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GluN2D-containing NMDA receptors in cholinergic interneurons control dopamine release in the mouse striatum

Xiaoqun Zhang and Karima Chergui

Department of Physiology and Pharmacology, Section of Molecular Neurophysiology, Karolinska Institute, Stockholm, Sweden

Introduction and aim of the study

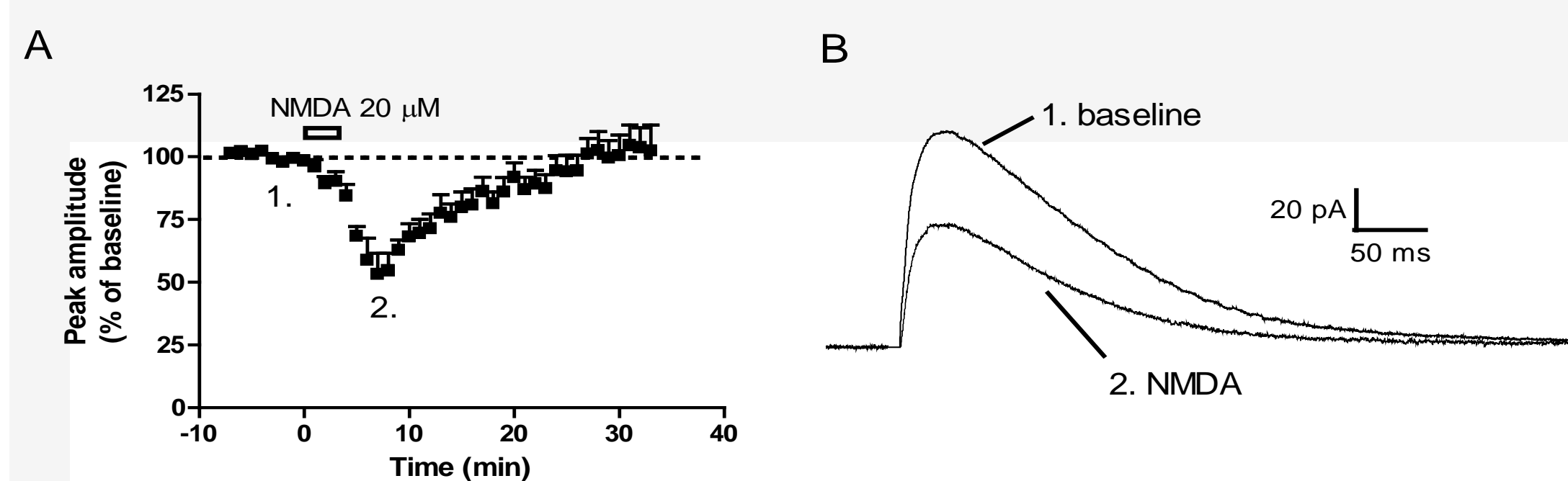
Dysfunctions of glutamatergic and dopaminergic neurotransmission play central roles in the pathophysiology of several neurological and psychiatric disorders. Because current therapeutic approaches are associated with severe side effects, it is essential to develop other tools for the treatment of these diseases. The GluN2 subunits of the N-Methyl-D-Aspartate (NMDA) receptor are attractive drug targets for therapeutic intervention in Parkinson's disease and drug addiction. However, the functional roles of the different GluN2 subunits, particularly in the regulation of dopamine release, is not clearly identified. We previously found that activation of NMDA receptors inhibits evoked, action potential-dependent, dopamine release in the mouse dorsal striatum and that this effect is not mediated by GluN2B-containing receptors. Given that dopaminergic neurons also express GluN2D, **our aim was to examine whether GluN2D-containing NMDA receptors modulate dopamine release in the striatum.**

Methods

- Male C57Bl6 mice were decapitated, their brains were rapidly removed and sagittal slices containing the striatum (400 μ m thick) were prepared with a microslicer. Slices were incubated for at least 1 h at 32°C in oxygenated artificial cerebro-spinal fluid (aCSF), before they were transferred to a recording chamber where they were continuously perfused with aCSF at 28°C.
- We monitored evoked-dopamine release through the use of carbon fiber electrodes combined with continuous amperometry. Single stimulation pulses were applied through a glass electrode filled with aCSF placed on the surface of the slice. Stimulation evoked a response corresponding to oxidation of dopamine at the surface of the carbon fiber electrode which was held at +500 mV.
- Drugs were applied in the perfusion solution in known concentrations.

