The role of alpha7 nicotinic receptors in the ventral hippocampus in heroin-induced conditioned place preference

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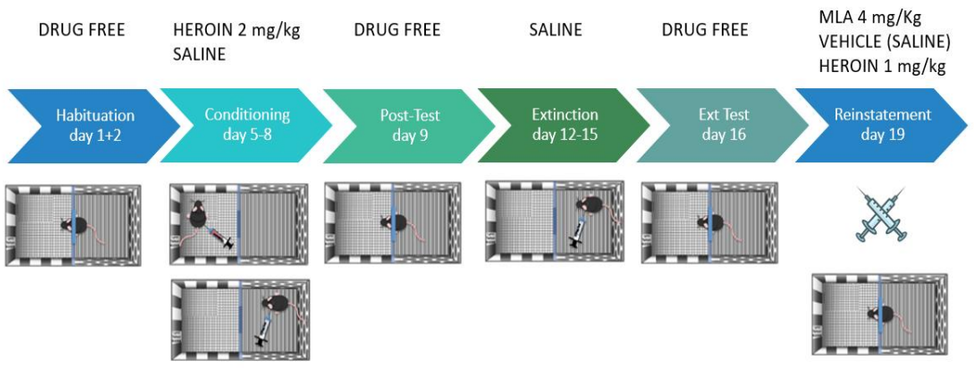
INTRODUCTION

The maintenance of maladaptive drug cue–context associations induces relapse. The ventral hippocampus (vHIP) processes associative memory, in which the cholinergic system can modulate synaptic plasticity. Inhibition of alpha7 nicotinic acetylcholine receptors (α7-nAChRs) in vHIP with the antagonist methyllycaconitine (MLA) attenuated reinstatement in morphine-conditioned place preference (CPP) [1]. The aims of the study are:

- To investigate if and how α7 nAChRs contribute to the reinstatement of the heroin- CPP in mice and to the c-Fos expression.
- To determine if MLA administered before heroin or saline affects c-Fos expression in the absence of drug-conditioning.
- To evaluate the actions of α7 nAChRs in the CA1 of vHIP slices with patch-clamp electrophysiology.

MATERIALS AND METHODS

- Time-line of heroin-CPP (male C57BL/6J)



- In the second experiment (Fig. 3), naive mice were treated as on the CPP reinstatement day and brains horizontally sliced.
- Whole-cell patch-clamp electrophysiology experiments were conducted in vHIP using α7 positive allosteric modulator (PAM; PNU-120596 (10 μM)), α7 agonist (PNU-282987 (300 nM)) and MLA (100 nM) [2]. Holding membrane potential was -60 mV, with voltage steps to 0 mV to permit recording of both EPSCs and IPSCs [3].].

RESULTS: C-FOS ACTIVATION IN vHIP IN NON-CONDITIONED MICE

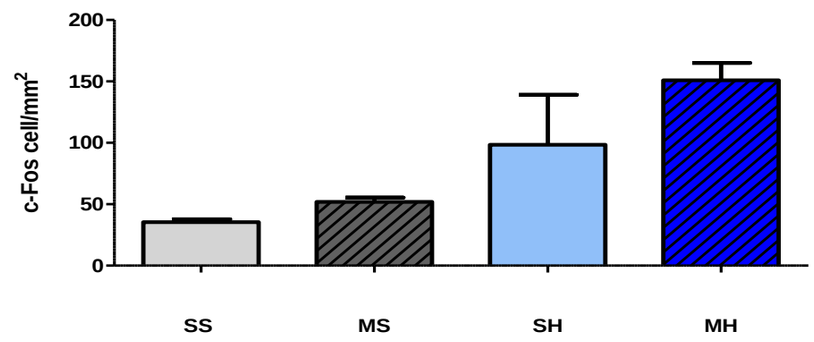


Figure 3 – MLA did not affect c-Fos expression in naive mice given a single injection of drug or saline

