Patch Clamp Electrophysiology

Modulation of spontaneous inhibitory synaptic transmission by pharmacological activation of alpha7 nicotinic acetylcholine receptors in mouse and human neocortex.

Introduction

The alpha7-type nicotinic acetylcholine receptor (α7nAChR) is a remarkable homopentameric ligand-gated ion channel, with relatively high calcium permeability. It is widely expressed in the brain with high levels in the hippocampus and the cerebral cortex. At the cellular level, they are found in pyramidal neurons, GABAergic interneurons, but also in non-neuronal cell types such as glial and immune cells. In neurons, its activation leads to Ca2+ influx, neurotransmitter release and regulates synaptic transmission and plasticity. They appear to be critical for learning and memory and attentional functions, but also to limit inflammatory neurotoxicity. Lower levels of α7nAChRs are observed in cerebral tissue from patients with Alzheimer's disease, schizophrenia, or Parkinson's disease, making them interesting and promising therapeutic targets.

Objective

It was reported that pharmacological activation of α7nAChRs increases spontaneous inhibitory transmission onto pyramidal neurons in mouse medial prefrontal cortex (Udakis et al., 2016). Hence, we wanted to develop such an assay in the cerebral cortex from mice and humans to have the capability of testing new compounds targeting α7nAChRs and their ability to modulate inhibitory synaptic transmission.

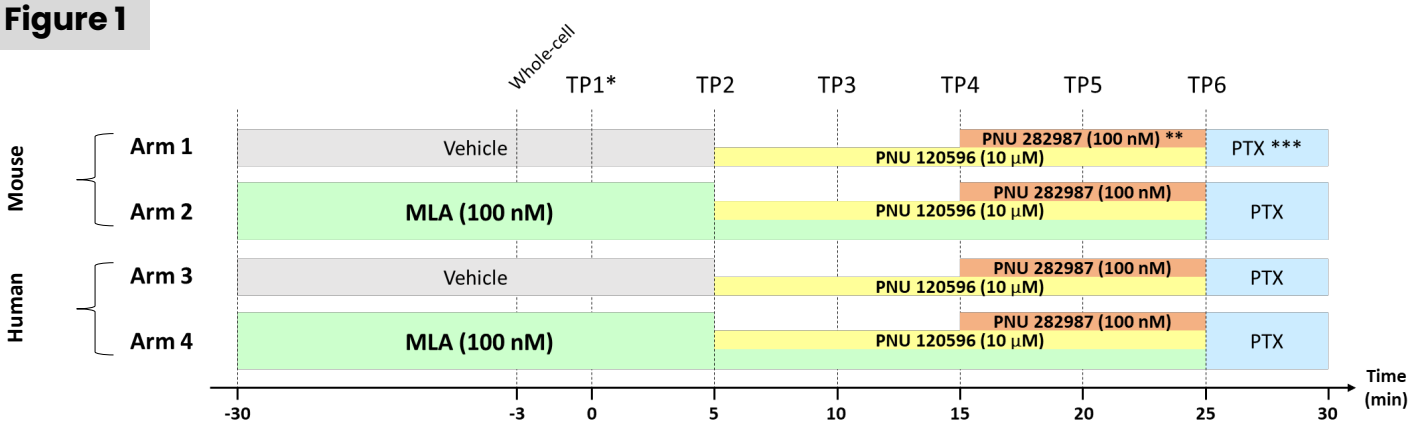
Methodology

Spontaneous inhibitory post-synaptic currents (sIPSCs) were recorded in whole-cell configuration and voltage-clamp mode from layer V pyramidal neurons, which represent the main efferent pathway to subcortical structures.
Recordings were performed in the medial prefrontal cortex (mPFC) from mice to reproduce data from the literature, and in the anterior medial temporal cortex from human epileptic patients. GABAAR-mediated sIPSCs were isolated using 40 µM D-AP5 and 10 µM NBQX and 5 µM CGP55845 to block glutamatergic and GABAB receptors, respectively, and held at resting membrane potential (-70 mV) using a CsCl-based internal solution. sIPSCs were detected using a custom-made script in Igor (10 pA static threshold) and sIPSC frequency and amplitude were quantified over time, within 30s-bins.

Experimental design

α7nAChR-mediated currents are known to desensitize very quickly and at very low agonist concentrations (Papke and Porter Papke, 2002). Hence, to counteract the desensitization of α7nAChRs, we used the selective positive allosteric modulator PNU 120596 (10 µM) alone (10 min) and in combination with the selective α7nAChR agonist PNU 282987 (100 nM, 10 min) after a vehicle control period (5 min) (Figure 1). We applied picrotoxin (PTX, 100 µM, 5 min) at the end of each experiment to confirm the GABAergic nature of the recorded sPSCs. In control arms, we used preapplication of the selective α7nAChR antagonist methyllycaconitine (MLA, 100 nM, 30 min) to prevent any sIPSC modulation by PNU 120596 and PNU 282987. This protocol was performed in mice and human (arms 1-2 and 3-4, respectively).