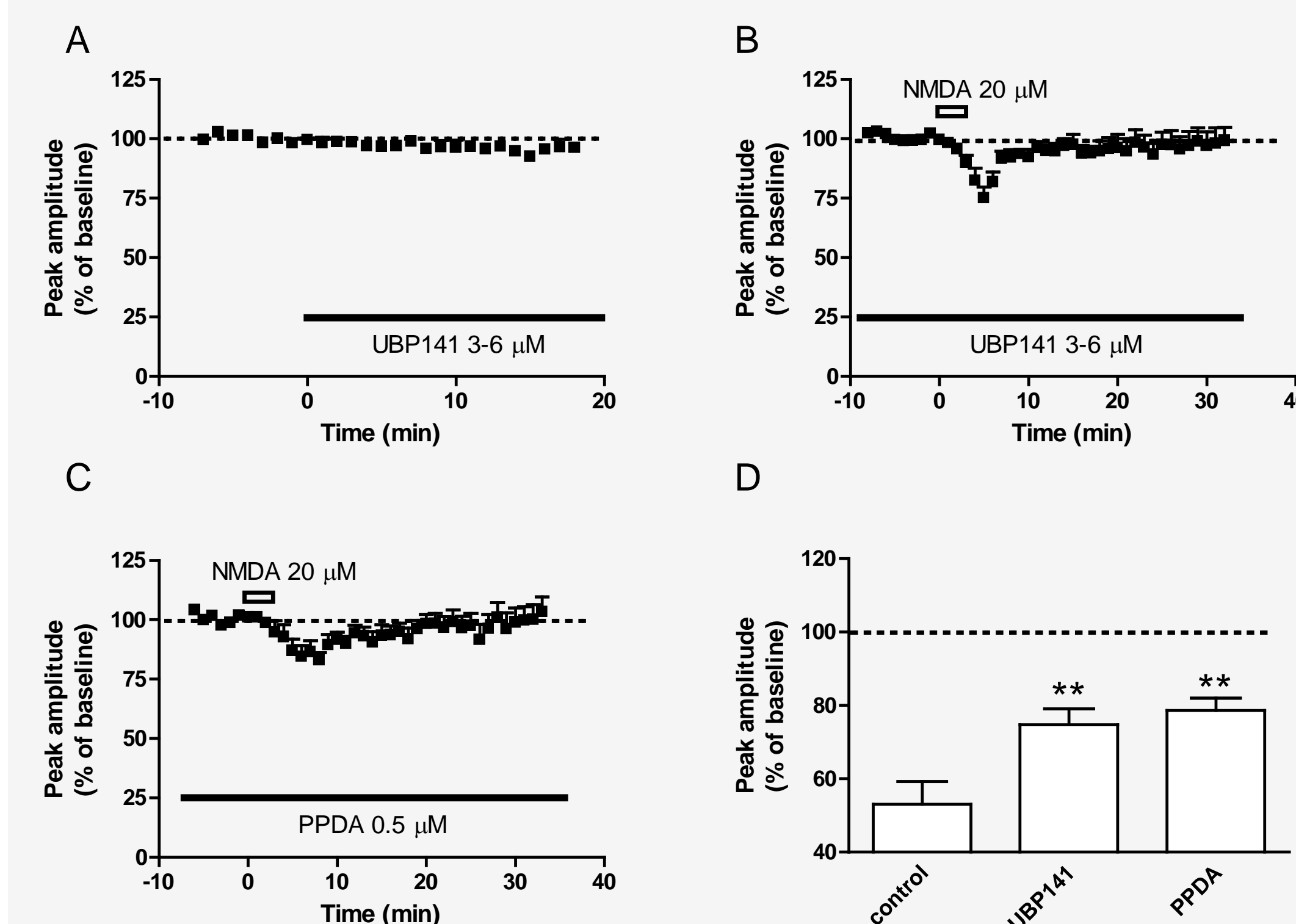
Results

I – NMDA depresses evoked-dopamine release in the dorsal striatum



A. Graph shows that NMDA (20 μ M), applied in the perfusion solution for 3 minutes, depresses the peak amplitude of the evoked-dopamine release in the dorsal striatum in control slices (n=8). B. Example of recording obtained in one slice before and after bath application of NMDA.