- No significant difference in mice pre-treated with MLA or SALINE 20 min before receiving SALINE or HEROIN**;** a single heroin injection (SH) increased c-Fos expression in the vHIP after and there is (SALINE-SALINE, SS: n=2; MLA-SALINE, MS: n=3; SALINE-HEROIN, SH: n=5; MLA-HEROIN, MH: n=2).
- MLA also did not decrease c-Fos expression per se in any of the following brain regions: infralimbic cortex, prelimbic cortex, dentate gyrus, CA2 & CA3 regions of vHIP, nucleus accumbens core and shell (data not shown)

RESULTS: HEROIN-INDUCED CPP REINSTATEMENT AND C-FOS EXPRESSION IN vHIP IN MLA-TREATED MICE AND SALINE CONTROLS

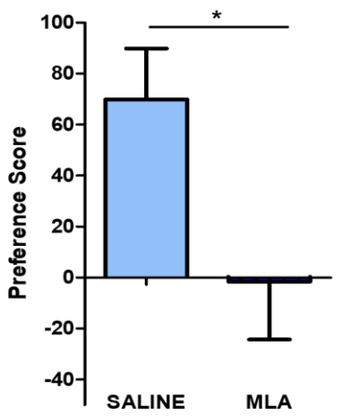


Fig. 1 – MLA abolished reinstatement of heroin-induced CPP – All mice acquired and extinguished heroin-CPP. MLA administered prior to heroin-priming abolished reinstatement of CPP (SALINE: N=8; MLA: N=8; *=p<0.05 Student's t-test).

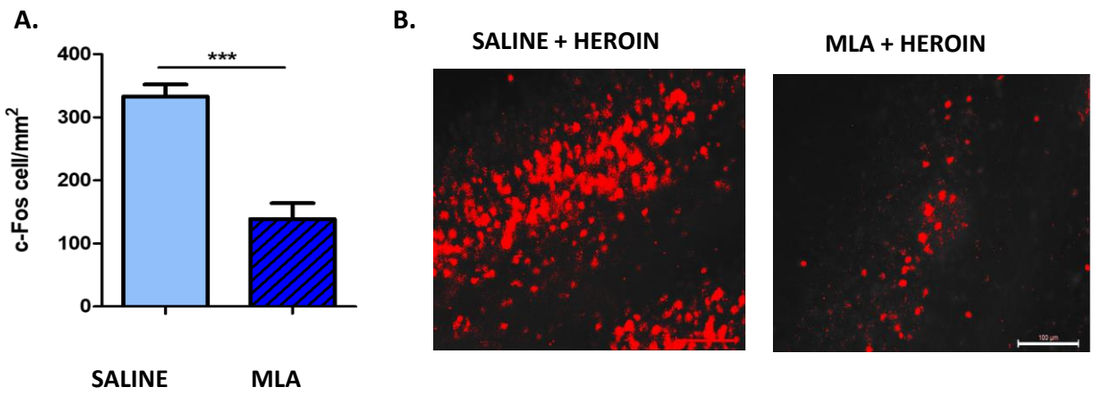
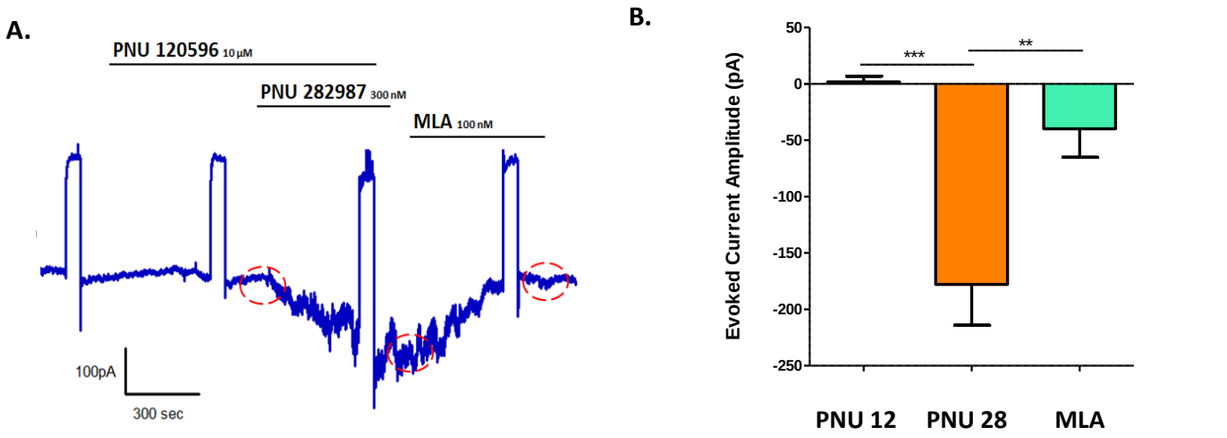


