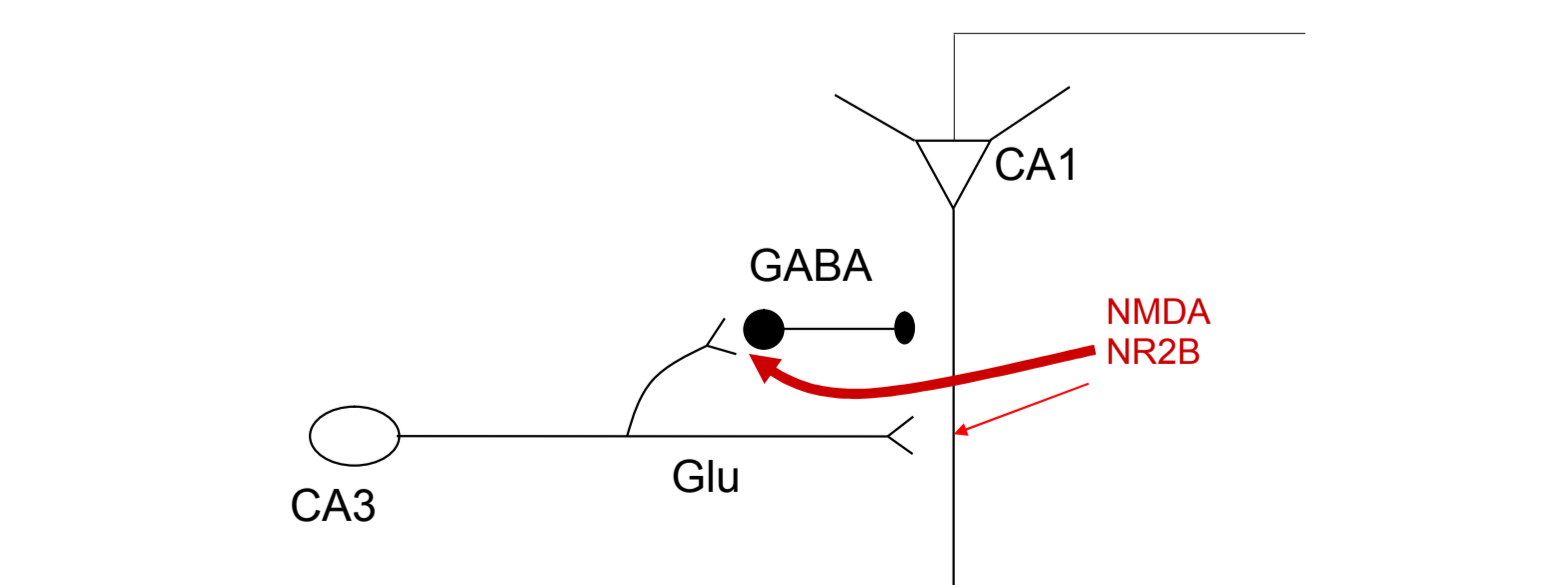
8 EVT 101 and EVT 105 produced a potentiation of submaximal LTP in CA1 pyramidal cells.



These results are consistent with previous studies indicating that, under the conditions used here of single shock stimulation, NR2A subunit containing receptors predominate in the mediation of NMDA receptor responses in mature Schaffer collateral - CA1 synapses and, furthermore, that NR2B antagonists do not block the induction of LTP. Our findings also suggest that the disinhibitory effects induced by NR2B subtype selective antagonists can lead to an enhancement submaximal LTP formation.

Conclusions

1. EVT 101 and EVT 105 increased fast excitatory synaptic responses in CA1 neurones following stimulation of the Schaffer collateral pathway through a disinhibitory effect
2. EVT 105 markedly inhibited NMDA receptor-mediated EPSPs and EPSCs on stratum radiatum interneurons but was without effect on CA1 pyramidal cells
3. In contrast to non-selective NMDA antagonists, EVT 101 & 105 potentiated sub-maximal LTP induction
4. These effects may underlie the cognitive enhancing effects of NR2B subtype selective antagonists seen in certain behavioural tests



References

Higgins, G.A., Ballard, T.M., Enderlin, M., Haman, M., Kemp, J.A. (2005) Evidence for improved performance in cognitive tasks following selective NR2B NMDA receptor antagonist pre-treatment in the rat. *Psychopharmacology*, 179: 85-98
 Preskorn, S.H., Baker, B., Kolluri, S., Menniti, F.S., Krams, M., Landen, J.W. (2008) An Innovative Design to Establish Proof of Concept of the Antidepressant Effects of the NR2B Subunit Selective N-Methyl-D-Aspartate Antagonist, CP-101,606, in Patients With Treatment-Refractory Major Depressive Disorder. *J Clin Psychopharmacol*, 28: 631-637