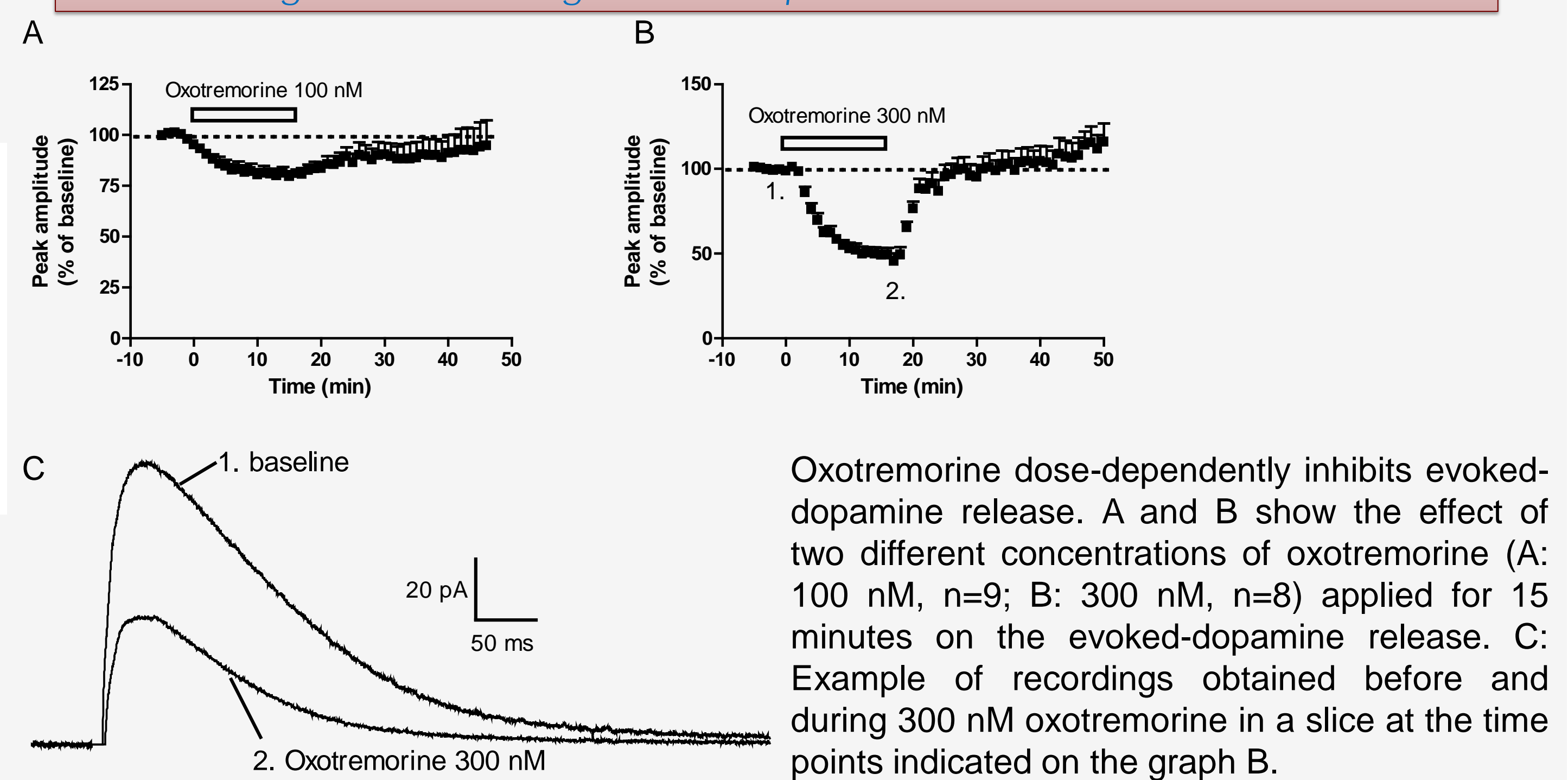
II – Effect of GluN2D antagonists on the depressant action of NMDA



The GluN2D antagonist, UBP 141 (3-6 μ M, n=4), has no effect on evoked dopamine release by itself (A), but it reduces the inhibitory effect of NMDA on evoked-dopamine release (B, n=8). The same observation was made with another GluN2D antagonist PPDA (0.5 μ M, C, n=8). D: Bar graph shows the averaged maximal effect of NMDA measured in individual slices for each treatment. **: p<0.01