Figure 2 – MLA decreased c-Fos expression after heroin-primed CPP reinstatement in the vHIP

A. Administration of MLA before to heroin-primed reinstatement significantly reduced c-Fos expression in vHIP compared to saline-treated mice (SALINE: n=4; MLA: n=4; ***=p<0.001 Student's t-test).

B. Representative coronal sections of mouse brain, at the level of the vHIP, stained for c-Fos.

RESULTS: POST-SYNAPTIC CURRENTS INDUCED BY α7 nAChR ACTIVATION IN CA1 PYRAMIDAL NEURONS IN vHIP

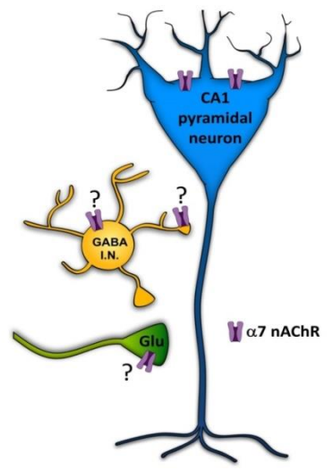


A. Representative recording trace **B.** Column chart of evoked currents

Fig. 4 A&B– Activation of α7 nAChRs induced significant changes in current - Co-administration of the α7 PAM (PNU 120596 (10 μM)) and α7 nAChR agonist (PNU 282987 (300 nM)) induced a significant inward current in vHIP CA1, reversed by the selective α7 nAChRs antagonist MLA (100nM). α7 PAM alone had no effect (n=8: 5 cells responded to PNU 282987, 3 cells did not. **p<0.001, ***p<0.001 One-Way Repeated Measures ANOVA with Turkey post-hoc test; n = 5; red circles indicate the point of the measurements).

CONCLUSIONS

- Reinstatement of heroin-CPP was inhibited by MLA.
- Reinstatement of heroin-CPP increases c-Fos expression in the vHIP, an effect that was also inhibited by MLA.
- MLA had not effect *per se* on c-Fos activity in vHIP in naïve mice. Its effect appears to be specific to reinstatement, highlighting the potential role of α7 nAChRs in recalling addiction-related memories.
- Activation of α7 nAChRs caused a significant inward current in CA1 pyramidal cells in vHIP, demonstrating postsynaptic localisation of α7 nAChRs. Further analysis of electrophysiological recordings will examine the effects of α7 nAChR activation on excitatory and inhibitory postsynaptic events, to identify potential presynaptic α7 nAChRs.
- Future work will determine how projections from vHIP to other brain regions are affected by MLA after heroin-CPP reinstatement.



REFERENCES: [1] Wright VL et al (2019) Addiction Biology 24:590-603; [2] Udakis et al (2016) Neuropharmaocology 105 618-629; [3] Semyanov and Kullmann (2000) Neuron *25*(3), 663-672