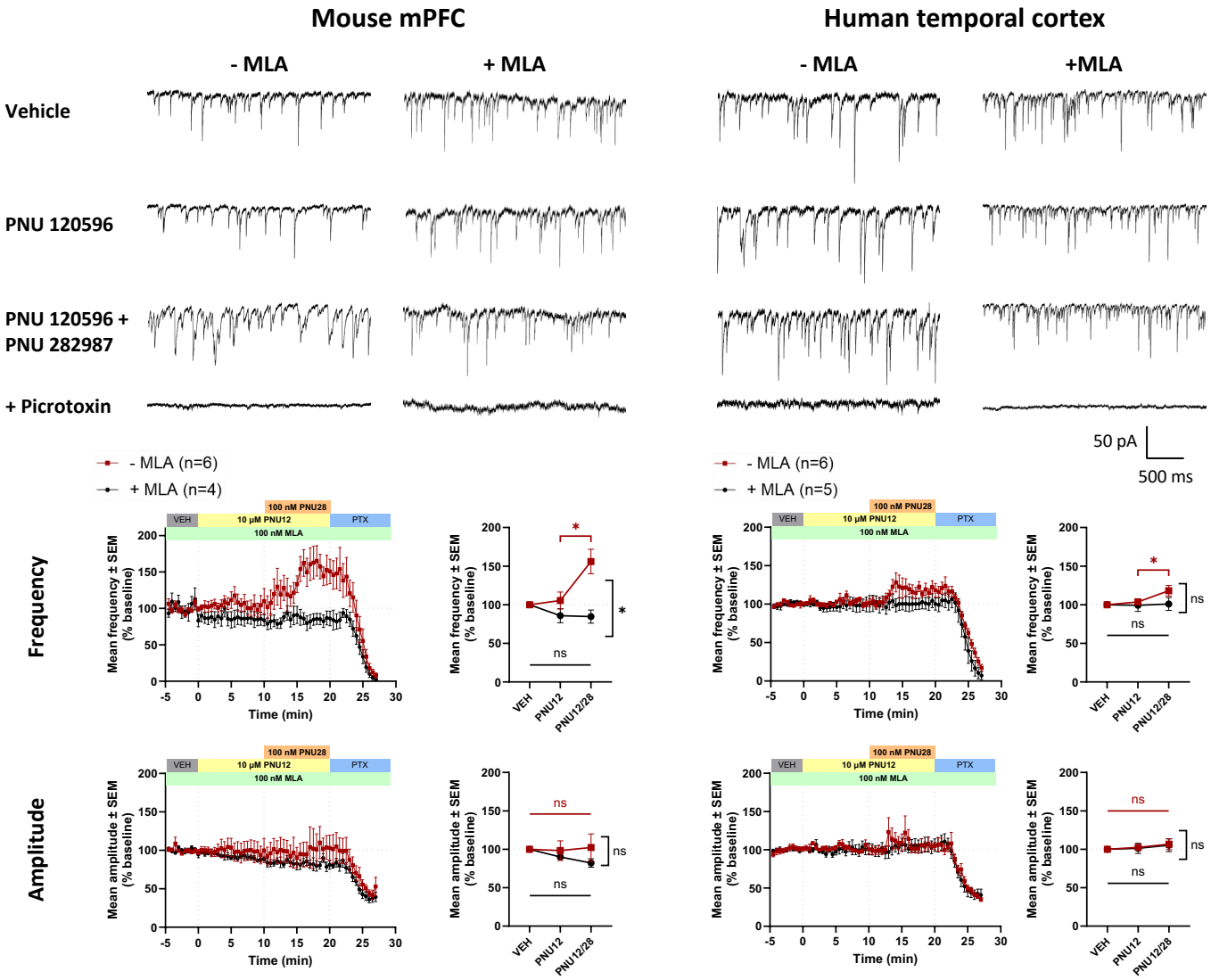
Figure 1



Results

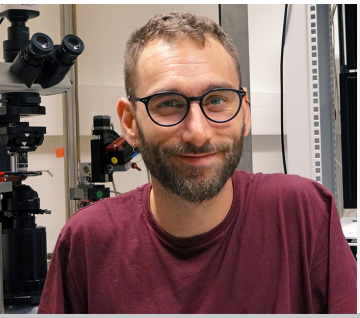
In mice, the combination of PNU 120596 and PNU 282987 increased sIPSC frequency by approximately 50% in mPFC L5 pyramidal neurons (Figure 2). sIPSC amplitude was not affected even if occasional bursts of large sIPSCs were observed. PNU 120596 alone did not alter sIPSC frequency or amplitude. Preapplication of MLA prevented α7nAChR-mediated upregulation of sIPSC frequency. Similar results were obtained in humans in which combining PNU 120596 and PNU 282987 induced a 20% increase of sIPSC frequency (Figure 2).

Figure 2



Conclusion

- Selective activation of α7nAChR by combining PNU 120596 and PNU 282987 increased inhibitory synaptic transmission in mouse mPFC and human temporal cortex through upregulation of sIPSC frequency.
- PNU 120596 alone did not alter sIPSC frequency or amplitude.
- α7nAChR-mediated upregulation of sIPSC frequency was prevented by preapplication of MLA.



About the author

Julien Artinian, Slice Electrophysiology Scientist at Neuroservices-Alliance, holds a Neuroscience Ph.D. from Marseille, France, focusing on epilepsy physiopathology. With a postdoc at the University of Montréal, he returned to France for further research in Bordeaux before joining Neuroservices-Alliance in 2019. Julien's transition from academia to the drug discovery industry was driven by his passion for collaborating closely with clients in experiment design, data interpretation, and research program decisions. Based in Bordeaux, Julien's work benefits from the unique access to human living brain samples, offering novel opportunities for drug discovery.