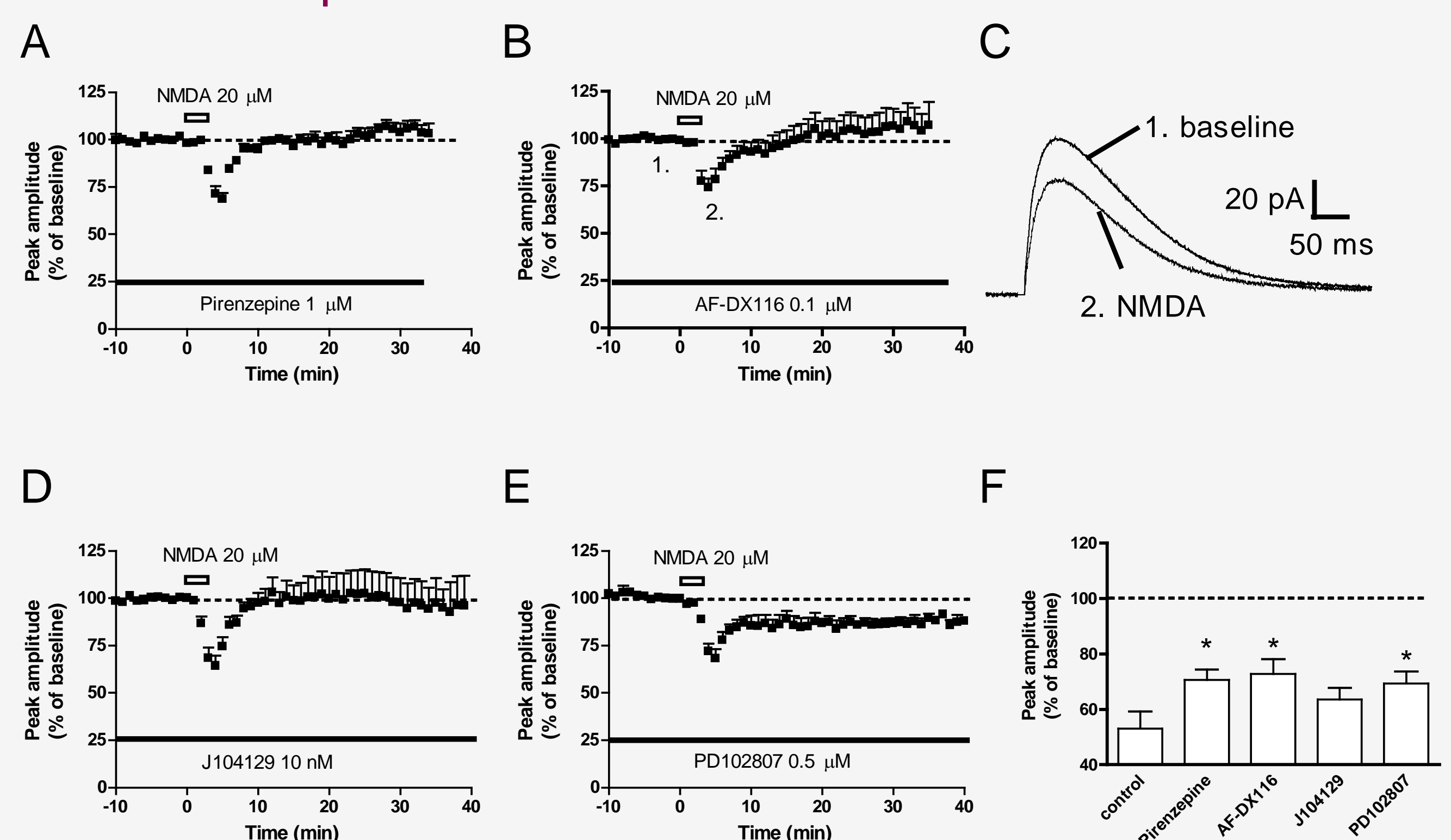
III – Effect of the muscarinic receptor agonist oxotremorine

To determine whether Cholinergic interneurons in the striatum, which also express GluN2D, contribute to the depressant action of NMDA, we tested the effect of muscarinic agonists and antagonists on dopamine release.



Oxotremorine dose-dependently inhibits evoked-dopamine release. A and B show the effect of two different concentrations of oxotremorine (A: 100 nM, n=9; B: 300 nM, n=8) applied for 15 minutes on the evoked-dopamine release. C: Example of recordings obtained before and during 300 nM oxotremorine in a slice at the time points indicated on the graph B.

IV – Effect of muscarinic receptor antagonist on the depressant action of NMDA.



Antagonists for M1 (A, Pirenzepine, n=11), M2 (B, AF-DX116, n=9), M3 (D, J104129, n=7) and M4 (E, PD102807, n=9) muscarinic receptors counteracted, to some degree, the effect of NMDA on evoked-dopamine release. C: Example of recordings obtained before and after NMDA in presence of AF-DX 116 at the time points indicated on the graph B. F: Bar graph shows the averaged maximal effect of NMDA for each treatment. *: p<0.05

Conclusion: This study identifies a critical role for cholinergic interneurons and for GluN2D-containing NMDA receptors in the presynaptic control of dopamine release in the striatum. GluN2D might thus constitute an alternative target for the development of novel pharmacological treatments.